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

By

Lakmini Gayathri Pathberiya

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

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 β -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. β -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 β - 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 β -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 3α -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 neurosperene. Other non-provitamin A carotenoids ζ -carotene, phytoene and phytofluene were also present. β -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⁻¹) were consistent with the extent of conjugated double bonds of the carotenoids concerned.

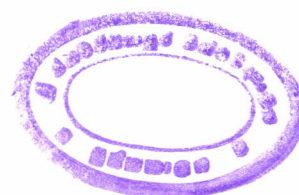
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