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SOME STUDIES ON BIOACTIVE COMPONENTS OF SPOTTED SARDINELLA (*Amblygaster sirii*) AND PALMYRAH (*Borassus flabellifer*) FLOUR

BY

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DECLARATION BY THE CANDIDATE

The work described in this thesis was carried out by me under the supervision of Professor E. R. Jansz (Head of the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Professor H. Peiris (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) 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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DECLARATION OF THE SUPERVISORS

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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ABBREVIATIONS

TLC	Thin Layer Chromatography
AOAC	Association of Official Analytical Chemists
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
WHO	World Health Organization
F-II	Bitter flabelliferin
F_B	Anti-microbial flabelliferin triglycoside
F_C	Inactive flabelliferin triglycoside
R_f	Retardation factor
kg	Kilogram
UV	Ultra Violet
ml	Millilitre
^oC	Centigrade
MW	Molecular Weight
L	Litre
ICR	Institute of Cancer Research
g	Grams
IU ml^{-1}	International Units per millilitre
h	Hours
cm	Centimetre
ppm	Parts per million
CE	Capillary Electrophoresis
LC	Liquid Chromatography

µl	Micro litre
DTH	Delayed Type Hypersensitivity
PDB	Palmyrah Development Board
HPLC	High Performance Liquid Chromatography
nm	Nanometre
kJ	Kilojoules
kV	Kilovolt
ID	Inner Diameter
pmoles	Pico moles
Sp.gr.	Specific gravity
CV	Coefficient of Variation
ELISA	Enzyme Linked Immunosorbent Assay
GPR	General Purpose Regent
AR	Analytical Grade

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ABSTRACT

This study comprises of two parts, namely the potentially toxic components of *Amblygaster sirm* (hurulla) and *Borassus flabellifer* (palmyrah) flour. Hurulla is considered by some to be a "heaty" (allergenic) fish. Past studies have indicated that the cause may be tyramine in hurulla. This study conclusively shows that tyramine content, which was either not detected or found only in trace quantities, is not the cause. Further tyrosine decarboxylation activity is not in evidence. Histamine was quantified by TLC-UV spectrophotometry, TLC-densitometry, ELISA system and histaminase assay. Histamine content in hurulla fish too, is low (< 0.9 mg/g after 24 h) and is produced as described previously, by bacterial action on keeping. Histidine decarboxylases are present. They are bacterial exoenzymes, which are rapidly destroyed by proteases. Solubilisation of histamine complex by lowering pH to 1 and retarding protease action by chelation of cofactors with EDTA increases histamine levels on keeping, but not to the levels seen in the well known heaty fish Skipjack (balaya) which is reported to have histamine. The bacteria from skin, intestine and gill of hurulla were found to contain histamine in the ranges of 32 to 314 ppm, 48 to 144 ppm and 11to 202 ppm respectively. Lysine content of fresh hurulla fish was sometimes high (0.28 ± 0.04 mg/g) and this may lead to the supposition that a gizzerosine type effect could lead to

allergenicity at low histamine levels. It is also possible that this symptom is manifested only in persons deficient in vitamin B_6, a coenzyme for histamine detoxification.

The second part of the study deals with the well known neurotoxic effect of palmyrah flour on Wistar rats. As expected, the crude water extract showed neurotoxicity (Test 1), but on complete separation of (I) amines and (II) neutral and negatively charged molecules, the two fractions resulting showed no toxicity. On recombining the two fractions, neurotoxicity was exhibited (Test 2). This confirms that toxicity is due to more than one component and that the components exhibited synergism. Comparison of neurotoxic rats (Test 1 and Test 2) with those not showing symptoms (controls) gave a significant difference in serum NH_4^+ content ($p=8 \times 10^{-5}$ and $p=1.5 \times 10^{-4}$) but no significant difference in serum creatinine ($p=0.52$ and $p= 0.32$). Serum urea appeared to decline. This supported previous studies on liver mitochondrial damage as evidence by electron microscopy and elevated serum aspartate aminotransferase levels.