

**Studies on the hypocholesterolaemic effect,  
*in vitro* antioxidant capacity and carotenoids  
of palmyrah fruit pulp.**

By

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**STUDIES ON THE HYPOCHOLESTEROLAEMIC EFFECT, *in vitro* ANTIOXIDANT CAPACITY AND CAROTENOIDS OF PALMYRAH FRUIT PULP**

**By**

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## DECLARATION BY THE CANDIDATE

The work described in this thesis was carried out by me under the supervision of Prof. E.R. Jansz (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayawardenapura) and a report on this has not been submitted in whole or in part to any University for another Degree/ Diploma.

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## **CERTIFICATION BY SUPERVISOR**

I 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.

  
.....  
Prof. E.R. Jansz

<b>Table of contents</b>	<b>i</b>
<b>List of tables</b>	<b>x</b>
<b>List of figures</b>	<b>xii</b>
<b>List of plates</b>	<b>xiv</b>
<b>Abbreviations</b>	<b>xv</b>
<b>Acknowledgements</b>	<b>xvii</b>
<b>Abstract</b>	<b>xix</b>
<b>1. Introduction</b>	<b>1</b>
1.1 General Introduction	1
1.2 Scope of study	4
<b>2. Literature Review</b>	<b>6</b>
2.1 Palmyrah Palm	6
2.1.1 Uses of palmyrah palm	6
2.1.1.1 Non-edible Products	6
2.1.1.2 Edible products of palmyrah	8
(i) Sap-based products	8
(ii) Added value palmyrah sweet toddy products	11
2.2 Palmyrah tuber	14
2.2.1 Tuber based products of palmyrah palm	14

2.2.1.1 Raw tuber	14
2.2.1.2 Tuber flour	15
2.2.1.3 Debittering of tuber flour (Odiyal)	15
2.2.1.4 Palmyrah tuber starch	15
2.2.1.5 Palmyrah flour based product	16
2.2.1.6 Boiled dried palmyrah tuber	16
2.2.1.7 Foods from “Plukodiyal”	16
2.2.1.8 Palmosha	16
2.2.1.9 Biscuits	17
2.2.2 Toxicity of palmyrah flour	17
2.2.2.1 Neurotoxic effects	17
2.2.2.2 Hepatotoxic effects	19
2.2.2.3 Immunosuppressive effects	19
2.2.2.4 Mutagenic and clastogenic effects	20
2.2.2.5 Other bioactivities caused by palmyrah flour	20
2.2.2.6 The flabelliferins of palmyrah flour	20
2.2.2.7 Detoxificaton	21
2.3 Palmyrah Fruit Pulp (PFP)	21
2.3.1 Past work on palmyrah fruit pulp	22
2.3.2 Components of PFP	23
2.3.2.1 Proteins	23
2.3.2.2 Lipids	23
2.3.2.3 Carotenoids	23
2.3.2.4 Pectins	25

2.3.2.5 Minerals and Vitamins	26
2.3.2.6 Steroidal saponins	26
2.4 Flabelliferins from PFP	26
2.4.1 Structural studies on flabelliferins	29
2.4.2 Debittering (removal of bitterness)	32
2.4.3 Studies on bioactivities of PFP	32
2.4.3.1 Anti-microbial effects	32
2.4.3.2 Weight gain studies	33
2.4.3.3 Hypoglycaemic effects	34
2.4.3.4 Hypocholesterolaemic effect	34
2.4.3.5 Anti-oxidant effects	35
2.4.3.6 Saponin like properties	35
(a) Foam stabilizing property	35
(b) Haemolytic activity	35
2.4.4 Separation techniques used for flabelliferins	35
2.4.5 Purification of flabelliferins	36
2.5 Steroids	36
2.5.1 Plant Sterols	37
2.5.2 Cholesterol	37
2.5.2.1 Cholesterol biosynthesis	39
2.5.2.2 Dietary cholesterol	39
(a) Digestion and absorption	39
(b) Transport	40
2.5.3 Factors affecting the serum cholesterol	40

2.5.4 Clinical aspects of hypercholesterolaemia	42
2.5.5 Drug therapy on reduction of cholesterol	42
2.5.5.1 Statins	43
2.5.5.2 Niacin	43
2.5.5.3 Bile Acid resins	43
2.5.5.4 Fibrates	43
2.5.6 Plant sterols and serum cholesterol reduction	43
2.6 Bile Acids	45
2.6.1 Enterohepatic circulation	45
2.6.2 Amphipathic nature of bile acids	45
2.7 Dietary fiber	46
2.7.1 Classification of dietary fiber	46
2.7.1.1 Insoluble dietary fiber	46
2.7.1.2 Soluble dietary fiber	49
2.7.2 Digestion of dietary fiber (DF)	50
2.7.3 Physiological action of DF and its metabolites on cholesterol	50
2.8 Carotenoids	52
2.8.1 Biosynthesis and chemical nature of carotenoids	53
2.8.2 Factors affecting the carotenoid composition	54
2.8.3 Biological activity	54
2.8.3.1 Carotenoids as pro-vitamin A	54
(a) Absorption	55
2.8.3.2 Carotenoids as antioxidants	55
2.8.3.3 Macular degeneracy	57



2.8.3.4 Protective effects of carotenoids in skin	58
2.9 Principles of animal model	58
2.9.1 Selection of animals	58
2.9.2 Formulation of animal diet	59
2.9.3 Suitable experimental conditions	60
<b>3. Materials and Methods</b>	<b>61</b>
3.1 Materials	61
3.1.1 Water	61
3.1.2 Solvents	61
3.1.3 Special chemicals	61
3.1.4 Enzymes	61
3.1.5 Collection of palmyrah fruits	62
3.1.5.1 Palmyrah fruits from Kalpitiya	62
3.1.5.2 Palmyrah fruits from Mannar	62
3.2 Methods	63
3.2.1 Extraction of palmyrah fruit pulp (PFP)	63
3.2.2 Determination of moisture content of PFP	63
3.2.3 Debittering of palmyrah fruit pulp	66
3.2.4 Isolation of crude flabelliferins from the PFP	66
3.2.4.1 Identification of flabelliferin fractions	67
(a) Thin layer chromatography	67
(b) Spray reagents	68
(c) Densitometry	68

3.2.4.2	Quantification of flabelliferin	68
3.2.4.3	Determination of flabelliferin profiles of 4 common types of palmyrah fruit pulps	69
3.2.4.4	Isolation and Quantification of crude flabelliferins of bitter and debitter palmyrah fruit samples	69
3.2.4.5	Quantification of flabelliferins of bitter and debitter palmyrah fruit samples	69
3.2.5	Animal studies	69
3.2.5.1	Animal model-1	69
3.2.5.2	Animal model-2	70
3.2.6	Collection of serum samples	72
3.2.7	Cholesterol determination	72
	(a) Principle of cholesterol kits	72
	(b) Procedure	72
	(c) Calculation	73
	(d) Procedure for HDL Cholesterol	73
	(e) Calculation	73
3.2.8	Tests on faeces	73
3.2.8.1	Collection	73
3.2.8.2	Separation of flabelliferins in faeces	74
3.2.8.3	Isolation of faecal bacteria	74
3.2.8.4	Hydrolysis of crude flabelliferins	74
3.2.9	Analysis of dietary fiber	75
3.2.9.1	Reagents	75

3.2.9.2 Analysis of dietary fiber	75
3.2.9.3 Isolation of soluble dietary fibre (SDF)	77
3.2.9.4 Analysis of pectin	78
3.2.9.5 Determination of profiles of pectin	79
3.2.10 Bile salts	80
3.2.10.1 Semi- quantitative analysis of bile salts	80
3.2.10.2 Analysis of faecal bile salts using 3 $\alpha$ -hydroxy steroid dehydrogenase enzyme and NAD <sup>+</sup>	83
3.2.11 Collection of faeces	86
3.2.11.1 Extraction of faecal bile salts	86
3.2.11.2 Quantification of bile salts in faeces	86
3.2.12 Analysis of carotenoids in PFP	89
3.2.12.1 Sample preparation	89
3.2.12.2 Determination of moisture content	89
3.2.12.3 Solvent extraction for isolation of crude carotenoids	89
3.2.12.4 Separation of Carotenoids	90
3.2.13 Anti-oxidant action of ABTS	91
3.2.13.1 Antioxidant activity of PFP	92
3.2.13.2 Antioxidant activity of mixed carotenoids	93
3.2.13.3 Antioxidant activity of isolated carotenoids	93
<b>4. Results</b>	<b>97</b>
4.1 Categorization of palmyrah fruits	97

4.1.1 Fruits from Kalpitiya	97
4.1.2 Fruits from Mannar	97
4.2. Hypocholesterolaemic effects of PFP	97
4.2.1 Effect on total serum cholesterol by 4 common types of PFP	97
4.2.2 Effect on HDL cholesterol and (Total-HDL) cholesterol by the 4 types of PFP	100
4.3 Weight gain and feed intake	100
4.4 Analysis of faeces from the mice fed on 10% PFP incorporated diet and the standard diet.	100
4.4.1 Extraction of flabelliferins from faeces ( <i>in vivo</i> )	101
4.5 Analysis of hydrolysis of flabelleferins by the faecal bacteria.	101
4.6 Flabelliferin profiles of 4 common types of PFP	105
4.7 Effect of bitter and debitter PFP on serum total cholesterol	114
4.7.1 Hydrolysis of bitter PFP	114
4.7.2 Effect of bitter and debittered PFP on serum cholesterol.	114
4.8 Dietary fiber of 4 common varieties of PFP	114
4.8.1 Soluble (SDF) and insoluble (IDF) dietary fiber of 4 common types of PFP	114
4.8.1.1 Sepharose gel chromatography	118
4.9 Effect of 10% PFP on bile salt of faeces.	118
4.9.1 Quick assay of faecal bile salts	118
4.9.2 Specific 3 $\alpha$ -hydroxy steroidal dehydrogenase enzyme assay	123
4.10 Carotenoids of PFP	123

4.11 Antioxidant activity of 4 types of PFP	125
4.11.1 Total and lipophilic antioxidants of PFP	125
4.11.2 Antioxidant properties of individual carotenoids	126
<b>5. Discussion</b>	132
<b>References</b>	149
<b>Appendix- I List of publications and communications</b>	169
<b>Appendix- II Curriculum vitae</b>	171

## List of Tables

Table 2.1	Palmyrah fruit pulp composition	24
Table 3.1	Feed formula of the test feed, based on the WHO recommended rat/mouse	71
Table 3.2	The volumes of reagents mixed in determining the standard curve for pectin and the absorbance values obtained	81
Table 3.3	The volumes mixed and the absorbance values of the semi-quantitative experiment	84
Table 3.4	Amounts and volumes used in determining the standard curve for bile salt	87
Table 3.5	Standard curve for the ABTS <sup>++</sup> activity	94
Table 4.1	Description of palmyrah fruit types from Kalpitiya.	98
Table 4.2	Description of palmyrah fruit types for Mannar	98
Table 4.3	Differences of palmyrah fruits from Mannar and Kalpitiya	99
Table 4.4	Effect on total cholesterol of 4 types of PFP on ICR mice	102
Table 4.5	Effect of 4 types of PFP on (Total-HDL) cholesterol & HDL cholesterol of serum of ICR mice.	103
Table 4.6	Feed intake and Weight gain of ICR mice fed with 4 types of Palmyrah fruit pulp	104
Table 4.7	Content of flabelliferin of 4 types of fruits	108
Table 4.8	The contents of flabelliferin bitter and debitter PFP	115
Table 4.9	Effect of incorporation of 10% bitter and debittered PFP on total and HDL serum cholesterol.	115

Table 4.10	Dietary fiber contents of 4 types of PFP	120
Table 4.11	Pectin content of the 4 types of PFP	120
Table 4.12	Effect of 10% PFP incorporated diet on bile salts excretion in faeces.	124
Table 4.13	Distribution and contents of carotenoids in 4 types of PFP	130
Table 4.14	Trolox equivalents of total and lipophilic antioxidants of 4 types of PFP.	131
Table 4.15	Antioxidant potentials of separated individual carotenoids.	131

## List of Figures

Figure 2.1	Structure of the aglycone (spirost-5en-3 $\beta$ ol) isolated by Jeyaratnam (1986)	27
Figure 2.2	Proposed structure of the free steroid isolated by Jeyaratnam (1986)	27
Figure 2.3	Probable sequence of the more bitter flabelliferin	31
Figure 2.4	Structure of F <sub>B</sub>	31
Figure 2.5	Alternative sequences of F <sub>C</sub>	31
Figure 2.6	Structure of F <sub>D</sub>	31
Figure 2.7	Structure of F <sub>N</sub>	31
Figure 2.8	Parent Structure of steroids	38
Figure 2.9	Structure of campesterol and sitosterol	38
Figure 2.10	Biosynthesis of cholesterol	41
Figure 2.11	Structure of cholesterol	41
Figure 2.12	Biosynthesis of bile acids	47
Figure 2.13	Amphipathic nature of a bile salt	47
Figure 2.14	Fragment of a pectin macromolecule	51
Figure 3.1	The standard curve for pectin	82
Figure 3.2	Standard curve for Taurocholate using conc. H <sub>2</sub> SO <sub>4</sub> and sucrose	85
Figure 3.3	Standard curve for cholate	88
Figure 3.4	Standard curve for ABTS <sup>++</sup> activity on trolox	95
Figure 4.1	Densitogram for flabelliferins at zero time	



	incubation with bacteria isolates	106
Figure 4.2	Densitogram for flabelliferins after 3 days incubation with bacterial isolates.	107
Figure 4.3	Densitogram for flabelliferins of type I PFP	110
Figure 4.4	Densitogram of flabelliferins of type II PFP	111
Figure 4.5	Densitogram of flabelliferins of type III PFP	112
Figure 4.6	Densitogram of flabelliferins of type IV PFP	113
Figure 4.7	Densitogram of flabelleferins of bitter PFP	116
Figure 4.8	Densitogram of flabelleferins of debitter PFP	117
Figure 4.9	Pectin profiles of four types	121
Figure 4.10	Profiles of Soluble dietary fiber	122
Figure 4.11	Spectrum of (a) Phytoene and (b) Phytofluene	127
Figure 4.12	Spectrum of (a) neurosperene (b) $\zeta$ -carotene	128
Figure 4.13	Spectrum of (a) $\beta$ -carotene (b) unidentified carotenoid	129
Figure 5.1	Entire Biosynthesis pathways of non-oxygenated carotenoids	142
Figure 5.2	Type I, II-A, III carotenoid patterns	143
Figure 5.3	Type II-B carotenoid patterns	143
Figure 5.4	ABTS free radical cation	145

## List of Plates

Plate 1.1	Juvenile palms in an estate of Kalpitiya	3
Plate 2.1	A mature female palmyrah palm	9
Plate 2.2	Palmyrah products	9
Plate 3.1	Morphological type from Kalpitiya	64
Plate 3.2	Morphological type II-B from Mannar	65
Plate 3.3	Palmyrah fruit	65
Plate 3.4	Extracted fruit pulp	65
Plate 4.1	Flabelliferin profiles of 4 types from Kalpitiya	109
Plate 4.2	Extracted pectin from Type II fruits – Kalpitiya	119

## Abbreviations

A.O.A.C	Association of Official Analytical Chemists
ABTS	2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
AC	Acetone
ACAT	Acetyl co A: Cholesterol acyltransferase
acetyl CoA	Acetyl Co enzyme A
Car	Carotenoid
CE	Cholesteryl Esters
DEE	Diethyl ether
DF	Dietary fiber
EI/MS	Electron Impact- Mass Spectrometry
FAB/MS	Fast Atom Bombardment- Mass spectrometry
F <sub>B</sub>	Flabelliferin-B, triglycoside
F <sub>C</sub>	Flabelliferin-C, triglycoside
F <sub>D</sub>	Flabelliferin-D, diglycoside
F <sub>E</sub>	Flabelliferin-E, diglucoside
F <sub>F</sub>	Flabelliferin-F, monoglucoside
F-I	Flabelliferin-I, tetraglucoside
F-II	Flabelliferin-II, tetraglycoside
GC/MS	Gas Chromatography - Mass Spectrometry
Glc	Glucose

HDL	High density lipoprotein
HMG CoA	3-hydroxy3-methylglutaryl Coenzyme A
HPLC	High Performance Liquid Chromatography
IDF	Insoluble dietary fiber
LCAT	Lecithin:Cholesterol acyltransferase
LDL	Low density lipoprotein
MPLC	Medium Pressure Liquid Chromatography
NAD	Nicotinamide Adenine Dinucleotide
OCC	Open Column Chromatography
PE	Petroleum ether
PF	Palmyrah flour
PFP	Palmyrah fruit pulp
PLB	Phospholipid bilayer
RE	Retinol equivalent
R <sub>f</sub>	Retardation factor
Rha	Rhamnose
SCFA	Short chain fatty acids
SDF	Soluble dietary fiber
TG	Triglycerides
TLC	Thin layer chromatography
VFA	Volatile fatty acids
VLDL	Very low-density lipoproteins
WHO	World Health Organization

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Lakmini Gayathri Pathberiya

## **ABSTRACT**

The palmyrah palm (*Borassus flabellifer* L.) mainly grows in the dry zone of the northwest, north and northeast of Sri Lanka. The fruit contains a yellow to orange pulp, which although having many potential uses, is underutilized. Past research on bioactivity of palmyrah fruit pulp (PFP) has been mainly focussed on a family of  $\beta$ -sitosterol containing saponins termed flabelliferins. This study showed that 10% PFP incorporated into standard WHO feed reduced serum cholesterol in ICR mice significantly by 24% to 34%. Serum cholesterol was determined by the cholesterol oxidase / peroxidase method.  $\beta$ -sitosterol is known to decrease cholesterol absorption but free sitosterol is not present in fresh PFP. Analysis of the faeces of mice fed on 10% PFP showed the presence of the antimicrobial flabelliferin (FB) indicating that the enzymes of the gastrointestinal tract cannot hydrolyse flabelliferins. *In vitro* studies on bacteria isolated from the faeces showed only partial hydrolysis of the carbohydrate moieties on flabelliferins. However, hydrolysis by anaerobic bacteria in the colon cannot be ruled out although this has no bearing on the effect of  $\beta$ - sitosterol on lowering of serum cholesterol. Serum cholesterol reduction by 4 types of PFP show no correlation with flabelliferin II (F-II) content even though F-II is an inhibitor of glucose uptake and could theoretically play an indirect role in lowering of cholesterol. Studies using naringinase to shorten the carbohydrate moiety

of flabelliferins did not provide evidence for small carbohydrate chain flabelliferins acting as  $\beta$ -sitosterol mimics. Use of metabisulphite stabilized stored PFP showed no hypocholesterolaemic effect ( $p=0.78$ ) indicating possibly an endoenzyme hydrolysis of the active agent.

Study of 4 types of PFP showed high insoluble dietary fiber (IDF) 6.8% to 16.3% and soluble dietary fiber (SDF) 5.5% to 10.6%. Nearly all the SDF was found to be pectin. Sepharose gel chromatography showed that pectins of all types of PFP were polydisperse. Type I and type IV indicating a MW greater than 2 million. Pectins lower serum cholesterol in many ways including by excretion of bile salts. Studies on the bile salt content of faeces by a specific  $3\alpha$ -hydroxy steroid dehydrogenase /  $NAD^+$  assay showed that mice fed on 10% PFP feed had significantly higher ( $p=0.0009$ ) bile salts in their faeces than that of control feed. This was consistent with dietary fiber playing a significant role in lowering of serum cholesterol.

Studies on the carotenoids PFP from Mannar showed that 3 types of pulp were dominated by neurospere. Other non-provitamin A carotenoids  $\zeta$ -carotene, phytoene and phytofluene were also present.  $\beta$ -Carotene was the only provitamin-A carotenoid detected. Type II-B had a similar profile with 2 additional carotenoids. Using 2,2' azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ABTS as an *in vitro* antioxidant assay, the antioxidant capacities of PFP were found to be both lipophilic and non-lipophilic. A method of use of  $ABTS^{++}$  for the assay of trolox equivalent of carotenoids separated by celite:MgO open column chromatography was worked out. Not all the *in vitro* lipophilic antioxidant values (Trolox equivalent.g<sup>-1</sup>) were consistent with the extent of conjugated double bonds of the carotenoids concerned.