INVESTIGATION OF SECRETORY PHOSPHOLIPASE A2 INHIBITORS FROM SELECTED SRI LANKAN MEDICINAL PLANTS AS POTENTIAL THERAPEUTIC AGENTS FOR DENGUE FEVER

by

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Investigation of Secretory Phospholipase A2 inhibitors from selected Sri Lankan medicinal plants as potential therapeutic agents for dengue fever

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Declaration of the candidate

“The work described in this thesis was carried out by me under the supervision of Emeritus Prof. A. M. Abeysekera, Prof. Gathsaurie Neelika Malavige, Prof. U. G. Chandrika and Dr. Chayanika Padumadasa and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma."

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“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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AdPLA2	Adipose phospholipase A2
ATR	Attenuated Total Reflection
CAT	Catalase
CCR	Chemokine Receptor
CRA	*Cyperus rotundus* aqueous extract
CRE	80% ethanol extract of *Cyperus rotundus*
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorraghic Fever
DMSO	Dimethyl Sulfoxide
DMEM	Dulbecco's Modofied Eagle Medium
DSS	dengue shock Syndrome
DTNB	5,5'-Dithiobis-(2-Nitrobenzoic acid)
EA	Ethyl Acetate
EDTA	Ethylene Diammine Tetra Acetic acid
FBS	Fetal Bovine Serum

FTIR	Fourier Transform Infrared Spectroscopy
GPx	Glutathione Peroxidase
GSH	Glutathione
HDL	High density Lipo protein
HPLC	High Performance Liquid chromatography
IC_{50}	Half maximal inhibitory concentration
IgG	Immunoglobulin G
IgM	Immunoglobulin M
iPLA2	Ca^{2+} independent PLA2
JAA	aqueous extract of *Justicia Adatoda*
JAE	80 % ethanol extract of *Justicia Adatoda*
LC_{50}	Lethal dose required to kill 50 % of the population
LDL	Low Density Lipoprotein
LYPLA2	Lysosomal PLA2
MTT	Assay using dimethyl thiazolyl diphenyl tetrazolium salt
NPR	Natural Product Reagent
NS1	Non Structural Protein
NSAIDs	Non Steroidal anti Inflammatory Drugs

PAF	Platelet Activating Factor
PAFR	Platelet Activating Factor Receptor
PAFAH	Platelet Activating Factor acetylhydrolase
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PE	Phosphatidyl ethanolamine
PEG	Polyethylene glycol
PG	Phosphatidylglycerol
PLA	Phospholipase A
PS	Phosphatidylserine
R_F	Retardation factor
sPLA2	Secretory Phospholipase A2
SOD	Superoxide dismutase
SRB	Sulfordhamine B
TCA	Tri chloro acetic acid
TG	Tri glycerides
TH	*Tragia hispida*
THA	Aqueous extract of *Tragia hispida*

THB	Butanol fraction of aqueous extract of *Tragia hispida*
THE	80 % ethanol extract of *Tragia hispida*
TLC	Thin Layer Chromatography
TNFα	Tumor Necrosis Factor alpha
UV	Ultra violet
VLDL	Very Low Density Lipoprotein

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Investigation of Secretory Phospholipase A2 inhibitors from selected Sri Lankan medicinal plants as potential therapeutic agents for dengue fever

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ABSTRACT

Secretory phospholipase A2s (sPLA2s) are small-secreted proteins, which has great importance in a large variety of disease conditions including inflammation, asthma, cancer, and bronchitis. sPLA2 is involved in the synthesis of a platelet-activating factor which is a predominant mediator of vascular leak in dengue hemorrhagic fever patients. It had been recently found that sPLA2 level is dramatically increased during the first five days of illness in dengue fever patients. This study is focused on the inhibition of sPLA2 enzyme activity of dengue patient sera and bee venom by three Sri Lankan medicinal plants, *Tragia hispida*, *Cyperus rotundus*, and *Justicia adathoda* which are used traditionally for bleeding and fever. In the initial screening, bee venom was used as the source of sPLA2. Three known sPLA2 inhibitors (CAY 10590, LY 311727 and LY 315920) were used as the positive controls in this study. The sPLA2 activity was measured using a commercial sPLA2 assay kit. The butanol fraction of the aqueous extract of *Tragia hispida* (THB) showed promising sPLA2 inhibitory activity with bee venom sPLA2 (sPLA2 group III, IC_{50} = 0.15 µg/µL). THB was further investigated with dengue patient serum sPLA2 (n=31) and showed a significant inhibitory effect ($p<0.0001$) compared to positive control, CAY 10590. Furthermore, cytotoxicity of THB was examined by Sulforhodamine B colorimetric assay using MRC 5 cells , and it was found that THB is nontoxic to the normal human cells

(IC_{50} =4400, 445, 199 µg/mL for 24, 48 and 72 hrs respectively). THB was further separated into individual phytoconstituents by bioactivity-guided fractionation. Initially, it was separated into phenolic and flavonoid fractions by column chromatography on silica gel. Although both fractions showed good sPLA2 inhibitory activity, the very low inhibitory effect was observed in the flavonoids which isolated from flavonoid fraction by column chromatography of polyamide 6 and sephadex LH 20. This suggests that flavonoids are acting synergistically. TLC of the phenolic fraction lead to the isolation of three fractions of which one (compound X) showed good sPLA2 inhibitory effect, IC_{50} =2.69 µg/µL with bee venom and IC_{50} =7.59 µg/µL with dengue patient serum (n=31). Although the sPLA2 inhibitory effect of compound X is not good as THB, X also has a relatively good sPLA2 inhibitory effect. Cytotoxicity of X was evaluated by MTT assay using vero cells evidenced that could be nontoxic to the mammalian cells (IC_{50} =1195, 496, 307 µg/mL for 24, 48 and 72 hrs respectively). Chromatographic investigation indicates that it is a pure compound whose structure elucidation is in progress.