DOI :10.31357/fapsmph.2000.00552

ASPECTS OF THE CHEMISTRY AND ANTIMICROBIAL ACTIVITY OF FLABELLIFERINS OF PALMYRAH FRUIT PULP

By

Janaka Keerthi Nikawala

M.Phil. 2000

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JANAKA KEERTHI NIKAWALA

Thesis submitted to the University of Sri Jayewardenepura for the award of the degree Master of Philosophy in Applied Chemistry

The work described in this thesis was carried out by me under the supervision of Prof. E. R. Jansz, Prof. A. M. Abeysekera and Dr. (Mrs) S. C. Wijeyaratne and a report on this has not been submitted to any University for another degree.

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J. K. Nikawala

26/06/2000.

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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Prof. E. R. Jansz

.....

Prof. A. M. Abeysekera

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Dr.(Mrs.) S. C. Wijeyaratne

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ABBREVIATIONS

AOAC	- Association of Official Analytical Chemists
PFP	- Palmyrah fruit pulp
F-I	- Flabelliferin tetraglucoside in Kalpitiya
F-II	- Bitter flabelliferin tetraglycoside
F_B	- Anti-microbial flabelliferin triglycoside
F_C	- Inactive flabelliferin triglycoside
F_D	- Inactive Flabelliferin diglycoside
F_X	- Low Rf naringinase flabelliferin product
F_Y	- High Rf naringinase flabelliferin product
FAB/MS	- Fast Atom Bombardment Mass Spectrometry
MW	- Moleculer weight
^{1}HNMR	- Proton Nuclear Magnetic Resonance
MPLC	- Medium Pressure Liquid Chromatography
Rf	- Retardation fractor
Tlc	- Thin layer chromatography
NCTC	- National Collection Typed Cultures
rha	- rhamnose
glu	- glucose
TMS	- Trimethylsilyl

ACKNOWLEDGEMENTS

The succesful completion of my post-graduate work was made possible by the efforts of many people. I take this opportunity to express my heartful gratitude to Prof. E.R. Jansz, my supervisor, who had been a tower of strength with his support and encouragement and proper guidance to complete my work succesfully.It is my bounden duty to show my deep appreciation and gratitude to him.I am privileged to receive the supervision of Prof. Jansz who is a pioneer and an expert in bioorganic experimental research in Sri-Lanka.

I especially wish to express my deepest gratitude to my supervisor Prof. A. M. Abeysekera for his invaluable advice, kind guidance and professional expertise throughout my study, inspite of his other commitments.

I am deeply indebted to Dr.(Mrs.) S.C. Wijeyaratne, also my supervisor, for the constant support and encouragement she offered during the course of this investigation.

I am very much indebted to the Mr.A. M. Paciyanathan, Chairman, Palmyrah Development Board for providing me the golden opportunity of undertaking this research project and also for granting me leave.

I extend my gratitude to NARESA (RG/95/C/13) for providing funds and Dr. (Mrs.) Malin Akerblom Director, International Programme In Chemical Sciences, Uppsala University, Sweden for providing facilities for experiments at Royal Institute of Technology, Stockholm, Sweden.

I am grateful to Prof. Peter Baeckstrom, Ass. Prof. (Mrs.) Ulla Jacobsson, Ass. Prof. (Mrs.) A. K. B. Karlsson and Prof. Per Erik Janssen for their guidance and assistance in various ways during my stay in Sweden.

To Dr. A. Amerasekera and Mr. Wimal Padmasiri I owe a deep debt of gratitude for the NMR interoperation at University of Colombo.

I also extend my thanks to Mr. R.Guhanesan, Research Officer,PDB for encouragement.

Special thanks are extended to Dr. (Mrs.) S. Jayasekara and the staff of the Animal center, Medical Research Institute, and Mr.T.M.S.G.Tennekoon and Mr.S. Rangoda all the non-academic staff members of the laboratories in the departments of Bio-chemistry, Chemistry, and Botany of the University of Sri Jayewardenepura.

I extend my gratitude to Mrs. Oranee Jansz for proof reading.

I am forever grateful to my parents and wife who helped in ways which are too many to list.

ABSTRACT

Palmyrah fruit pulp is available in excess of 15 –20 Metric tons.per. annum. It is under-utililized due to a bitter principle, which had been tentatively identified as a saponin, that is a tetraglycoside of spirost-5en-3βol (24R). The aim of this study was to investigate the bitter principle and debittering of palmyrah fruit pulp obtained from Hambantota.

The flabelliferins of palmyrah fruit pulp collected from Hambantota were isolated by methanol extraction, cleaned with petroleum ether and extracted into acetone, followed by dry cellulose chromatography and flash chromatography. Four Flabelliferins were obtained and were called F-II, F_B , F_C, and F_D. F-II the bitter compound was confirmed as saponin tetraglycoside M.W. 1030 with a rha. terminus. This saponin showed average haemolysis, slight foam stabilization and was an inhibitor of yeast at 250 μg/ml. Also isolated were F_B and F_C saponin triglycosides M. W. 868 with a rhamnose terminus. Both were haemolytic and foam stabilizing (more by F_B), F_B was also highly active against growth of yeast and alcoholic fermentation by *Saccharomyces cerevisiae* strain S11-F_3 and six strains of bacteria. F_C a saponin triglycoside did not show such bio-activity. F_D was a saponin diglycoside that also did not show bio-activity.

The crude flabelliferins could be debittered by naringinase which yielded two saponin spots termed F_X and F_Y. F_X was a triglycoside possibly identical or very similar to F_C. F_Y was an impure mixture containing at least 3 to 4 compounds. On separation by MPLC, one of these compounds was a steroid M.W. 414. The MS and NMR spectra of the steriod were consistant with stigmast -5 en - 3βol (24 α Et).

The other compound F_Y probably arose from F-II, F_B and F_D, (smaller glycosides). It is considered that debittering not only hydrolyses F-II but also F_B,thus destroying both bitterness and anti-microbial activity. In addition it is possible to debitter some samples of palmyrah fruit pulp with a cheap enzyme heat stable α - amylase and mixture of amylo glucosidase and pullulanase.