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SOME BIOACTIVE STEROIDAL SAPONINS OF PALMYRAH

By

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DECLARATION

The work described in this thesis was carried out by me under the supervision of Professor E. R. Jansz and Dr. S. Ekanayake and a report on this has not been submitted in whole or in part to any University or any other Institution for another Degree/Diploma.

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A. A. P. Keerthi

..04/09/2008...

Date

CERTIFICATION

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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Professor E. R. Jansz

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Dr S. Ekanayake

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Abbreviations

^{13}C-NMR	Carbon Nuclear Magnetic Resonance spectrometry
1D-TOCSY	One Dimensional - Total Correlation Spectroscopy
^{1}H-NMR	Proton Nuclear Magnetic Resonance spectrometry
2D-TOCSY	Two Dimensional - Total Correlation Spectroscopy
AOAC	Association of Official Analytical Chemists
AR	Analytical Reagent (chemical grade)
BEN	Butanol:Ethanol:Ammonia
CSTH	Colombo South Teaching Hospital
DMSO	Dimethyl Sulfoxide
FAB/MS	Fast Atom Bombardment – Mass Spectrometry
F_A	New flabelliferin triglycoside
F_B	Anti bacterial flabelliferin triglycoside
F_C	Flabelliferin triglycoside
F_D	Flabelliferin diglycoside
GC	Gas Chromatography
GC-MS	Gas Chromatography – Mass Spectroscopy
Glc	Glucose
GPR	General Purpose Reagent (chemical grade)
HMBC	Heteronuclear Single Quantum Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromtography
ICR	Institute of Cancer Research
MPLC	Medium Pressure Liquid Chromatography

MRI	Medical Research Institute
MRSA	Methicillin resistant *Staphylococcus aureus*
MW	Molecular Weight
PDB	Palmyrah Development Board
PF	Palmyrah flour
PFP	Palmyrah fruit pulp
R_f	Retardation Factor
Rha	Rhamnose
RPM	Rounds per minute
RPR-HPLC	Reverse Phase Recycle - High Performance Liquid Chromtography
TEM	Transition Electron Microscopy
TLC	Thin Layer Chromatography
TMS	Tetra methyl silane
TOF-Mass	Time Of Flight– Mass Spectrometry
UV	Ultra Violet

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A. A. P. Keerthi
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ABSTRACT

TITLE: SOME BIOACTIVE PRINCIPLES OF PALMYRAH

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Palmyrah fruit pulp is known to have flabelliferins (steroidal saponins) which have hypoglycemic, hypocholesterolaemic and anti-microbial properties. The anti-microbial property was studied further and compound F_B (a β sitosterol glycoside) was found to have an IC_{50} of 31 μmol/L on *Escherichia coli* ATCC 25922 in liquid medium. Sitosterols and all flabelliferins bind florescent carotenoids, which make the complex less soluble in aqueous medium. Therefore, the Bauer-Kerby method was used to test anti-microbial activity of the complex (F_B and UV active binder). The complex had higher activity than the pure glycoside both on equimolar and equal-weights basis against *Escherichia coli* ATCC 25922. The complex on application to human non-ulcerous wounds was as good as the normal hospital treatment.

Palmyrah flour (PF) contained a number of other bioactivities. One flabelliferin with a linear Glc.Rha.Rha sugar moiety caused lethality to larvae of the dengue mosquito *Aedes albopictus* and *Aedes aegypti* at a LC_{50} ranging from 60 mg/L to 75.8 mg/L by forming a layer on the surface of water, which the short breathing siphons of these larvae could not penetrate. PF also contains a hyperhemolytic steroidal saponin (MW= 1534) with 5 Rha and 1 Glc and a fragment of 228. Another tetraglycoside (MW= 1014) with 1 Glc and 3 Rha had cytotoxic activity on melanoma cells at a concentration of 100 μg/mL. It has a aglycone of spirostane and its the structure has been elucidated.

PF is known to exert a neurotoxic effect on rats and mice. The nature of this neurotoxin had evaded classification since 1971. This was found to be due to the toxin being in a

mixture of steroidal saponins. Separation of the mixture resulted in loss of activity on oral administration. Intravenous administration in a bioactivity directed separation on Wistar rats by MPLC, ion exchange chromatography and preparative TLC of suspected neurotoxin narrowed the range to a mixture of 3 compounds, which exhibited the neurotoxic effect. They were two primary amines (A and B) found in mixture to be toxic and a non amine which was non-toxic. It was found that there is a synergistic effect between the two amines. The amines were found to be tetraglycosides of spirostane contaning 3 α Rha and β pyranoside which is likely to contain a NH_2 group. It is possible that this NH_2 group is at position 6 in A and in position 3 in B of a first pyranoside moiety. More data is needed to confirm this. Knowing the chemical nature of the toxin along with the data previously gathered, it is possible to hypothesize the mechanism of uptake of the neurotoxin and its neurotoxic effects. Some understanding has been reached to explain why humans have never be reported to show the neurotoxic effect.