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**EVALUATION OF THE PLANT EXTRACTS OF
AN ANTI-TUBERCULAR HERBAL REMEDY**

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PhD

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**EVALUATION OF THE PLANT EXTRACTS OF AN ANTI-
TUBERCULAR HERBAL REMEDY**

PATRICIA OCHEIMINUN ODUMOSU

**A thesis submitted in partial fulfilment of the requirements of the
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Dedicated to El gibbor..... Faint yet pursuing!

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ABBREVIATIONS

API	Atmospheric pressure ionisation
ASAP	Atmospheric Pressure Solids Analysis Probe
ATCC	American Type Culture Collection
BSAC	British Society for Antimicrobial Chemotherapy
COSY	COrrrelation SpectroscopY
CPZ	Chlorpromazine
DEPT	Distortionless Enhancement by Polarization Transfer
EI	Electron Impact
ELSD	Evaporative Light Scattering Detector
EtBr	Ethidium bromide
FID	Flame ionisation detector
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
ISA	Iso - sensitest agar
MIC	Minimum Inhibitory Concentration
MIC	Minimum Inhibitory Concentration
MtpB	Mycobacterium protein tyrosine phosphatidase B
MS	Mass Spectrometry
MTB	<i>Mycobacterium tuberculosis</i>

NCIMB	National Collection of Industrial and Marine Bacteria Ltd
NCPF	National Collection of Pathogenic Fungi
NCTC	National Collection of Type Cultures
NCYC	National Collection of Yeast Cultures
NMR	Nuclear Magnetic Resonance
NPLC	Normal Phase Liquid Chromatography
pca	Pyruvate carboxylase enzyme
PCM	<i>Pavetta crassipes</i> methanol extract
PDA	Photodiode array
RID	Refractive Index Detector
RPLC	Reversed Phase Liquid Chromatography
SFC	Supercritical Fluid Chromatography
STM	Streptomycin
TB	Tuberculosis
UoS	University of Sunderland
UV-VIS	Ultraviolet - Visible
XAM	<i>Ximenia americana</i> methanol extract
XAT	<i>Ximenia americana</i> total water extract
XDR-TB	Extensively Drug resistant Tuberculosis

LIST OF TABLES

- Table 1.1: Description of antigenic protein
- Table 1.2: Estimated TB Incidence, Prevalence and Mortality, 2008
- Table 1.3: Drug used in treating Drug Sensitive TB, Options for Treating MDR - TB and XDR -TB as well as new drugs under development
- Table 1.4: Summary of antimicrobial action of some clinically important antibiotics
- Table 2.1: Summary of chemical constituents reported in *X. americana* from literature
- Table 2.2: Summary of chemical constituents reported in *P. crassipes* from literature
- Table 3.1: Antimicrobial testing of *X. americana* root extracts
- Table 3.2: Antimicrobial testing of *P. crassipes* leaf extracts
- Table 3.3: Antifungal testing of *X. americana* root extracts
- Table 3.4: Anti-dermatophyte/acne testing of *X. americana* root extracts
- Table 3.5: Antimicrobial susceptibility of *P. crassipes* leaf extract to *P. acnes*
- Table 4.1: *M. aurum* testing at test concentrations of 1000 – 125 µg/mL
- Table 5.1: Mastring S M - 14 antibiotic discs content with code
- Table 5.2a: Data of *in vitro* drug interaction studies using *S. aureus*
- Table 5.2b: Data of *in vitro* drug interaction studies using *P. aeruginosa*
- Table 5.2c: Data of *in vitro* drug interaction studies using *E. coli*

- Table 5.3a: Summary of *in vitro* interaction studies of antibacterials with *P. crassipes* methanol extract
- Table 5.4: Schematic drawing to show methods used to calculate FIC index for each well
- Table 5.5: Checkerboard results used for interpretation of synergy
- Table 5.6: Table of value showing inhibition zone diameters for *P. crassipes* and *X. americana* against *E. coli* streptomycin resistant strain
- Table 6.1: Table of results showing the anti-TB activity of plant fractions
- Table 7.1: Preparative LC data of XAH
- Table 7.2a: ^{13}C and ^1H NMR assignments of stigmasterol at 75 and 300 MHz respectively
- Table 7.2b: ^1H assignments of XAD (500 MHz) and XAH1 (300 MHz)
- Table 7.3: LC semi – preparative data for XAD
- Table 7.4: Properties of some of the predicted compounds
- Table 7.5a: Preparative LC data of *P. crassipes* methanol extract
- Table 7.5b: ^1H and ^{13}C NMR spectral assignment of compound 5 from *P. crassipes* leaf dissolved in CD_3OD
- Table 7.5c: ^1H and ^{13}C NMR spectral assignment of compound 7 from *P. crassipes* leaf dissolved in CD_3OD

LIST OF FIGURES

- Fig. 1.0: *Mycobacterium tuberculosis* acid fast bacilli
- Fig. 1.1: Schematic diagram of mycobacterial cell wall
- Fig. 1.2: Colonies of mycobacterium tuberculosis on Lowenstein-Jensen medium
- Fig. 1.3: Diagram showing the distribution of soluble antigens in the genus mycobacterium
- Fig. 1.4.1: Chart illustrating estimated incidence of TB globally in 2008
- Fig. 1.4.2: Chart illustrating estimated prevalence of TB in 2008
- Fig. 1.4.3: Chart illustrating estimated mortality of TB in 2008
- Fig. 1.5: Chemical structures of first line drugs used in therapy
- Fig. 1.6a: First – line treatment of TB for drug-sensitive TB with proposed sites of action
- Fig. 1.6b: Drugs inhibited in MDR – TB (isoniazid and rifampicin) and possible effect treatments using other drugs in combination
- Fig. 1.6c: Drugs inhibited in XDR-TB (isoniazid, rifampicin, fluoroquinolones and at least one of three second line injectable) with possible effective treatment using other drugs in combination
- Fig. 1.6d: New TB drugs under development with their proposed mechanisms of action
- Fig. 1.7: Chemical structures of plant derived anti-tubercular compounds
- Fig. 2.1: Photos of ximenia plant in its habitat and as shown in Kew gardens herbarium catalogue

- Fig. 2.2: Photos of *Pavetta crassipes* in its habitat and as shown in Kew gardens herbarium catalogue
- Fig. 3.1: i) Guide showing how to read the MIC of antibiotic E- test gradient strip. ii) Examples of plates prepared experimentally showing ampicillin test strips
- Fig. 4.1: Conversion of MTT to formazan by succinic dehydrogenase enzyme
- Fig. 4.2: A 96 multi-well plate for conducting redox colorimetric assay
- Fig. 4.3: (i) Plates Showing *M. aurum* Control Plate (ii) Plates Showing growth inhibition zones for STM, XAD, XAH and solvent control disc
- Fig. 4.4: (i) TLC Plate (5 x7cm) Spotted with crude XAD (ii) Duplicate TLC Bio-autogram Visualised with MTT Solution (iii) TLC Plate (20 x 20cm) Spotted with XAD, XD1, XD2, XD3 and XD4, (TLC Fractions) XAH, XH4, XH5 (LC Fractions) and STM (iv) TLC Bio-autogram
- Fig. 4.5: (i) TLC Bio-autogram Control Plate (ii) TLC Bio-autogram Spotted with LC Fractions
- Fig. 5.1: a. Chart showing percentage differences in zone diameter of inhibition against *S. aureus* b. Chart showing percentage differences in zone diameter of inhibition against *P. aeruginosa* c. Chart showing percentage differences in zone diameter of inhibition against *E. coli*
- Fig. 5.2: i) Plates showing *X. americana* disc assay with *S. aureus* ii) with *E. coli* iii) with *P. aeruginosa*
- Fig. 5.3: Plates showing *P. crassipes* disc assay with *S. aureus*, *E. coli* and *P. aeruginosa*

- Fig. 5.4: i, ii) MIC determination of *P. crassipes* and *X. americana* methanol extracts by disc assay
- Fig. 5.5: Plates showing examples of *E. coli* inoculated plates
- Fig. 5.6: i) Plates show examples of *E. coli* inoculated control plates containing no plant extracts ii) with 10 mg/mL PCM incorporated into agar iii) with a combination of PCM and XAM in a 1:1 ratio
- Fig. 5.7: i) Streptomycin vs. *X. americana* methanol extract micro-dilution synergy assay (8 x 8) with *S. aureus* ii) wells with STM alone iii, iv) with a combination of streptomycin and *X. americana* extract in duplicate
- Fig. 5.8: Plates showing XAM and PCM MIC determination at high concentrations
- Fig. 5.9: Chemical structure of ethidium bromide
- Fig. 5.10: Graphs showing different concentrations of ethidium bromide with different ratios of *E. coli* and glucose control
- Fig. 5.11: Graph showing *E. coli* cells loaded with ethidium bromide
- Fig. 5.12: Graph of efflux experiment showing the influence of different concentrations of EPI (CPZ) on fluorescence
- Fig. 5.13: a. Graphs showing *M. aurum* cells loaded with ethidium bromide (1 – 6 µg/mL) in PBS and in glucose b. *M. aurum* cells loaded with 3 µg/mL ethidium bromide in PBS and glucose
- Fig. 7.1: Agilent 1200 preparative LC system with Chemstation data processing unit
- Fig. 7.2: Xevo QToF mass spectrometer

- Fig. 7.3: i) UV spectrum of hexane extract and fractions obtained from elution with DCM on an ACE 5 CN semi-preparative column ii) UV spectrum of DCM extract iii) UV spectrum of methanol extract iv) UV spectrum of water extract
- Fig. 7.4: i, ii) Normal phase analytical LC profile of *X. americana* extracts on ACE 5 SIL column, 250 x 4.6 mm i.d on HP 1090 LC system with PDA detector iii) Normal phase semi-preparative LC profile of *X. americana* hexane extract separated with 100 % DCM on ACE 5 CN column iv) Analytical LC profile of peak 1 from XAH on ACE 5 SIL column v) Analytical LC profile of peak 2 from XAH on ACE 5 SIL column vi) Analytical LC profile of peak 3 from XAH on ACE 5 SIL column
- Fig. 7.5: Reversed phase analytical profile of *X. americana* methanol extract on ACE 5 C18 column
- Fig. 7.6: a. Proton NMR spectrum of DCM extract of *X. americana* (XAD) b. Proton NMR spectrum of XAH peak 1 c. Proton NMR spectrum of β -sitosterol (reference standard) d. Proton NMR spectrum of stigmasterol (reference standard) e. HSQC spectrum of *X. americana* DCM fraction f. Carbon 13 NMR spectrum of XAH peak1 g. EI mass spectrum of crude XAD extract h. Mass spectrum of *X. americana* hexane extract
- Fig. 7.7: i) Analytical and preparative LC profile of XAD on ACE 5 CN column, 250 x 4.6mm i.d at 1 and 5 mL/min ii) EI mass spectrum of *X. americana extract*, XD1 iii) EI mass spectrum of *X. americana extract*, XD2 iv) EI mass spectrum of *X. americana extract*, XD3 v) EI mass spectrum of *X. americana extract*, XDW
- Fig. 7.8: LC chromatogram of XAH on ACE 5 SIL column using DCM

- Fig. 7.9: i) TLC plate sprayed with 2-DNP and H₂SO₄ ii) H₂SO₄ sprayed TLC plate with crude and LC fractions from XH and XD in hexane-chloroform-methanol (4:4:1 v/v/v) solvent system visualized with UV lamp at 254 and 366 nm
- Fig. 7.10: Chemical structures of suggested compounds from *X. americana*
- Fig. 7.11a: i) Mass spectrum of XD1 compound 1 ii) mass spectrum of XD1 compound 2
- Fig. 7.11b: i) Mass spectrum of XD2 compound 1 ii) mass spectrum of XD2 compound 2 iii) mass spectrum of XD2 compound 3 iv) mass spectrum of XD2 compound 4 v) mass spectrum of XD2 compound 5
- Fig. 7.11c: i) Mass spectrum of XD3 compound 1 ii) mass spectrum of XD3 compound 2
- Fig. 7.11d: i) Proton NMR spectrum of TLC spot 1, XD1 (300 MHz) ii) ¹³C DEPT NMR spectrum of XD1 iii) ¹³C DEPT NMR spectrum of XD1 (zoomed) iv) Proton NMR spectrum of TLC spot 2, XD2 (300 MHz) v) Proton NMR spectrum of TLC spot 3, XD3 (300 MHz)
- Fig. 7.12a: GC analysis of a mixture of 1 - stearic acid, 2 - cholesterol, 3 - stigmasterol, 4 - sitosterol
- Fig. 7.12b: GC profiles for i) crude XH ii) crude XD iii) XH1 enriched fraction iv) XD1 enriched fraction v) enriched fraction XH2 vi) enriched fraction XD2 vii) spiked fraction XH1 viii) spiked fraction XD1 ix) spiked fraction XH2 x) spiked fraction XD2
- Fig. 7.13a: i) LC profiles of samples analysed on Agilent 1290 Infinity system with RID detector on ACE 5 SIL column at 230 nm ii) sample containing 1 mg/mL XAD iii) sample containing 1 mg/mL sitosterol iv) LC profile of XAT (1 mg/mL) on ACE 5 SIL column at 210 nm v) LC profile of XAT 1 mg/mL on HYPERCARB column run with DCM at 210 nm vi) LC

profile of XAD 10 mg/mL run on HYPERCARB column with DCM at 254 nm

Fig. 7.14a: i) Waters analytical SFC system, Method station II equipment ii) schematic overview of the components of SFC coupled to PDA detector and mass spectrometer

Fig. 7.14b: i, ii) SFC profiles of standards; lanosterol, stigmasterol and sitosterol on 2-ethylpyridine column with ELSD and UV detection iii) SFC profile of a mixture of standard compounds, lanosterol, stigmasterol and sitosterol on different columns using UV detection

Fig. 7.14c: i) SFC profile of a mixture of lanosterol, stigmasterol and sitosterol on 2-ethylpyridine column ii) SFC profile of XAD using the same gradient conditions as the standards iii) SFC profile of XAD with modified gradient condition iv) SFC profile of XAD on 2-ethylpyridine column using isocratic conditions v) SFC profile of XAD on 2-ethylpyridine column with pressure modification

Fig. 7.14d: i) SFC profile of XD3 on 2-ethylpyridine column using gradient conditions with EtOH as co-solvent ii) SFC profile of XD3 on 2-ethylpyridine column using a modified flow rate with MeOH as co-solvent

Fig. 7.14e: i) SFC profile of XD4 on 2-ethylpyridine column using gradient conditions with EtOH as co-solvent ii) SFC profile of XD4 on 2-ethylpyridine column using isocratic conditions with EtOH as co-solvent iii) SFC profile of XD4 screened with different columns using UV detection and gradient conditions with EtOH as co-solvent iv) SFC profile of XD4 screened with different columns using ELSD detection and gradient conditions with EtOH as co-solvent

- Fig. 7.15a: Semi-preparative LC profiles of *P. crassipes* methanol extract (10 mg/mL) on ACE 5 C18 column, 250 x 10 mm i.d with gradient elution using methanol-water mobile phase
- Fig. 7.15b: Structures of 5-O-caffeoyl quinic acid methyl esters and quercetin-3-rutinoside
- Fig. 7.15c: i) LC-MS UV trace of *P. crassipes* methanol extract obtained on Esquire 3000+ mass spectrometer at 225 nm ii) mass spectrum of *P. crassipes* methanol extract in positive ion mode iii) mass spectrum of methanol extract in positive ion mode zoomed 1 iv) spectrum of methanol extract in positive ion mode zoomed 2
- Fig. 7.15d: i) Accurate mass spectrum of PCM 5 from *P. crassipes* leaf by ESI-MS (negative ion mode) ii) proton NMR spectrum of PCM 5 from *P. crassipes* leaf iii) carbon 13 NMR spectrum (125 MHz) for PCM 5 from *P. crassipes* leaf
- Fig. 7.15e: i) Accurate mass measurement for PCM 7 from *P. crassipes* leaf by ESI-MS (negative ion mode) ii) proton NMR spectrum (500 MHz) of PCM 7 from *P. crassipes* leaf iii) Carbon 13 NMR spectrum of PCM 7 from *P. crassipes* leaf
- Fig. 7.16: i) Proton NMR spectrum of PCM 2 from *P. crassipes* leaf ii) HSQC spectrum of PCM 2 from *P. crassipes* leaf
- Fig. 7.17: i) Proton NMR spectrum of PCM 3 from *P. crassipes* leaf ii) Proton NMR spectrum of PCM 3 from *P. crassipes* leaf (zoomed) iii) HSQC spectrum of PCM 3 from *P. crassipes* leaf
- Fig. 7.18: i) Proton NMR spectrum (300 MHz) of PCM 4 from *P. crassipes* leaf
- Fig. 7.19: i) Proton NMR spectrum (300 MHz) of PCM 6 from *P. crassipes* leaf ii) HSQC spectrum of PCM 6 from *P. crassipes* leaf

- Fig. 7.20: LC profile of *P. crassipes* leaf hexane extract on ACE 5 SIL column, 250 x 4.6 mm i.d using DCM-hexane (70:30 v/v) mobile phase
- Fig.7.21: SFC profile of *P. crassipes* total water extract solvent screening on fluorophenyl column, 150 x 4.6 mm i.d using methanol-water (98:2) mobile phase
- Fig. 8.1: Flow chart showing the analytical steps taken with *X. americana* extract
- Fig. 8.2: Flow chart showing the analytical steps taken with *P. crassipes* extract

ABSTRACT

Ximenia americana root bark (Olacaceae) and *Pavetta crassipes* (Rubiaceae) leaf used in Nigerian traditional medicine were tested individually against clinical isolate of *Mycobacterium tuberculosis* by Lowenstein - Jensen method. Crude aqueous extracts of *X. americana* and *P. crassipes* exhibited minimum inhibitory activity (MIC) of 100 µg/mL and 200 µg/mL respectively.

Sequential screening with solvents of different polarities was used in evaluation tests to readily locate the source of the activity against tuberculosis and for conditions related to skin diseases since it was readily available. In general, antimicrobial screening of crude extracts gave MICs ranging from 31.25 µg/mL to > 5 mg/mL, with *X. americana* methanol extract being most active at 31.25 µg/mL against *Staphylococcus aureus*. In an effort to determine possible mechanisms of action, synergistic interaction studies between standard antibiotics and plant extracts were carried out with some synergy being observed between *X. americana* extract and streptomycin.

Hexane (MIC 60.6 µg/mL) and dichloromethane (MIC 30.5 µg/mL) fractions of *X. americana* exhibited 94.3 % and 96.4 % inhibition against *M. tuberculosis* H37HRv (virulent strain) while *P. crassipes* hexane fraction had 86.7% inhibition at > 64 µg/mL. Using HPLC, TLC, GC, 1D and 2D-NMR as well as mass spectral analyses it was possible to identify rutin and 5-O-caffeoyl quinic acid methyl ester from *P. crassipes*. It proved extremely difficult to identify compounds from LC and TLC fractions from the non-polar extracts of *X. americana* responsible for anti-TB activity. There was some spectroscopic evidence from these fractions for closely related phytosterol esters and individual compounds such as stigmast-3, 5-diene, stigmastane oleate and β-sitosterol.

Subsequent LC work with refractive index detection and SFC with evaporative light scattering data confirmed that the difficulties in assignment arose from the presence of non-UV absorbing non-volatile co-eluting compounds. Preparative

SFC or SFC-MS with the aid of the NIST database would have been needed for identification.

Overall, these results lend some credence to the claims of the Nigerian remedy and potentially could be a source of assay biomarkers for monitoring its safety, efficacy and quality as required by IRCH (International Regulatory Co-operation for Herbal Medicines).

TABLE OF CONTENTS

	Page
Title Page - - - - -	i
Dedication - - - - -	ii
Acknowledgements - - - - -	iii
Abbreviations - - - - -	v
List of Tables - - - - -	vii
List of Figures - - - - -	ix
Abstract - - - - -	xvii
Table of Contents - - - - -	xix

1.0 INTRODUCTION

1.1 History - - - - -	1
1.2 General description of <i>Mycobacterium tuberculosis</i> genus -	3
1.2.1 Physiology and morphology of mycobacteria - - - - -	3
1.2.2 Growth and cultural characteristics - - - - -	5
1.2.3 Antigenic structure - - - - -	6
1.2.4 Pathogenicity and virulence determinants - - - - -	8
1.2.5 Genetics of <i>M. tuberculosis</i> - - - - -	9
1.2.6 Clinical findings/diagnosis - - - - -	10

1.2.7	Immunity and vaccination	-	-	-	-	-	-	11
1.3	Epidemiology (global incidence)	-	-	-	-	-	-	13
1.3.1	TB and HIV	-	-	-	-	-	-	17
1.3.2	Principles of therapy	-	-	-	-	-	-	17
1.3.3	Drugs used in treatment and mechanisms of action	-	-	-	-	-	-	20
1.3.4	Antibiotic and molecular basis of resistance	-	-	-	-	-	-	26
1.4	Need for new drugs	-	-	-	-	-	-	26
1.4.1	Natural products in drug discovery	-	-	-	-	-	-	28
1.4.2	Plant derived anti-tubercular plant compounds	-	-	-	-	-	-	29
1.4.3	Drugs development from plant sources	-	-	-	-	-	-	31
1.4.3.1	Commercial consideration	-	-	-	-	-	-	31
1.4.3.2	Biological consideration	-	-	-	-	-	-	33
1.4.4	Rationale for choosing plants used in the research study	-	-	-	-	-	-	33
1.5	Research Aims and Objectives	-	-	-	-	-	-	34

2.0 LITERATURE REVIEW: MEDICINAL PLANTS

2.1	Introduction							
2.1.1	Description of <i>X. americana</i>	-	-	-	-	-	-	35
2.1.2	Medicinal uses	-	-	-	-	-	-	36
2.1.3	Constituents and pharmacology	-	-	-	-	-	-	37
2.1.4	Description of <i>Pavetta crassipes</i>	-	-	-	-	-	-	39

2.1.5	Medicinal uses	-	-	-	-	-	-	-	40
2.1.6	Constituents and pharmacology	-	-	-	-	-	-	-	40
3.0	GENERAL ANTIMICROBIAL SCREENING OF PLANT EXTRACT								
	AGAINST BACTERIA AND FUNGI								
3.1	Background	-	-	-	-	-	-	-	42
3.1.1	Introduction	-	-	-	-	-	-	-	42
3.1.2	Agar-diffusion methods	-	-	-	-	-	-	-	43
3.1.3	Dilution methods	-	-	-	-	-	-	-	44
3.1.4	Bio-autographic methods	-	-	-	-	-	-	-	47
3.1.5	Inoculum	-	-	-	-	-	-	-	48
3.2	Aims and Objectives	-	-	-	-	-	-	-	48
3.3	Experimental								
3.3.1	Materials	-	-	-	-	-	-	-	49
3.3.2	Equipments	-	-	-	-	-	-	-	49
3.3.3	Bacterial and fungal strains	-	-	-	-	-	-	-	49
3.3.4	Preparation of reagents and media	-	-	-	-	-	-	-	49
3.3.4.1	Turbidity standard for inoculum preparation – McFarland								
	standard	-	-	-	-	-	-	-	49
3.3.4.2	Nutrient agar/broth	-	-	-	-	-	-	-	50
3.3.4.3	Iso-sensitest agar/broth	-	-	-	-	-	-	-	50

3.3.5	Isolation and cultivation of bacterial and fungal species	-	50
3.3.6	Determination of minimum inhibitory concentration by agar dilution	-	50
3.3.7	Antifungal susceptibility testing by agar dilution method	-	51
3.3.4	Results and Discussion	- - - - -	52
3.5	Conclusion	- - - - -	56

4.0 DRUG SUSCEPTIBILITY TESTING OF PLANT EXTRACT

AGAINST *Mycobacterium aurum*

4.1	Background	- - - - -	57
4.1.1	Introduction	- - - - -	57
4.2	Aims and objectives	- - - - -	59
4.3	Experimental		
4.3.1	Materials	- - - - -	59
4.3.2	Equipment	- - - - -	59
4.3.3	Preparation of reagents and media	- - - - -	59
4.3.3.1	McFarland standard	- - - - -	59
4.3.3.2	Glucose agar	- - - - -	59
4.3.3.3	Stock storage medium for bacterial culture	- - - - -	60
4.3.4	Isolation and cultivation of <i>M. aurum</i>	- - - - -	60
4.3.5	Determination of MIC by micro broth dilution assay	- -	60

4.3.6	Determination of drug susceptibility by disc diffusion assay	-	-	-	-	-	-	-	-	61
4.3.7	Determination of active fractions by agar overlay bio-autography	-	-	-	-	-	-	-	-	62
4.4	Results and Discussion	-	-	-	-	-	-	-	-	62
4.5	Conclusion	-	-	-	-	-	-	-	-	69
5.0	SYNERGY AND EFFLUX STUDIES ON METHANOL EXTRACTS									
	OF <i>X. americana</i> ROOT AND <i>P. crassipes</i> LEAF									
5.1	Background	-	-	-	-	-	-	-	-	70
5.1.1	Introduction	-	-	-	-	-	-	-	-	71
5.1.2	Synergy studies	-	-	-	-	-	-	-	-	71
5.1.2.1	Philosophy of herbal therapy	-	-	-	-	-	-	-	-	72
5.1.3	Synergy testing	-	-	-	-	-	-	-	-	72
5.2	Aims and objectives	-	-	-	-	-	-	-	-	73
5.3	Experimental									
5.3.1	Materials	-	-	-	-	-	-	-	-	73
5.3.2	Determination of minimum inhibitory concentration by broth dilution method	-	-	-	-	-	-	-	-	73
5.3.3	Determination of minimum inhibitory concentration by disc assay	-	-	-	-	-	-	-	-	74
5.3.4	Drug interaction assay using Mastring- S antibiotic strips	-	-	-	-	-	-	-	-	74

5.3.5	Checkerboard (FIC) assay of <i>X. americana</i> methanol extract and streptomycin	-	-	-	-	-	-	75
5.3.6	Computation of drug interactions	-	-	-	-	-	-	75
5.3.7	Statistical analysis	-	-	-	-	-	-	76
5.4	Results and Discussion	-	-	-	-	-	-	76
5.4.1	Preliminary synergy testing	-	-	-	-	-	-	76
5.4.2	Synergy test with combination of streptomycin and XAM against <i>S. aureus</i>	-	-	-	-	-	-	78
5.4.3	Synergy testing of combination of gentamicin and methanol extract (PCM and XAM) against <i>E. coli</i> SR	-	-	-	-	-	-	78
5.4.4	Statistical analysis	-	-	-	-	-	-	79
5.5	Evaluation of efflux pumps inhibitory property by Rotor – gene 3000	-	-	-	-	-	-	93
5.6	Aims and Objectives	-	-	-	-	-	-	95
5.7	Experimental							
5.7.1	Materials	-	-	-	-	-	-	96
5.7.2	Equipment	-	-	-	-	-	-	96
5.7.3	Determination of MIC by E -test and macro – dilution	-	-					96
5.7.4	One concentration synergy testing	-	-	-	-	-	-	96
5.7.5	EtBr concentration curve	-	-	-	-	-	-	97
5.7.6	EtBr accumulation assay	-	-	-	-	-	-	97

5.7.7	EtBr efflux assay	-	-	-	-	-	-	-	98
5.8	Results and Discussion								
5.8.1	MIC determination against <i>E. coli</i> streptomycin resistant strain	-							98
5.8.2	One concentration synergy testing	-	-	-	-				98
5.8.3	EtBr concentration curve	-	-	-	-	-	-		99
5.8.4	Demonstration of accumulation of EtBr	-	-						100
5.8.5	Demonstration of efflux of EtBr mediated by <i>E. coli</i> efflux pumps	-							101
5.8.6	Demonstration of accumulation and efflux in <i>M. aurum</i>	-							102
5.9	Conclusion	-	-	-	-	-	-	-	103

6.0 DRUG SUSCEPTIBILITY TESTING OF PLANT EXTRACTS

AGAINST *M. tuberculosis*

6.1	Background	-	-	-	-	-	-	-	105
6.1.1	Introduction	-	-	-	-	-	-	-	105
6.2	Aims and Objectives	-	-	-	-	-	-	-	107
6.3	Experimental								
6.3.1	Materials	-	-	-	-	-	-	-	107
6.3.2	Equipment	-	-	-	-	-	-	-	108
6.3.3	Preparation of reagents and media	-	-	-	-	-	-	-	108
6.3.4	Methods								
6.3.4.1	Isolation and cultivation of <i>M. tuberculosis</i> H37R _v	-	-	-	-	-	-	-	108

6.3.4.2	Determination of MIC against <i>M. tuberculosis</i> H37R _v	-	-	-	-	-	-	-	108
6.4	Results and Discussion	-	-	-	-	-	-	-	109
6.5	Conclusion	-	-	-	-	-	-	-	111

7.0 CHROMATOGRAPHIC AND SPECTRAL CHARACTERISATION OF PLANT EXTRACTS

7.1	Background	-	-	-	-	-	-	-	112
7.1.1	Introduction	-	-	-	-	-	-	-	112
7.1.2	Hyphenated techniques	-	-	-	-	-	-	-	115
7.2	Aims and Objectives	-	-	-	-	-	-	-	116
7.3	Experimental								
7.3.1	Materials	-	-	-	-	-	-	-	117
7.3.2	Equipment and accessories	-	-	-	-	-	-	-	117
7.3.3	Reagents for TLC	-	-	-	-	-	-	-	117
7.3.4	Methods								
7.3.4.1	Collection and preparation of plants materials	-	-	-	-	-	-	-	118
7.3.4.2	Preparation of mobile phases	-	-	-	-	-	-	-	119
7.3.4.3	UV analysis	-	-	-	-	-	-	-	119
7.3.4.4	HPLC analysis	-	-	-	-	-	-	-	119
7.3.4.5	Fractionation of <i>X. americana</i> crude extract and isolation of active constituents by HPLC	-	-	-	-	-	-	-	120

7.3.4.6 Isolation of active fraction from <i>X. americana</i> hexane and dichloro- methane LC fractions by Thin - layer chromatography	-	-	-	-	121
7.3.4.7 Gas Chromatography of <i>X. americana</i> hexane and dichloromethane extracts	--	-	-	-	121
7.3.4.8 HPLC analysis using refractive index detector	-	-	-	-	122
7.3.4.9 HPLC analysis of XD3 and XD4 (Sub-fraction of <i>M. tuberculosis</i> active fraction monitored with <i>M. aurum</i>)	-	-	-	-	122
7.4 Results and Discussion					
7.4.1 UV analysis of Crude <i>X. americana</i> extract	-	-	-	-	124
7.4.2 Initial separation of <i>X. americana</i> extracts by HPLC	-	-	-	-	126
7.4.3 Purity checks for crude hexane and dichloromethane fractions of <i>X. americana</i> and identification	-	-	-	-	129
7.4.4 LC separation and mass spectral analysis of XAD fractions	-	-	-	-	142
7.4.5 TLC analysis of LC, crude hexane and dichloromethane fractions	-	-	-	-	149
7.4.5.1 LC analysis	-	-	-	-	149
7.4.5.2 TLC analysis of fractions (LC and crude samples)	-	-	-	-	150
7.4.5.3 Identification of compounds from crude dichloromethane <i>X. americana</i> root extract by HR TOF – MS EI +	-	-	-	-	152
7.4.5.4 Gas chromatographic analysis of crude hexane, dichloromethane and column fractions of <i>X. americana</i>	-	-	-	-	171

7.4.5.5	LC analysis of XAH, XAD, XAT, XD3 and XD4 (active sub-fractions)								
	using refractive index detector and photodiode array detector	-							178
7.5	Supercritical Fluid Chromatography	-	-	-	-	-	-	-	183
7.6	Experimental conditions	-	-	-	-	-	-	-	183
7.7	Results and Discussion	-	-	-	-	-	-	-	185
7.8	Identification of compounds from <i>P. crassipes</i> leaf	-	-	-	-	-	-	-	195
8.0	CONCLUSION AND SUGGESTIONS FOR FURTHER WORK								
8.1	Further Work	-	-	-	-	-	-	-	224
	References								