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The Diversity, Bioactivity and Structural studies of flabelliferins from palmyrah (*Borassus flabellifer* L.) fruit pulp

By

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M. Phil. 2002

Declaration

"The work described in this thesis
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and professor A. M. Abeysekera
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I certify that this thesis does not incorporate without acknowledgement, any material previously submitted for a degree or diploma in any university or higher educational institution in Sri Lanka or abroad and to the best of my knowledge and brief, it does not contain any material previously published or written by another person except where due reference is made in the text"

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We certify that the above statement
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Professor E. R. Jansz

Professor A. M. Abeysekera

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Abbreviations

AOAC	Association of Official Analytical Chemists
^{13}C-NMR	Carbon Nuclear Magnetic Resonance spectrometry
EI/MS	Electron Impact - Mass Spectrometry
ESI-MS	Electron Spray Ionization - Mass Spectrometry
FAB/MS	Fast Atom Bombardment - Mass Spectrometry
F-I	Flabelliferin tetraglucoside in Kalpitiya
F-II	Bitter flabelliferin tetraglycoside
F_B	Anti bacterial flabelliferin triglycoside
F_C	Inactive flabelliferin triglycoside
F_D	Inactive flabelliferin diglycoside
F_E	New flabelliferin
F_F	New flabelliferin
F_M	New flabelliferin
F_N	New flabelliferin
F_O	New flabelliferin
GC	Gas Chromatography
Glc	Glucose
^{1}H-NMR	Proton Nuclear Magnetic Resonance spectrometry
HMQC	Heteronuclear Multiple Quantum Coherence
HSQC	Heteronuclear Single Quantum Coherence
MPLC	Medium Pressure Liquid Chromatography
MW	Molecular Weight
PFP	Palmyrah fruit pulp
R_f	Retardation Factor
Rha	Rhamnose
TMS	Tetra Methyl Silane
TLC	Thin Layer Chromatography

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Abstract

Palmyrah fruit pulp (PFP) has a total potential production of 15- 20 k tonnes. annum^{-1}. However, the bulk of PFP goes to a waste on account of: (1) inadequate basic knowledge in processing and (2) the presence of a bitter principle and bioactive factors now collectively known as flabelliferins. These are steroidal saponins. The overall objective of this study was to collect chemical data of flabelliferins from PFP to promote its wider utilization. There are two possible major avenues for utilization: (1) debittering the fruit pulp to give jams, cordials etc., (2) fermenting the fruit pulp to obtain potable alcohol.

The bitter flabelliferin, F-II, a tetraglycoside is an inhibitor to the Na^+/K^+ pump. The triglycoside F_B is a microbial inhibitor (yeast and selected bacteria). The triglycoside F_C and the diglycoside F_D are inactive flabelliferins. F-I was reported to be a tetraglucoside. In order to support the utilization of PFP, it was important to study the flabelliferin profiles of varying fruit pulps and attempt to correlate them to an easily distinguishable feature of the fruit or the fruit pulp so as to aid in the selection of the best mode of processing for utilization.

The study showed that flabelliferin profiles of specimens collected varied considerably in morphology. There was a common type, type- I (size, medium to big; colour, black; pericarp, rough with brown longitudinal striations; distal side, black) and three other types, type- II, type- III and type- IV. There was no correlation between flabelliferin profile and morphological type, colour of fruit pulp, carotenoid content (total absorbance)

and the carotenoid present (λ_{max}). As expected bitter fruit pulps contained large amount of F-II. There was great diversity in flabelliferin profile ranging from 2 flabelliferins (for example, Chilaw) to more than 10 flabelliferins (for example, Polonnaruwa). There appeared to be a correlation with location but this line of study was not pursued.

The diversity of flabelliferin profiles made previously documented procedures of separation not universally applicable to all PFP's. When the flabelliferin mixture is complex (for example, the Polonnaruwa sample containing at least 10 flabelliferins) a better separation technique was needed. Therefore other procedures; solvent gradient chromatography, chromatotron, selective solvent extraction and medium pressure liquid chromatography (MPLC) were employed. MPLC was the most successful technique and flabelliferins could be separated from the most complex mixtures and also from a specific florescent flabelliferin-binding agent. However, depending on flabelliferin profile, other techniques had specific value.

The flabelliferin F_B (antimicrobial triglycoside) and the flabelliferin F_D (inactive diglycoside) and three new flabelliferins (F_N, triglycoside of MW 884; F_E, diglycoside of MW 738 and F_F, monoglucoside of MW 579) were separated from pooled specimens of PFP collected from Ampara, Anamaduwa, Polonnaruwa and Mannar. Starting from 200 g of PFP, 88.8 mg of F_B, 52.3 mg of F_D, 7.8 mg of F_E, 30.6 mg of F_F and 5.6 mg of F_N were isolated by an MPLC technique.

Starting from 200g of PFP from Jaffna, 300mg of F-II was isolated using a chromatotron. Selective solvent extraction using ethyl acetate from a methanol extract is a simple method of isolating F_D from flabelliferin profiles containing triglycosides and tetraglycosides. A technique based on direct MPLC (without methanol extraction, petroleum ether cleaning, acetone extraction and dry cellulose chromatography) was worked out to separate not only the flabelliferins in their pure state but also the carotenoids and the free sugars in PFP. This has the advantage of not subjecting materials to heat and usage of a lesser amount of chemicals. In addition it is less time consuming and gives a yield 2.4 fold that of the indirect method of isolation.

The flabelliferins were hydrolyzed by trifluoro acetic acid (0.1M) and the aglycone was confirmed as β-sitosterol by GC/EI/MS studies of the trimethyl silyl derivative and also by ^{1}H-NMR and ^{13}C-NMR.

Elucidation of the structure of F_B was important due to its potential for use as an antimicrobial agent. The F_B was analyzed by Hakomori method followed by GC/EI/MS and also by NMR. It showed that the sugar chain was linked to β- sitosterol by the anomeric C of glucose (β configuration), which is linked to two rhamnoses (both were of α anomeric configuration) by 1→2 and 1→4 linkages. F_B therefore has a branched glycosidic moiety. Similarly, the structure of F_D (diglycoside) showed that the sugar chain was linked to β- sitosterol by the anomeric C of glucose. Glucose is linked to a rhamnose by α-1→4 linkages. The anomeric C of glucose (β configuration) was linked to the β-

sitosterol of F_F (monoglucoside). Though the linkage positions of F_E, and F_N could not be determined due to insufficient data. It was found that in F_N (MW 884), the carbohydrate moiety consists of two glucoses and a rhamnose with a glucose terminus. The sugar moiety is attached to the sapogenin (β- sitosterol) by the other glucose. Similarly, F_E (MW 738) showed a carbohydrate moiety of two glucoses.

The last part of the study concerned the effect of debittering with naringinase on the nutritive status of ICR mice. Results showed that incorporation of bitter PFP at 10 % level in WHO recommended standard rat/ mice breeding feed, reduced weight gain significantly compared to the control ($p=0.029$). Debittering of PFP reversed the effect ($p=0.88$) with respect to control. As debittering not only hydrolyzed F-II but also F_B, this was not proof that F-II was the causative agent for reducing weight gain. Using data from the morphological study, two special natural PFP's were detected. One contained F-II but not F_B (bitter PFP) and the other contained only F_B and not F-II (non- bitter PFP). Results showed that the bitter PFP reduced weight gain compared to control ($p=0.021$) but the non-bitter increased weight gain compared to control ($p=0.014$). It is concluded that, provided F-II is absent, PFP at 10 % level does not have a weight reducing effect. On the contrary it appears to be growth promoting. This may be due to its carotenoid content.

The Diversity, Bioactivity, Chromatographic and Spectroscopic studies of palmyrah (*Borassus flabellifer* L.) fruit pulp

Darshika Dilleny Ariyasena

ABSTRACT