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

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

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A thesis submitted in partial fulfilment of the requirements of the University of Sunderland for the degree of Doctor of Philosophy

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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	COrrelation 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
MptpB	Mycobacterium protein tyrosine phosphatidase B
MS	Mass Spectrometry
MTB	*Mycobacterium tuberculosis*

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	*Pavetta crassipes* 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	*Ximenia americana* methanol extract
XAT	*Ximenia americana* total water extract
XDR-TB	Extensively Drug resistant Tuberculosis

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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).

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