

# **SOME BIOACTIVE STEROLDAL SAPONINS OF PALMYRAH**

**Bv**

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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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..04/07/2008...

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.



.....

Professor E. R. Jansz



.....

Dr S. Ekanayake

# TABLE OF CONTENTS

|                         |              |
|-------------------------|--------------|
| <b>List of tables</b>   | <b>xi</b>    |
| <b>List of figures</b>  | <b>xiii</b>  |
| <b>List of plates</b>   | <b>xv</b>    |
| <b>Abbreviations</b>    | <b>xvi</b>   |
| <b>Acknowledgements</b> | <b>xviii</b> |
| <b>Abstract</b>         | <b>xxii</b>  |

|          |   |    |
|----------|---|----|
| <b>1</b> | <b>INTRODUCTION</b>                           |    |
| 1.1      | Palmyrah cultivation and its importance       | 1  |
| 1.2      | Justification of the study                    | 2  |
| 1.3      | Scope and objectives of the thesis            | 4  |
| <b>2</b> | <b>LITERATURE REVIEW</b>                      |    |
| 2.1      | Palmyrah palm                                 | 7  |
| 2.2      | Uses of palmyrah palm                         | 7  |
| 2.2.1    | Palmyrah fruit based products                 | 8  |
| 2.2.2    | Palmyrah flour                                | 8  |
| 2.2.3    | Palmyrah sap based products                   | 10 |
| 2.2.4    | Non edible palmyrah products                  | 11 |
| 2.2.5    | Palmyrah plant in conservation of environment | 11 |
| 2.3      | Flabelliferins of palmyrah fruit pulp (PFP)   | 12 |
| 2.3.1    | Steroid molecules from palmyrah               | 12 |

|            |   |           |
|------------|---|-----------|
| 2.3.2      | Debittering of palmyrah fruit pulp                                | 12        |
| 2.3.3      | Flabelliferins and the UV active binder                           | 13        |
| <b>2.4</b> | <b>Bioactivity of flabelliferins of Palmyrah Fruit Pulp (PFP)</b> | <b>13</b> |
| 2.4.1      | Hypoglycemic effect   | 16        |
| 2.4.2      | Hypocholesterolaemic effect                                       | 16        |
| 2.4.3      | Antimicrobial activity  | 17        |
| <b>2.5</b> | <b>Bioactive molecules from palmyrah flower</b>                   | <b>18</b> |
| <b>2.6</b> | <b>Toxicity on palmyrah flour (PF)</b>                            | <b>19</b> |
| 2.6.1      | Hepatotoxicity  | 20        |
| 2.6.2      | Immunosuppression   | 24        |
| 2.6.3      | Neurotoxic effects  | 26        |
| 2.6.3.1    | Toxic symptoms  | 26        |
| 2.6.3.2    | Internal changes  | 26        |
| 2.6.3.3    | Biochemical changes   | 28        |
| 2.6.3.4    | Neurotoxic compounds in PF  | 28        |
| <b>2.7</b> | <b>Clastogenic, mutagenic and other bioactive effects</b>         | <b>30</b> |
| <b>2.8</b> | <b>Separation of flabelliferins</b>                               | <b>31</b> |
| 2.8.1      | Separation of flabelliferins from palmyrah fruit pulp (PFP)       | 31        |
| 2.8.2      | Separation of the compounds from palmyrah flour                   | 32        |
| <b>2.9</b> | <b>Effects of palmyrah flour components on mosquitoes</b>         | <b>32</b> |

|             |  |           |
|-------------|--|-----------|
| 2.9.1       | Distribution of mosquitoes                   | 32        |
| 2.9.2       | Effects of palmyrah flour on mosquito        | 32        |
| 2.9.3       | Mosquito life cycle and identification keys  | 33        |
| <b>2.10</b> | <b>Antibiotics and wound healing</b>         | <b>35</b> |
| 2.10.1      | History of wound care                        | 35        |
| 2.10.2      | Wounds and medicine                          | 35        |
| 2.10.2.1    | Identification of ulcers and treatments      | 35        |
| 2.10.2.2    | Natural products in drug discovery           | 35        |
| 2.10.2.3    | Antibiotics containing carbohydrate moieties | 36        |
| 2.10.2.4    | Antibiotic resistance                        | 37        |
| 2.10.2.5    | New bacterial diseases                       | 37        |
| 2.10.3      | Wound healing                                | 37        |
| <b>3</b>    | <b>MATERIALS &amp; METHODS</b>               |           |
| <b>3.1</b>  | <b>Materials</b>                             | <b>41</b> |
| 3.1.1       | General chemicals and solvents               | 41        |
| 3.1.2       | Water  | 41        |
| 3.1.3       | Glassware                                    | 41        |
| 3.1.4       | Laboratory instruments                       | 41        |
| 3.1.5       | Palmyrah fruits and fruit pulp               | 41        |
| 3.1.6       | Palmyrah flour                               | 42        |
| 3.1.7       | Palmyrah seed shoots                         | 42        |
| 3.1.8       | Microbiological media                        | 42        |
| 3.1.9       | Bacterial samples                            | 44        |



|            |   |           |
|------------|---|-----------|
| 3.1.10     | Animals   | 44        |
| 3.1.11     | Volunteers  | 45        |
| <b>3.2</b> | <b>Methods</b>  | <b>45</b> |
| 3.2.1      | Flabelliferin separation  | 45        |
| 3.2.2      | Cellulose column chromatography for crude<br>flabelliferins           | 45        |
| 3.2.3      | Medium pressure liquid chromatography (MPLC)                          | 45        |
| 3.2.4      | Chromatatron  | 47        |
| 3.2.5      | Thin layer chromatography   | 47        |
| 3.2.6      | Re-crystallization  | 50        |
| 3.2.7      | Spray reagents  | 50        |
| 3.2.7.1    | Ninhydrin   | 50        |
| 3.2.7.2    | Anisaldehyde  | 50        |
| 3.2.7.3    | Phosphomolybdic acid  | 51        |
| 3.2.7.4    | Vanillin / sulfuric acid reagent                                      | 51        |
| 3.2.7.5    | Iodine vapor  | 51        |
| 3.2.8      | Anti-bacterial flabelliferin B (F <sub>B</sub> )                      | 51        |
| 3.2.8.1    | Separation of F <sub>B</sub>  | 51        |
| 3.2.8.2    | General microbiological procedures                                    | 51        |
| 3.2.8.3    | Liquid medium experiments   | 52        |
| 3.2.8.4    | IC <sub>50</sub> determination of the F <sub>B</sub> in liquid medium | 52        |
| 3.2.8.5    | Bauer Kirby method (Bauer <i>et al.</i> , 1966)                       | 53        |
| 3.2.8.6    | Animal trials   | 53        |
| 3.2.8.7    | Flabelliferin B (F <sub>B</sub> ) test on humans                      | 56        |



|          |  |    |
|----------|--|----|
| 3.2.9    | Palmyrah flour and mosquito larvae   | 60 |
| 3.2.9.1  | Collection of mosquito larvae samples  | 60 |
| 3.2.9.2  | Identification of the mosquito larvae samples  | 61 |
| 3.2.9.3  | Effect of methanolic extractives on <i>Aedes albopictus</i> mosquito larvae, is there any pupation enhancer? | 62 |
| 3.2.9.4  | Froth test   | 62 |
| 3.2.9.5  | LC <sub>50</sub> for palmyrah flour methanol extractives   | 62 |
| 3.2.9.6  | Bioactivity directed fractionation of compounds  | 63 |
| 3.2.9.7  | Effects of crude methanolic extractives on <i>A. albopictus</i> mosquito larvae                              | 63 |
| 3.2.9.8  | Effect of different solvent extractives on <i>A. albopictus</i> mosquito larvae                              | 63 |
| 3.2.9.9  | Effect of MPLC fractions on <i>A. albopictus</i> mosquito larvae   | 65 |
| 3.2.9.10 | Identification of the mosquito larvaecidal flabelliferin   | 65 |
| 3.2.9.11 | Testing for reasons for larvaecidal activity   | 65 |
| 3.2.9.12 | Re-crystallization and spectroscopic studies   | 67 |
| 3.2.9.13 | Structural elucidation of the compounds  | 67 |
| 3.2.10   | Isolation of the neurotoxin  | 67 |
| 3.2.10.1 | Separation of the volatiles from the palmyrah flour  | 69 |
| 3.2.10.2 | Animal Experiment I  | 69 |
| 3.2.10.3 | Samples preparation for histopathology   | 71 |

|          |   |    |
|----------|---|----|
| 3.2.10.4 | Animal Experiment II  | 71 |
| 3.2.10.5 | Volatile analysis using gas chromatography and<br>mass spectrometry (GC-MS) | 72 |
| 3.2.11   | Separation of toxic compounds from raw palmyrah<br>seed shoots              | 72 |
| 3.2.11.1 | Crude seed shoot extraction   | 72 |
| 3.2.11.2 | Method of following the bioactive compound on<br>TLC                        | 72 |
| 3.2.11.3 | Fractionation of MeOH: H <sub>2</sub> O, 1:1 extraction using<br>MPLC       | 74 |
| 3.2.11.4 | Ion exchange column chromatography  | 74 |
| 3.2.11.5 | Preparative thin layer chromatography (Prep. TLC)                           | 76 |
| 3.2.11.6 | Rat toxicity through intra-venous injections                                | 77 |
| 3.2.11.7 | Recycle HPLC  | 80 |
| 3.2.11.8 | Spectroscopy NMR/ MS/ IR  | 81 |
| 3.2.11.9 | Effect of heat on separated compound  | 81 |
| 3.2.12   | Isolation of the hyper-haemolytic compound                                  | 81 |
| 3.2.12.1 | Haemolytic assay  | 82 |
| 3.2.12.2 | Haemolytic effects of the amine and saponin<br>fractions                    | 82 |
| 3.2.12.3 | Haemolytic index  | 82 |
| 3.2.12.4 | Mass spectroscopy   | 83 |
| 3.2.13   | Cytotoxic compound  | 83 |
| 3.2.13.1 | Isolation and crystallisation   | 83 |

|            |          |   |           |
|------------|----------|---|-----------|
|            | 3.2.13.2 | Bioassay  | 83        |
|            | 3.2.13.3 | Spectroscopic studies                                       | 83        |
|            | 3.2.14   | Statistical Analysis  | 84        |
| <b>4</b>   |          | <b>RESULTS</b>  | <b>85</b> |
| <b>4.1</b> |          | <b>Separation of F<sub>B</sub> from palmyrah fruit pulp</b> | <b>85</b> |
|            | 4.1.1    | Liquid medium experiments                                   | 86        |
|            | 4.1.1.1  | Liquid medium experiments with the UV active binder         | 86        |
|            | 4.1.1.2  | Liquid medium experiments without the UV active binder      | 86        |
|            | 4.1.1.3  | Bauer-Kirby method experiments                              | 89        |
|            | 4.1.1.4  | MRSA studies  | 89        |
|            | 4.1.2    | Animal studies using flabelliferin B (F <sub>B</sub> )      | 89        |
|            | 4.1.2.1  | Wistar rat wound healing study                              | 89        |
|            | 4.1.2.2  | Toxicity test on Wistar rat eye                             | 91        |
|            | 4.1.2.3  | Toxicity test on New-Zealand white rabbit eye               | 93        |
|            | 4.1.2.4  | Patch test  | 93        |
|            | 4.1.2.5  | Pilot study on human wounds                                 | 93        |
| <b>4.2</b> |          | <b>Mosquito Larvicidal compound of palmyrah</b>             | <b>97</b> |
|            | 4.2.1    | Mosquito larvae samples                                     | 97        |
|            | 4.2.2    | Is there any pupation enhancer in EtOH extractives?         | 97        |
|            | 4.2.3    | Effects of EtOH extractives on <i>Aedes albopictus</i>      |           |

|            |  |            |
|------------|--|------------|
|            | mosquito larvae  | 98         |
| 4.2.4      | The LC <sub>50</sub> for palmyrah flour methanol extractives                       | 98         |
| 4.2.5      | Bioactivity directed fractionation of compounds                                    | 98         |
| 4.2.6      | Clastrogenic effect  | 99         |
| 4.2.7      | Effects of palmyrah flour MeOH extractives on<br>breathing siphons                 | 99         |
| 4.2.8      | Effects of crude flour methanolic extractives on<br>other aquatic organisms        | 103        |
| 4.2.9      | Spectroscopic studies of purified mosquito<br>larvaecidal compound                 | 105        |
| <b>4.3</b> | <b>Neurotoxic compounds of palmyrah</b>  | <b>109</b> |
| 4.3.1      | Tests using palmyrah flour volatiles   | 109        |
| 4.3.1.1    | Oral administration and inhalation of volatiles                                    | 109        |
| 4.3.1.2    | Post mortem appearance and histopathology  | 110        |
| 4.3.2      | Palmyrah seed shoot  | 110        |
| 4.3.3      | Chromatographic separation of bioactive principles                                 | 110        |
| 4.3.4      | Medium pressure liquid chromatography using<br>different solvents                  | 110        |
| 4.3.5      | Ion exchange chromatography  | 113        |
| 4.3.6      | Preparative thin layer chromatography (TLC) for<br>separation of cationic fraction | 113        |
| 4.3.7      | Rat toxicity through intravenous injections  | 116        |
| 4.3.7.1    | MeOH: H <sub>2</sub> O (1:1) extraction  | 116        |
| 4.3.7.2    | Extraction using different solvents  | 116        |

|            |  |            |
|------------|--|------------|
| 4.3.7.3    | Fractions from cation exchange column  | 116        |
| 4.3.7.4    | Compounds from preparative TLC   | 120        |
| 4.3.7.5    | Tests on individual compounds  | 120        |
| 4.3.8      | IR spectroscopy for the separated compounds                                      | 121        |
| 4.3.9      | Recycle reverse phase high performance liquid chromatography (HPLC)              | 124        |
| 4.3.10     | Spectroscopic studies of the separated pure compounds                            | 124        |
| 4.3.10.1   | Neurotoxic compound A and B  | 124        |
| <b>4.4</b> | <b>The hyper-haemolytic and cytotoxic steroidal saponins from palmyrah flour</b> | <b>135</b> |
| 4.4.1      | The hyper-haemolytic steroidal saponin   | 135        |
| 4.4.1.1    | Isolation and the purification   | 135        |
| 4.4.1.2    | Structure elucidation  | 135        |
| 4.4.2      | Cytotoxic steroidal saponin from palmyrah flour                                  | 138        |
| 4.4.2.1    | Separation of the compound   | 138        |
| 4.4.2.2    | Cytotoxicity on cancer cells   | 138        |
| 4.4.3      | Structure elucidation  | 138        |
| <b>5</b>   | <b>DISCUSSION</b>  | <b>147</b> |
| <b>6</b>   | <b>REFERENCES</b>  | <b>188</b> |
| <b>7</b>   | <b>APPENDICES</b>  | <b>226</b> |



|  |            |
|--|------------|
| <b>APPENDIX I - Awards, Publications and<br/>communications</b>  | <b>226</b> |
| <b>APPENDIX II - Spectra representing two<br/>dimensional experiments conducted on<br/>neurotoxic compound A</b> | <b>229</b> |
| <b>APPENDIX III - Spectra representing two<br/>dimensional experiments conducted on cytotoxic<br/>compound</b>   | <b>236</b> |

## List of Tables

|            |   |     |
|------------|---|-----|
| Table-1.1  | Turnover from the palmyrah industry during January to October, 2007   | 3   |
| Table-2.1  | Summary of some of the identified flabelliferins  | 15  |
| Table-2.2  | Some carbohydrate containing drugs  | 38  |
| Table-2.3  | The development of drug resistance  | 38  |
| Table-2.4  | New bacterial diseases after 1977   | 39  |
| Table-4.1  | Nephelometric readings for different concentrations of F <sub>B</sub>   | 87  |
| Table-4.2  | Inhibition zones of F <sub>B</sub> with and with out UV active binder   | 90  |
| Table-4.3  | Effects of F <sub>B</sub> on rat wounds   | 92  |
| Table-4.4  | Effects of F <sub>B</sub> on human wounds   | 94  |
| Table-4.5  | Colony counts obtained from human wound swabs   | 95  |
| Table-4.6  | Liquid medium experiments for human wound swabs   | 95  |
| Table-4.7  | Effect of different concentratives of ethanolic extractives on 4 <sup>th</sup> instar <i>Aedes albopictus</i> mosquito larvae | 100 |
| Table-4.8  | LC <sub>50</sub> obtained for different mosquito larvae, at different larval stages   | 100 |
| Table-4.9  | R <sub>f</sub> value of anisaldehyde positive spots yields with different solvents  | 101 |
| Table-4.10 | Effects of different solvent extractive on 3 <sup>rd</sup> instar <i>Aedes albopictus</i> larvae                              | 101 |
| Table-4.11 | Effects of the MPLC fractons on 3 <sup>rd</sup> instar <i>Aedes albopictus</i> larvae   | 102 |
| Table-4.12 | R <sub>f</sub> values for fractions from ion exchange column  | 114 |
| Table-4.13 | Compounds obtained from preparative TLC of cationic fraction  | 114 |



|            |  |     |
|------------|--|-----|
| Table-4.14 | Animals experiment for MeOH extract of palmyrah seed shoot on MPLC                           | 117 |
| Table-4.15 | Animal experiment for various solvent extracts of palmyrah seed shoot                        | 118 |
| Table-4.16 | Animals experiment for fractions from cation exchange column                                 | 119 |
| Table-4.17 | Animal experiments using extractions of preparative TLC fractions                            | 122 |
| Table-4.18 | Animal experiment data for different doses of compound A                                     | 123 |
| Table-4.19 | Animal experiment for compound B   | 123 |
| Table-4.20 | Animal experiment for compound C   | 125 |
| Table-4.21 | Animal experiment data for mixture of compound A & B   | 125 |
| Table-4.22 | $^{13}\text{C}$ and $^1\text{H}$ NMR comparison for aglycones of two molecules               | 130 |
| Table-4.23 | Haemolysis observed in human RBC using two different concentrations.                         | 137 |
| Table-4.24 | Absorbance of partially purified compound at different concentrations                        | 137 |
| Table-4.25 | Chemical shift assignments of the cytotoxic aglycone   | 145 |
| Table-4.26 | Chemical shift assignments of the carbohydrate moieties                                      | 146 |
| Table-5.1  | Differences between the human and rabbit eye   | 156 |
| Table-5.2  | Comparison of the spectral data of mosquito larvaecidal $^{13}\text{C}$ -NMR with literature | 165 |
| Table-5.3  | Spectral data for the carbohydrate moieties of compound A                                    | 176 |
| Table -5.4 | Chemical shift comparison of carbohydrate moieties   | 184 |

## List of Figures

|             |  |     |
|-------------|--|-----|
| Figure-2.1  | Diagrammatic representation of some uses of palmyrah flour                                 | 9   |
| Figure-2.2  | Aglycone structures isolated by Jeyaratnam in 1986   | 14  |
| Figure-2.3  | The flabelliferins (R= Glc and Rha different sequences)                                    | 14  |
| Figure-2.4  | New compounds isolated from palmyrah flower  | 21  |
| Figure-2.5  | New compounds isolated from palmyrah flower  | 22  |
| Figure-2.6  | Aglycones of molecules from palmyrah flower  | 23  |
| Figure-2.7  | Structure of (17 $\alpha$ )-23-(E)-dammara-20, 23-diene-3 $\beta$ , 25-diol                | 27  |
| Figure-3.1  | Flow diagram for flabelliferin separation  | 49  |
| Figure-3.2  | Separation of mosquito larvaecidal compound from palmyrah flour                            | 64  |
| Figure-3.3  | Sample preparation for TEM   | 68  |
| Figure-3.4  | Experimental set-up with cold finger to trap volatiles                                     | 70  |
| Figure-3.5  | Constant composition chamber to evaluate the effect of palmyrah volatiles <i>via</i> lungs | 73  |
| Figure-3.6  | Flow diagram for separation of toxic compound/s  | 75  |
| Figure-4.1  | Growth rates of <i>E. coli</i> – Inhibition of bacteria by F <sub>B</sub>                  | 87  |
| Figure-4.2  | IC <sub>50</sub> determination of flabelliferin B  | 88  |
| Figure-4.3  | Microscopic observation of breathing siphons of mosquito larvae                            | 104 |
| Figure-4.4  | <sup>1</sup> H-NMR of the mosquito larvaecidal compound                                    | 106 |
| Figure-4.5  | <sup>13</sup> C-NMR of the mosquito larvaecidal compound                                   | 107 |
| Figure-4.6  | MicroToff mass spectrum of the mosquito larvaecidal compound                               | 108 |
| Figure-4.7  | Steps in separation of compounds from palmyrah flour                                       | 112 |
| Figure-4.8  | Seven bands separated from preparative TLC   | 115 |
| Figure-4.9  | Three compounds separated from preparative TLC   | 115 |
| Figure-4.10 | IR spectra obtained for compound A, B and C.   | 126 |

|             |  |     |
|-------------|--|-----|
| Figure-4.11 | $^1\text{H}$ NMR spectrum of the compound A (Rf – 0.65)                            | 128 |
| Figure-4.12 | $^{13}\text{C}$ - NMR spectrum of the compound A (Rf – 0.65)                       | 129 |
| Figure-4.13 | Comparison of $^1\text{H}$ -NMR of compound A before and after the heat treatment. | 131 |
| Figure-4.14 | $^1\text{H}$ NMR spectrum of the compound B (Rf – 0.72)                            | 132 |
| Figure-4.15 | $^{13}\text{C}$ -NMR spectrum of the compound B (Rf – 0.72)                        | 133 |
| Figure-4.16 | Comparison of $^1\text{H}$ -NMR of compound B before and after the heat treatment  | 134 |
| Figure-4.17 | MicroTOF mass spectrum of the hyper haemolytic compound                            | 140 |
| Figure-4.18 | FAB (Neg) Mass spectrum for cytotoxic compound                                     | 142 |
| Figure-4.19 | $^1\text{H}$ -NMR spectrum of the cytotoxic compound                               | 143 |
| Figure-4.20 | $^{13}\text{C}$ -NMR spectrum of the cytotoxic compound                            | 144 |
| Figure-5.1  | Structure of the anti-bacterial flabelliferin ( $\text{F}_\text{B}$ )              | 150 |
| Figure-5.2  | Different aglycones with similar carbohydrate moieties (all are anti-bacterial)    | 152 |
| Figure-5.3  | Comparison of Streptomycin and carbohydrate moiety of $\text{F}_\text{B}$          | 153 |
| Figure-5.4  | Model for the saponin layer on the water air interface                             | 163 |
| Figure-5.5  | Sitosterol and spirostane aglycone where both having MW of 414                     | 166 |
| Figure -5.6 | Structure of spirosolane and possible mechanism due to heat                        | 174 |
| Figure-5.7  | HMBC correlations of neurotoxic compound A   | 177 |
| Figure-5.8  | Structure of the cytotoxic compound  | 185 |

## List of Plates

|           |   |     |
|-----------|---|-----|
| Plate-3.1 | Palmyrah fruits and seed shoots   | 43  |
| Plate-3.2 | Animals housed in the university animal house   | 46  |
| Plate-4.1 | <i>E. coli</i> Inhibition zones of F <sub>B</sub> on agar plate   | 90  |
| Plate-4.2 | Rat wounds before and after the treatment with F <sub>B</sub>   | 92  |
| Plate-4.3 | Wound area observed on a graph paper and wound photographs<br>for patient No 4 with a 16.7% wound healing rate per week | 96  |
| Plate-4.4 | Affected fish from the MeOH extractives   | 104 |
| Plate-4.5 | Microscopic view of livers of rats  | 111 |
| Plate-4.6 | TLC containing hyper haemolytic compound  | 136 |
| Plate-4.7 | Haemolytic effect on red blood cells with intense red colour in<br>fraction 8   | 136 |
| Plate-4.8 | Cytotoxic compound on a TLC (BEN: 7:3:4)  | 141 |



## Abbreviations

|                     |  |
|---------------------|--|
| <sup>13</sup> C-NMR | Carbon Nuclear Magnetic Resonance spectrometry   |
| 1D-TOCSY            | One Dimensional - Total Correlation Spectroscopy |
| <sup>1</sup> 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 <sub>A</sub>      | New flabelliferin triglycoside                   |
| F <sub>B</sub>      | Anti bacterial flabelliferin triglycoside        |
| F <sub>C</sub>      | Flabelliferin triglycoside                       |
| F <sub>D</sub>      | 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 <i>Staphylococcus aureus</i>             |
| MW             | Molecular Weight   |
| PDB            | Palmyrah Development Board                                     |
| PF             | Palmyrah flour   |
| PFP            | Palmyrah fruit pulp  |
| R <sub>f</sub> | Retardation Factor   |
| Rha            | Rhamnose   |
| RPM            | Rounds per minute  |
| RPR-HPLC       | Reverse Phase Recycle - High Performance Liquid Chromatography |
| 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

A. A. P. Keerthi

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<sub>B</sub> (a  $\beta$  sitosterol glycoside) was found to have an IC<sub>50</sub> of 31  $\mu$ mol/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<sub>B</sub> 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<sub>50</sub> 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  $\mu$ g/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 containing 3  $\alpha$  Rha and  $\beta$  pyranoside which is likely to contain a  $\text{NH}_2$  group. It is possible that this  $\text{NH}_2$  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 been reported to show the neurotoxic effect.