ISOLATION AND CHARACTERIZATION OF PRIVILEGED STRUCTURES FROM *Hunteria umbellata* (APOCYNACEAE) AND THE EVALUATION OF THEIR CANCER MULTIDRUG RESISTANCE INHIBITORY ACTIVITIES.

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BY

AJALA, OLUSEGUN SAMSON
B. Pharm. (Ife), M. Sc. (Lagos)

A thesis submitted to the School of Postgraduate Studies, University of Lagos, in fulfilment of the requirements for the award of the degree of Doctor of Philosophy (PhD) in Pharmaceutical Chemistry.

NOVEMBER, 2011

SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS

CERTIFICATION

This is to certify that the thesis:

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Submitted to the
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DOCTOR OF PHILOSOPHY (Ph.D.)
Is a record of original research carried out

By:

AJALA, OLUSEGUN SAMSON
In the Department of Pharmaceutical Chemistry

AUTHOR'S NAME	SIGNATURE	DATE
1ST SUPERVISOR'S NAME	SIGNATURE	DATE
2ND SUPERVISOR'S NAME	SIGNATURE	DATE
1ST INTERNAL EXAMINER	SIGNATURE	DATE
2ND INTERNAL EXAMINER	SIGNATURE	DATE
EXTERNAL EXAMINER	SIGNATURE	DATE
SPGS REPRESENTATIVE	SIGNATURE	DATE

DEDICATION

This thesis is dedicated to the **LION OF THE TRIBE OF JUDAH,** and to all those who know who **He** is and are waiting for **His** appearing.

AKNOWLEDGEMENTS

First and foremost, my profound gratitude goes to the Lord God Almighty for the gifts of life and good health, divine provision and protection throughout the course of the PhD programme.

My special appreciation goes to my supervisor, Prof. H.A.B. Coker, whose contributions to the successful completion of this work can neither be qualified nor quantified by mere words: In his usual characteristics as voice for the voiceless, feet for the feeble , and eyes for the blind, Prof. literally lent his full weight into lifting me above all hurdles and bottlenecks that could have prevented me from travelling abroad to do the part of this work that could not be done in Nigeria. His personal financial involvement in the work was unquantifiable. His moral and spiritual supports, as Rabbi and Bishop, and inspiring words provided the much needed internal vigour for moving forwards even when there seemed to be no way forward. Prof. Single-handedly taught me how to create and work at small interphases between natural product chemistry and molecular pathology, emphasising always the importance of working with nature or natural materials without necessarily losing the primary focus of being a medicinal chemist. His constructive criticisms of the work itself gave it direction and a logical conclusion. Prof., I doubt if anyone has ever or will ever again contribute to my life as you have done in these few years I have come to know you. I feel so privileged to have known you and I say a big thank you, sir, for all you have done for me.

Special appreciation also goes to my co-supervisor, Dr. S.A. Adesegun, for his pieces of advice and technical assistance without which this work would never have been completed. Thank you very much sir.

Prof. Robert J. Capon of the institute for Molecular Bioscience (IMB), University of Queensland, Australia, nominated me for the Occupational trainee programme of the University of Queensland, and hosted me in his laboratory for my entire stay in Brisbane, Queensland, Australia as an occupational trainee. Thank you very much.

I received financial supports from the Science and Technology Education Post Basic (STEP-B) project, and from the Education Trust Funds (ETF), both of which facilitated my trip and stay in Australia, and are hereby thankfully acknowledged. I also use this opportunity to thank all the UNILAG accounting and administrative staff that saw to the prompt disbursements of these funds.

I cannot forget the entire staff members of the department of pharmaceutical chemistry for their numerous and diverse contributions towards the successful completion of this work.

Finally but by no means the least, I appreciate specially the unflinching love, support and understanding of my darling wife and loving children, especially during my long period of absence from them.

To all the afore-mentioned and several others that time and space would not permit mentioning, I say thank you and God bless.

Olusegun Samson Ajala

Lagos, 2011

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ABSTRACT

Chemotherapy remains the best means of treatment of metastatic cancer. It is however bedevilled by the phenomenon of multidrug resistance (MDR) whereby cancer cells evade the cytotoxic effects of chemotherapeutic agents of diverse chemical structures and mechanisms of action. The MDR phenomenon has been found to be principally mediated by the over experession, by cancer cells, of the ATP-Binding Cassette (ABC) superfamily of trans membrane transporter proteins, the most important member of which is the Permeability glycoprotein (P-gp).

The biogenetic and structural analogy between serotonin, an established substrate of a known ATP-dependent transmembrane transporter (i.e. serotonin reuptake pump), and monoterpenoid indolealkaloids (MTIAs) provided the rationale for a structure-guided exploration of the leaf, stem-bark, and seeds of the plant *Hunteria umbellata* (Apocynaceae) for MTIAs as highly probable inhibitors of the P-gp. Furthermore, an observation of the fact that MTIAs are essentially privileged structures prompted similar structure-guided exploration of the same plant materials for iridoid- and terpenoid- privileged structures to enhance the chances of isolating putative P-gp inhibitors from the plant.

The main structural-guide was ^{1}H NMR profiling at 600MHz; isolation and purification were largely by High Performance Liquid Chromatography (HPLC); gross structure elucidation was done by a combination of 1D and 2D NMR (^{1}H , ^{13}C , ^{1}H-^{1}H gCOSY, DEPT-edited ^{1}H-^{13}C HSQC and ^{1}H-^{13}C gHMBC) data acquisition with subsequent interpretation aided by molecular formula information from High Resolution ElectroSpray Ionization Mass Spectrometry (HRESIMS). Assignment of relative configuration, where necessary, was done by coupling constants analysis, supported in a few cases by the ^{1}H-^{1}H ROESY 2D NMR

spectral analysis. P-gp inhibition evaluation was by slightly modified standard procedures; using calcein (a fluorescing dye) accumulation in SW620 AD300 (a MDR subline of human colon carcinoma cells selected at 300ng/ml of adriamycin) as indicator.

In all, three new MTIAs (N-[2-(2-carbomethoxy-3-indolyl)ethyl]-3-ethylpyridinium, 4-chloromethylakuammineammonium ion, and desmethylserpentinine), four known MTIAs (serpentine, pseudoakuammigine, 4-methylhuntrabrineammonium ion and strictosidinic acid), one known triterpenoid (ursolic acid) and one new lactone iridoid glucoside (1-glucopyranosyl-8-methyliridan-3-en-4,7-carbolactone) were isolated and chemically characterized. Four of the compounds (N-[2-(2-carbomethoxy-3-indolyl)ethyl]-3-ethylpyridinium, serpentine, strictosidinic acid and pseudoakuammigine) demonstrated highly significant P-gp inhibitory activities ($P < 0.01$), each at 25µg/ml.

The discovered afore-mentioned four compounds with highly significant P-gp inhibitory activities could be employed as leads the development of clinically applicable P-gp inhibitors for co-administration as adjuncts with anticancer drugs for effective cancer chemotherapy.