

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

BY

AJALA, OLUSEGUN SAMSON

NOVEMBER, 2011

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**A thesis submitted to the School of Postgraduate Studies, University of
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Doctor of Philosophy (PhD) in Pharmaceutical Chemistry.**

NOVEMBER, 2011

**SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS**

CERTIFICATION

This is to certify that the thesis:

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

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Is a record of original research carried out

By:

AJALA, OLUSEGUN SAMSON

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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.

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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 expression, 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 ^1H 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 (^1H , ^{13}C , ^1H - ^1H gCOSY, DEPT-edited ^1H - ^{13}C HSQC and ^1H - ^{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 ^1H - ^1H 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 25 μ g/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.