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University of Colombo

Our Vision

"To be an International Centre of Excellence In Molecular Life Sciences "

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PROGRAMME

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Message from the Director - IBMBB

Professor Shiroma Handunnetti
Director & Professor in Immunology
Chairperson, Organizing Committee, IBMBB

It is my pleasure to send this message to mark the occasion of the 8th Annual Scientific Sessions of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo which is being held as the IBMBB completes its 12th Anniversary.

IBMBB is the only Institute in Sri Lanka dedicated to postgraduate training and research in Molecular Life Sciences and allied fields which was established in 2004. This was the culmination of a SAREC supported research programme led by its Founder Director, Professor Eric H Karunanayake with financial support from Swedish Government. Once the IBMBB was established, SAREC supported research programme together with its MPhil and PhD trainees were relocated to the IBMBB from the Faculty of Medicine, University of Colombo. Since then the MPhil/PhD programme has expanded to have about thirty students conducting their research at the IBMBB, supported by competitive research funding received by academics of the IBMBB. There have been several firsts in Sri Lanka among its MPhils and PhDs including the first molecular genetics study on breast cancer, first molecular study on phytoplasma, first genomic and proteomic study on insulin-like growth factors in relation to foetal growth, first study on the tea genome, first study to genetically characterize traditional and weedy rice. Several MPhil/PhD students who have completed studies at IBMBB are now established and serving as academic staff members of other universities in Sri Lanka, ie., University of Jaffna, Eastern University, University of Kelaniya and University of Sri Jayawardenapura. Thus, the IBMBB has made a considerable contribution to capacity building and strengthening of research training for the higher educational sector.

IBMBB commenced two MSc programmes in 2005, one in Molecular Life Sciences and the other in Cellular and Molecular Immunology. A third MSc programme in Bioinformatics was begun in 2012. At IBMBB, the MSc courses have a >80% completion rate and to date 122 students have graduated. Currently, IBMBB has about 75 post-graduate (MSc, MPhil & PhD) students are continuing their studies.

The Annual Scientific Sessions organized by the IBMBB provides a forum for Dissemination of research findings of IBMBB students and postgraduates from other Higher Educational Institutes.

IBMBB has held seven Annual Scientific Sessions starting from 2006 and two International conferences in 2009 and 2014 to mark the 5th and 10th anniversaries.

High point of the 8th Annual Scientific Sessions will be the Professor Stanley Wijesundera Memorial lecture on "Sri Lankan CKDu Epidemic – Research & Service Challenges" to be delivered by Vidyajyothi Emeritus Professor Rezvi Sheriff, Professor of Medicine, General Sir John Kotelawala Defence University, Sri Lanka. Prof. Sheriff has made enormous contribution to medicine in Sri Lanka in various capacities and also been a pioneer and an entrepreneur in medicine, nephrology, dialysis and transplantation both locally and internationally. This year there will be seventeen oral presentations and eight poster presentations including two from students/researchers from other Higher Educational/ Research Institutes. From the authors and Institutes given in the abstracts, wide collaboration of IBMBB with other Academic and Research Institutes, Health Sector and Agricultural Sector is clearly evident. The presentations will in different research areas including research on Medicinal plants, Molecular Biology, Biotechnology, Nanotechnology, Immunological aspects & Infectious diseases.

I take this opportunity to thank Professor Lakshman Dissanayake, Vice Chancellor, University of Colombo for his continued support, inspiration and specially, for gracing the inauguration ceremony, Prof Rezvi Sheriff for accepting the Professor Stanley Wijesundera Memorial lecture, all our collaborators for facilitating research studies, all those from other Higher Educational Institutes presenting their research at our Scientific Sessions, sponsors for their generous support and, the students and staff of the IBMBB for their dedication and hard work. A special thank to the reviewers, both external reviewers and IBMBB staff members. I specially thank Mr Kanchana Senanayake, Mr Sashika Niranjan and Mr Nipuna Ratnayake for designing the invitation cards and certificates, and coordinating the printing of the abstract book, Ms Anoma Jayasoma for coordinating sponsorships, Ms Thanuja Atapattu for arrangements on refreshments, Ms Sumali Champika and Ms Nadeesha Jayawardena for secretarial assistance, Ms Narmada Fernando, Ms Daniya Edward, Ms Ruwandi Ranasinghe, Dr Sumadi de Silva and Ms Nilupa Gunaratna for editorial help & for support in planning the abstract book and Ms Nishara Batagoda, compere for this event. Finally, I thank all academic, administrative and other IBMBB staff members who contributed to organizing this 8th Annual Scientific session.

I wish you all a successful meeting

Oral Communications

Session I - Medicinal Plants

OP1	**10.45 – 11.00 h**	Cytotoxic potential of new compounds isolated from *Mangifera zeylanica* on breast (MCF-7 and MDA-MB-231) and ovarian (SKOV-3) cancer cells	Ediriweera MK, Tennekoon KH, Samarakoon SR, Thabrew I, de Silva ED, Adhikari A
OP2	**11.00 – 11.15 h**	Possible mechanisms mediating anti-proliferating effects in breast cancer phenotypes by *Vernonia zeylanica* (less)	Mendis AS, Thabrew MI, Samarakoon S, Tennekoon KH
OP3	**11.15 – 11.30 h**	Anti-proliferative and proapoptotic effects of govaniadine in human breast cancer (MCF-7) cells	Sivakumaran N, Samarakoon SR, Adhikari A, Ediriweera MK, Tennekoon KH, Thabrew I, Malavige N
OP4	**11.30 – 11.45 h**	Anti-proliferative effect of five Sri Lankan endemic plants on breast cancer stem cells	Rajagopalan U, Samarakoon SR, Tennekoon KH, de Silva ED
OP5	**11.45 – 12.00 h**	Induction of apoptosis in response to improved gedunin by liposomal nano-encapsulation in human small-cell lung cancer (NCI-H292) cell line	Nwokwu CDU, Samarakoon SR, Karunaratne DN, Katuvawila KANP, Pamunuwa KMGK, Ediriweera MK, Tennekoon KH
OP6	**12.00 – 12.15 h**	Anti-filarial activity of *Dipterocarpus zeylanicus:* bioactivity guided isolation of active compounds and induction of apoptosis in filarial parasite *Setaria digitata in vitro*	Senathilake KS, Karunanayake EH, Samarakoon SR, Tennekoon KH, Adhikari A, de Silva ED

Session II – Immunology & Infectious Diseases

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OP8	**14.00 – 14.15 h**	Diagnostic accuracies of six different immunoassays compared to the gold standard tests real-time quantitative PCR and isolation of pathogenic *Leptospira*	Niloofa MJR, Karunanayake L, H Janaka de Silva, Premawansa S, Rajapakse S, Handunnetti SM
OP9	**14.15 – 14.30 h**	Effects of pathogenic *Leptospira* on functions and viability of vascular endothelial cells and kidney epithelial cells	Fernando N, Handunnetti SM, Rajapakse S, de Silva HJ, Premawansa S
OP10	**14.30 – 14.45 h**	Community based sero-prevalence of human leptospirosis infection in the Colombo District	Balaji K, Karunanayake L, Handunnetti S, Fernando SD, Rajapakse S
OP11	**14.45 – 15.00 h**	Contribution of different types of antioxidants to dengue hemorrhagic fever patients in Sri Lanka	Mapalagamage MS, Handunnetti SM, De Silva A, Premawansa G, Premawansa S

Session III – Molecular Biology & Biotechnology

OP12	**15.00 – 15.15 h**	Screening of selected Sri Lankan cancer patients for somatic mutation of *TP53* gene	Vahinipriya M, De Silva S, Karunanayake EH, Tennekoon KH, De Silva K
OP13	**15.15 – 15.30 h**	Identification of AFLP marker/s to differentiate herbicide resistant Sri Lankan rice (*Oryza sativa*) varieties	Ekanayaka EMSI, Weerakoon SR, Weerasena OVDSJ , Somaratne S
OP14	**15.30 – 15.45 h**	Screening of novel chitinolytic *Bacillus thuringiensis* strains isolated from Sri Lankan soil	Baragamaarachchi RY, Weerasena OVDSJ, Samarasekera RR
OP15	**15.45 – 16.00 h**	Effect of Pinacidil on the expression of SUR2A in rat embryonic heart-derived H9c2 cells	Mohammed Abdul KS, Vikram R, Perera HASD, Ramachandran R, Jovanović S, Jovanović A
OP16	**16.00 – 16.15 h**	Sequence variants in the promoter region of *GH1* gene in a cohort of growth hormone deficient (GHD) children in Sri Lanka	Sundralingam T, Tennekoon KH, de Silva KSH, De Silva S, Hewage AS
OP17	**16.15 - 16.30 h**	Draft Genome Sequence of *Lactobacillus jensenii* Strain MD IIE-70(2)	Karlyshev A, Nadarajah S, Abramov V

Poster Presentation

Medicinal Plants

PP1 Screening of selected natural compounds for anti cancer activity regulated via induced differentiation on cancer stem cells (NT2)

Iromi HSPC, Samarakoon SR, Ediriweera MK, Adhikari A, Rajagopalan U, Tennekoon KH

PP2 Evaluation of the effects of proanthocyanidins extracted from the bark of *Thespesia populnea* (L.) Sol.ex Correa, on cell survival and apoptosis of human hepatoma (HepG2) cells

Tharmarajah L, Thabrew I, Samarakoon SR, Mendis AS, Padumadasa C

Immunology

PP3 Immunochemical characterization of venom of medically important ants in Sri Lanka and determination of venom cross-reactivity with Western ant species

De Silva BD, Handunnetti SM, Premawansa S, Dias RKS, de Silva NR

Molecular Biology & Bioinformatics

PP4 A pilot study on selected point mutations of *CYP21A2* gene among a cohort of Sri Lankan children with Congenital Adrenal Hyperplasia

Jayasundara MVML, Tennekoon KH, de Silva KSH, Hewage AS

PP5 A molecular phylogenetic analysis of six unknown amphibian specimens of the genus *Pseudophilautus* in Sri Lanka

Lowe JN, Samarakoon SR, Wickramasinghe LJM, Tennekoon KH, Wickramasinghe N

PP6 Biological signatures across seven dominant human cancers in Sri Lanka

Senadheera SPBM, Karunanayake EH, Weerasinghe AR, Wijesinghe CR

PP7 Mutational analysis of B cell activating factor receptor (BAFF-R) and inducible costimulator (ICOS) in patients with common variable immunodeficiency (CVID) in Sri Lanka

Gayathri K, Weeraman CPK, Sathkumara HD, Handunnetti SM, De Silva AD, De Silva R

PP8 DNA barcoding and latex protease activity of Sri Lankan mountain papaya

Fonseka GGM , Weerasena OVDSJ , Kottearachchi NS

Professor Stanley Wijesundera Memorial Lecture

Professor Stanley Wijesundera Memorial Lecture is an annual event of the IBMBB held to commemorate late Professor Stanley Wijesundera, who served as Professor of Biochemistry and as First Vice Chancellor, University of Colombo from 1978-1987. Professor Wijesundera was a visionary leader during whose tenure, infrastructure development and academic progress in the University of Colombo was at its peak. He played a key role in the development of the discipline of Molecular Biology in the University of Colombo.

Previous Professor Stanley Wijesundera Memorial Lectures were delivered by Professor Rune Liminga, Former Director, International Programme in Chemical Sciences, University of Uppsala, Sweden; Professor W D Lakshman, Former Vice Chancellor, University of Colombo; Professor Eric Karunanayake, Founder Director, IBMBB & Emeritus Professor of Biochemistry, University of Colombo; Professor Lal Chandrasena, Senior Professor of Biochemistry, University of Kelaniya and Professor E Dilip De Silva, Senior Professor of Organic Chemistry, University of Colombo;. Professor Kamani Tennekoon, Senior Professor of Molecular Life Sciences, IBMBB, University of Colombo, Former Director, IBMBB & former Professor of Physiology, Faculty of Medicine, University of Colombo. This year Professor Stanley Wijesundera Memorial Lecture will be delivered by Vidyajyothi Professor Rezvi Sheriff, Senior Professor of Medicine, Kotelawala Defence University, Sri Lanka and Former Senior Professor of Medicine, Faculty of Medicine, University of Colombo.

Professor Stanley Wijesundera Memorial Lecture

Sri Lankan CKDu Epidemic – Research & Service Challenges

Vidyajyothi Professor Rezvi Sheriff

MD FRCP FRCPE FRACP FCCP FNASSL FSLCGP FIMACGP

Senior Professor of Medicine, Kotelawala Defence University, Sri Lanka

An epidemic of chronic kidney disease of unknown origin (CKDu) was noticed by the University Medical unit, Colombo and presented to the Sri Lanka Medical Association in 1994. For a few years descriptive research directed by Dr. Abeysekare of Kandy General Hospital led to increased awareness and realization of its increasing incidence and prevalence. Initially North Western and North Central Provinces in the dry zone were identified and now Uva and Eastern Provinces have been added to endemic areas. Such CKDu "Hotspots" have now been identified in several areas in the world predominantly in poor farmers – rice farmers in Sri Lanka and Uddanam district in Andra Pradesh, India and Sugar cane workers in the Meso-American region of El Salvador, Costa Rica and Nicaragua. The clinical features and aetiopathogenesis seem similar everywhere and it is suggested that there may be some common associated factors as the pathology is found to be a chronic interstitial nephropathy.

As the case numbers increased, a vast amount of research was undertaken in Sri Lanka in several sectors – Agriculture, Water, Geology, Fertilizer use, Health, Public Health, Social Science etc. Many Academic Centres took part in these studies. University of Peradeniya has given leadership. The World Health Organization (WHO) and Epidemiology Unit of Ministry of Health with funding largely from the National Science Foundation (NSF) undertook a massive project bringing together many scientists and their teams including scientists from relevant sectors. Water sources, water contaminants, pesticide residues, heavy metals eg: Arsenic, Cadmium etc. have all been implicated as associated with aetiology. A multi-factorial origin has been proposed. No clear agreed hypothesis has yet emerged.

There is now enthusiastic political leadership and many committees, and many sets of recommendation have been partially implemented. Health Ministry has set up several screening clinics; dialysis and transplant centers to mitigate the effects of the illness on suffering families. Several measures to supply clean portable water is in place. Social service measures to counsel persons and offer financial help is in place. There is no definite aetiology yet in sight. A recent International Expert Consultation organized by the WHO, NSF and Presidential Task Force did detailed analysis of all factors and literature presented & published. Most scientists from all sectors took part in these discussions. These recommendations will examine the research and clinical challenges ahead promoting our medical and scientific personnel to come together, get this epidemic under control locally and also collaborate & learn lesson from the global epidemic.

What was needed is to join hands and do research at a national level to achieve goals needed. We hope the National Science Foundation will provide the necessary platform.

Abstract No: OP1

Cytotoxic potential of new compounds isolated from *Mangifera zeylanica* on breast (MCF-7 and MDA-MB-231) and ovarian (SKOV-3) cancer cells

Ediriweera MK[1], Tennekoon KH[1], Samarakoon SR[1], Thabrew I[1], de Silva ED[2], Adhikari A[3]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo
[2]Department of Chemistry, Faculty of Science, University of Colombo
[3]HEJ Research Institute of Chemistry, University of Karachi, Pakistan

Mangifera zeylanica (Anacardiaceae) is a plant endemic to Sri Lanka. Bark of this plant has been used in traditional medicine for the treatment of some cancers. The present study was planned to isolate active cytotoxic compounds from the chloroform extracts of the bark of *M. zeylanica*. Preliminary screening of extracts of *M. zeylanica* separated by sequential extraction confirmed that the chloroform extract of bark exerted cytotoxic effects to breast and ovarian cancer cells. Chloroform extract of the bark was subjected to chromatographic techniques including silica gel column chromatography and reversed phase preparative HPLC to isolate possible cytotoxic compounds. Two new halogenated compounds, bromomangiferic acid and chloromangiferamide along with two known flavonoids, quercetin and catechin were isolated from the chloroform extract. The structures of active compounds were elucidated by means of combination of 1H-NMR, 13C-NMR, 2D-NMR (COSY, HSQC, HMBC) and ESI-MS techniques. MDA-MB-231 (triple negative breast cancer cells), MCF-7 (ER positive breast cancer cells), SKOV-3 (ovarian epithelial cancer cells) and MCF-10A (normal mammary epithelial) cells were used to evaluate cytotoxic properties of isolated compounds. Among the two isolated halogenated compounds, only chloromangiferamide showed cytotoxicity to triple negative breast cancer cells (IC_{50}: 73. 06±0.37 μM) and it was not cytotoxic to normal mammary epithelial cells at 24 h post incubation. Quercetin and catechin showed cytotoxic activity in all three cancer cells.

This work was supported by the National Research Council of Sri Lanka (Grant No 11-018) and constitutes a part of PhD studies of MKE.

Abstract No: OP2

Possible mechanisms mediating anti-proliferating effects in breast cancer phenotypes by *Vernonia zeylanica* (L.) Less

Mendis AS, Thabrew MI, Samarakoon S, Tennekoon KH

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Vernonia zeylanica (L.) Less (Pupula), (Family Compositeae) is traditionally used in Sri Lanka to treat boils. Previous studies by the authors has shown that chloroform and ethyl acetate fractions (CE fraction) of *V. zeylanica* can exert significant cytotoxic effects in MCF-7 (ER positive / PR positive, Her2 negative), and MDA-MB-231 (Triple negative) breast cancer cells. Main aim of the present study was to determine the possible mechanisms by which the CE fraction of *V. zeylanica* mediates cytotoxic effects in MCF-7 and MDA-MB-231 cells. Modulation of heat shock protein (HSP) 70 & 90 were evaluated by (a) Real time reverse transcription PCR and (b) Immunofluorescence analysis. Apoptosis was evaluated by (a) fluorescent microscopic examination of apoptosis related morphological changes and (b) DNA fragmentation and (c) Caspase 3/7 activities. Significant ($p<0.05$) inhibitions of HSP 90 and HSP 70 expression were mediated by CE fraction in MCF-7 and MDA-MB-231. Clear apoptotic morphological changes on Acridine orange/Ethidium bromide staining and DNA fragmentation were observed in both breast cancer cell lines. Caspase 3/7 were significantly ($p<0.05$) activated only in MDA-MB-231 cells indicating caspase dependent apoptosis in these cells and caspase independent apoptosis in MCF-7 cells. Modulation of HSP 90 and HSP 70 expressions is a possible mechanism by which the CE extract of *V. zeylanica* mediates anti-proliferating effects in MCF-7 and MDA-MB-231 cells. This effect appears to correlate with enhanced apoptosis in these cells. Overall findings suggest that the *V. zeylanica* has the potential to be exploited further for effective treatment of breast cancer.

This work was supported by the Ministry of Higher Education, Sri Lanka and constitutes a part of PhD studies of ASM.

Abstract No: OP3

Anti-proliferative and proapoptotic effects of govaniadine in human breast cancer (MCF-7) cells

Sivakumaran N[1], Samarakoon SR[1], Adhikari A[2], Ediriweera MK[1], Tennekoon KH[1], Thabrew I[1], Malavige N[3]

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[2]HEJ Research Institute of Chemistry, University of Karachi, Pakistan

[3]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayawardanapura

Breast cancer is the most common cancer in women worldwide. Induction of apoptosis is one of the major mechanisms by which cancer cells are eliminated by anti- cancer agents. The present study was carried out to evaluate the anti- cancer activity of govaniadine extracted from *Corydalis govaniana* Wall roots, against MCF-7 breast cancer cells. Anti-proliferative effects of govaniadine in MCF-7 and normal mammary epithelial (MCF-10A) cells were evaluated by Sulforhodamine B assay; distinct morphological changes of apoptosis by light and fluorescence microscopy; apoptosis related gene expression (*Bax*, *Survivin* and *p53*) using real time PCR; quantification of caspase-7 levels by Caspase-Glo® 3/7 Assay; quantification of apoptosis by flow cytometry and DNA fragmentation by gel electrophoresis. Govaniadine caused significant anti-proliferative effects against MCF-7 cells in a dose- and time-dependent manner with IC_{50} of 0.9579±0.7, 0.7573±0.89 and 0.5273±0.75 µg/mL at 24, 48 and 72 h respectively and no significant cytotoxicity against MCF-10A cells. Gene expression analysis confirmed that govaniadine-induced apoptosis was accompanied by significant increase ($p<0.05$) in the expression levels of *Bax* and *p53* in a dose dependent manner. *Survivin* expression was also significantly upregulated at the lowest dose. Flow cytometric analysis evidenced that the early and late apoptotic populations of treated MCF-7 cells significantly increased in a dose-dependent manner. Microscopic observations of treated MCF-7 cells confirmed morphological changes as characteristic of apoptosis. DNA laddering and caspase-7 activation were also shown by treated MCF-7 cells suggesting the involvement of intrinsic and extrinsic signaling pathways. Overall results of this study suggest that govaniadine has the potential to be developed as an anti-cancer agent against breast cancer.

This work was supported by the IBMBB and constitutes a part of the MSc studies of NS

Abstract No: OP4

Anti-proliferative effect of five Sri Lankan endemic plants on breast cancer stem cells

Rajagopalan U[1], Samarakoon SR[1], Tennekoon KH[1] and de Silva ED[2]

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[2]Department of Chemistry, Faculty of Science, University of Colombo

Breast cancer is the most common female cancer and cause of cancer-related deaths among women in the world. The cancer stem cell (CSC) hypothesis proposes that a small subset of cells is responsible for the initiation, extensive proliferation and metastasis of cancer. CSCs have the capacity to generate the heterogeneous population of cells that constitute a tumor and resistance to conventional radiation and chemotherapies. Therefore it is an urgent need to search persuasive drug leads which can target CSCs. In the present study, five potential Sri Lankan endemic plants; *Schumacheria castaneifolia* (Family: Dilleniaceae), *Garcinia zeylanica* (Family: Clusiaceae), *Garcinia quaesita* (Family: Clusiaceae); which have been used in traditional medicines and two plants *Memecylon rostratum* (Family: Melastomataceae) and *Doona nervosa* (Family: Dipterocarpaceae), were selected to test on breast cancer stem cells (bCSCs). BCSCs belonging to $CD24^{-/low}$ $CD44^{+}$ molecular phenotypic family concealed amongst MCF-7 cell population were isolated by magnet assisted cell sorting technique and propagated *in-vitro* as tumorspheres to perform functional assays. Extracts prepared from dried bark and leaves of selected plants with hexane, chloroform, ethyl acetate and methanol by ultra-sound assisted sequential maceration method were tested on bCSCs. Anti-proliferative activities of each extracts (25-400 µg/mL) were assessed by water soluble tertazolium-1 (WST1) cell viability assay after 24 h of exposure. Hexane and chloroform extracts of *G. zeylanica* bark exerted highest anti-proliferative effect on bCSC with IC_{50} of 13.65 µg/mL and 5.12 µg/mL respectively. Hexane crude of *G. quaesita* and *M. rostratum* barks exhibited somewhat less anti-proliferative activity (IC_{50}: 21.70 µg/mL and 34.77 µg/mL). Therefore, hexane and chloroform extracts of *G. zeylanica* were identified as the extracts with highest activity among the extracts tested.

This work was supported by National Research Council of Sri Lanka (Grant NRC-14-067) and constitutes a part of MPhil/PhD studies of UR.

Abstract No: OP5

Induction of apoptosis in response to improved gedunin by liposomal nano-encapsulation in human small-cell lung cancer (NCI-H292) cell line

Nwokwu CDU[1], Samarakoon SR[1], Karunaratne DN[2], Katuvawila KANP[3], Pamunuwa KMGK[2], Ediriweera MK[1] and Tennekoon KH[1]

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[2]Department of Chemistry, Faculty of Science, University of Peradeniya
[3]Sri Lanka Institute of Nanotechnology, Nanotechnology and Science Park, Homagama

Nano-encapsulation has proved to be effective in circumventing the limitations of conventional cancer chemotherapeutics. There are no documented evidences on the effect of gedunin, derived from the neem plant on lung cancer. Due to the hydrophobicity of gedunin, we sought to enhance its pharmacokinetic profile, and hence, its anti-cancer property. Gedunin was successfully encapsulated in liposomes by thin film hydration method with 86.82 % efficiency, and average particle size and zeta potential (mean±SD) of 394.1±89.4 nm and – 54.1±5.91, respectively. Both free and encapsulated gedunin were investigated for anti-proliferative activities by Sulphorhodamine B (SRB) assay against NCI-H292 cells. Liposomal gedunin (LG) exhibited dose- and time-dependent 10-fold inhibitory potential (Mean IC_{50} of 3, 2, 1.9 µg/mL at 24, 48 and 72 h) compared to the free gedunin (26, 23, and 20 µg/mL), while displaying considerable safety profile on normal MRC-5 cells relative to the clinically used drug, paclitaxel. Apoptosis detection assays showed that LG triggered caspase-mediated apoptosis ($p<0.05$) as verified by Caspase-Glo 3/7 assay, and significantly modulated ($p<0.0001$) mRNA expression of apoptosis-related genes (*p53, Bax* and *survivin*) as determined by real-time PCR. While *survivin* recorded a down-regulation by 0.14 fold, *p53* and *Bax* were up-regulated by as much as 33 and 7 folds respectively in the 1 µg/mL treatment. Further apoptotic evidences garnered by cytomorpho-logical assessment and DNA fragmentation reinforced our hypothesis. Because of its anticancer efficacy with minimal side effects, liposomal gedunin may therefore be considered a drug-able system for lung cancer that deserves optimization and clinical development.

This work was supported by the IBMBB and constitutes a part of the MSc studies of CDUN

Abstract No: OP6

Anti-filarial activity of *Dipterocarpus zeylanicus:* Bioactivity guided isolation of active compounds and induction of apoptosis in filarial parasite *Setaria digitata in vitro*

Senathilake KS[1], Karunanayake EH[1], Samarakoon SR[1], Tennekoon KH[1], Adhikari A[3], de Silva ED[2]

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[2]Department of Chemistry, Faculty of Science, University of Colombo

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Absence of a drug that kills adult parasites remains the major challenge in eliminating human lymphatic filariasis. Thus, the discovery of novel anti-filarial compounds with adulticidal activity is an urgent need. The current study was aimed at the isolation of anti-filarial saponins from seeds of *Dipterocarpus zeylanicus* (Dipterocarpaceae) and the investigation of apoptosis induced by their less toxic aglycones in cattle filarial parasite *Setaria digitata*. The dried methanol extract of seeds of *D. zeylanicus* was dissolved in water and partitioned in to hexane, dichloromethane and ethyl acetate sequentially. The active ethyl acetate extract was subjected to bio-assay guided isolation of antifilarial compounds using chromatographic techniques. Isolated compounds were identified using mass spectrometry and NMR techniques. Cytotoxicity of compounds to human cells was investigated by using peripheral blood mononuclear cells (PBMC) and WST-1 assay. Two antifilarial saponins were isolated and identified as Asperosaponin C and Hederoside A2. Since these compounds were toxic to PBMC, they were acid hydrolyzed to obtain oleonolic acid. Oleonolic acid had reduced toxicity against PBMC but retained good adulticidal and microfilaricidal activity (IC_{50} of 38.20±3.18 μM and 22.00±3.16 μM respectively). The apoptotic features in parasites treated with oleonolic acid were visualized by Hoechst 33342 staining, TUNEL assay and DNA ladder assay. Condensation of nuclear DNA, apoptotic body formation and DNA laddering were observed in treated parasites. In conclusion, Oleonolic acid obtained from acid hydrolysis of antifilarial saponins of *D. zeylanicus* induced apoptosis in adult and microfilaria of *S. digitata* suggesting its potential as a good drug lead against filarial parasites.

This work was supported by the Ministry of Higher Education of Sri Lanka and constitutes a part of PhD studies of KSS.

Abstract No: OP7

Comparison of HPLC profiles of venom of *Apis darsata* Fabricius (Giant Asian honey bee) and *Apis mellifera* Linnaeus (Western honey bee)

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Anaphylaxis due to *Apis dorsata* Fabricius (Giant Asian honey bee) venom is a recognized cause of death among people predominantly in rural areas of Sri Lanka. Characterization of venom components of *A. dorsata* is necessary as the data is limited. This study was conducted to determine the venom components of *A. dorsata* and to compare with venom components of *Apis mellifera* Linnaeus (Western honey bee). The venom of *A. dorsata* was collected by mild electrical stimulation of worker bees resulting in ejection of venom from its stinger. Commercially available crude venom and two pure venom components: phospholipase A_2 and melittin; from *A. mellifera* were used for comparison. High Performance Liquid Chromatography (HPLC) methodology for separation of venom components was established using a C18 (100 Å) chromatographic column and two mobile phases: A-0.1% trifluoroacetic acid (TFA) in deionized water and B-0.1% TFA in acetonitrile: 0.1% TFA in deionized water (80:20). The separated venom components were detected using photo diode array (PDA) detector at 220 nm. The best separation for venom components from both species were obtained with gradient elution 5% B-80% B for 40 min at flow rate of 2.0 mL/min. Similar HPLC profiles were obtained with a total of 7 peaks for both species. Two of the peaks were identified as phospholipase A_2 and mellitin from the crude venom of both species. Melittin gave the highest peak for both *A. dorsata* and *A. mellifera*. The similarities in venom components identified may suggest similar reactivities in patients who have had anaphylaxis due to venom components of *A. dorsata* and *A. mellifera*.

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Abstract No: OP8

Diagnostic accuracies of six different immunoassays compared to the gold standard tests, real-time quantitative PCR and isolation of pathogenic *Leptospira*

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Leptospirosis is mostly diagnosed on clinical grounds and laboratory confirmation is made by the microscopic agglutination test (MAT). Leptospirosis Burden Epidemiology Reference Group (LERG) recommends using MAT on or after eight days of fever or test paired sera for detection of antibody rise or seroconversion. Detection of pathogenic *Leptospira* organism by culture or the DNA in the clinical sample is the gold standard tests recommended by LERG. We evaluated the diagnostic performance of six immunoassays against the microbiological gold standard tests. The immunoassays tested were MAT against genus specific *Leptospira*, commercial IgM ELISA (Virion-Serion), two in-house IgM-ELISAs developed to detect IgM against saprophytic and pathogenic *Leptospira*, two IgM-based immunochromatography tests (Leptocheck-WB, Zephyr Biomedicals and Standard diagnostics (SD)). Real-time quantitative PCR for LipL32 gene of pathogenic *Leptospira* was performed. Sixty patients whose day of sampling was <8 were tested out of which 71.7% (n=43) patients showed positive results by qPCR. This group had 14 pathogenic *Leptospira* isolations. The immunoassays were compared against the qPCR/culture. Sensitivity of acute MAT was very low (39.5%) compared to paired MAT (74.4%) and their specificities were 88.2% and 76.5% respectively. Serion, in-house IgM-ELISAs (saprophytic and pathogenic) showed sensitivities of 66.5%, 69.8% & 53.5% and specificities of 82.4%, 82.4% & 88.2%. Rapid IgM tests Leptocheck-WB and SD test produced sensitivities of 69.8% & 51.2% and specificities of 82.8% & 58.8%. Acute MAT showed low sensitivity than paired MAT, therefore paired MAT may be more suitable for laboratory confirmation. Both Leptocheck-WB and in-house saprophytic IgM-ELISA established showed similar diagnostic accuracies compared to gold standard.

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Abstract No: OP9

Effects of pathogenic *Leptospira* on functions and viability of vascular endothelial cells and kidney epithelial cells

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Leptospirosis is the commonest zoonotic infection worldwide. Knowledge on its pathogenesis is very limited. Objective of the study was to determine the effect of the interaction between a pathogenic *Leptospira* and i) human vascular endothelial cells (VEC) and ii) kidney proximal convoluted tubule epithelial cells (KEC). *L. interrogans* (pathogenic) and as a control, *L. biflexa* (saprophytic) were grown in Ellinghausen-McCullough-Johnson-Harris media. Washed *Leptospira* organisms were interacted with both VEC and KEC monolayers at 0.2, 1 and 2 multiplicity of infection (MOI) separately for 1, 2, 4 and 6 hour durations at 37 °C. Cell viability and function were determined using SRB and MTT assay respectively at each time point. Percentage viabilities of both VEC and KEC even at 6 hours of incubation were >90 % for all three MOIs of *L. biflexa*. Also, no significant reduction of NADPH oxidase activity was observed at any of three MOIs of *L. biflexa* interaction with both VEC and KEC. In experiments with *L. interrogans,* MOI 1 and 2 after 6 hours had significant low percentage viabilities (13 % and 12 % respectively) and loss of NADPH oxidase activity (79 % reduction) in VEC ($p<0.001$). No such significant loss of viability or NADPH oxidase activity was observed in KEC for all three MOIs of *L. interrogans* even after 6 hours. The results indicate that 6 hours exposure of *L. biflexa* does not affect function or viability of VEC and KEC. In contrast, after 6 hours of exposure, *L. interrogans* causes significant cell death and loss of cell function in VEC. However, KEC were not affected by them. These results may explain partly the sequestration and long-term survival ability of *L. interrogans* in the kidney and also its ability to damage the vascular endothelium contributing to pathogenesis of leptospirosis.

This work was supported by National Research Council (Grant no. 12-077), Sri Lanka and constitutes a part of the PhD studies of NF.

Abstract No: OP10

Community based sero-prevalence of human leptospirosis infection in the Colombo District

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Leptospirosis is considered as the most globally widespread zoonotic illness. Despite the high prevalence of leptospirosis in Sri Lanka, there is a paucity of data on ser-prevalence of the disease. This study was carried out to determine the community based sero-prevalence of leptospirosis in the Colombo district for accurate identification of epidemiological associations in local communities. A community based cross sectional study was carried out in the Colombo District. A representative sample of the district was selected by a two stage cluster sampling technique with probability proportion to size. A total of 27 Grama Niladhari Divisions (GNDs) were selected. A cluster of 30 households was selected from each of the 27 GNDs. Five mL of blood was collected from a participant above the age of 18 years from each household. Serological assays were done at the Medical Research Institute, Colombo to determine the Microscopic Agglutination Test (MAT) titers using a panel of 11 common pathogenic serovars for leptospirosis found in Sri Lanka. A MAT titer of ≥40 was considered as indicative of sero-positive in this community based study. To date, 300 samples have been analyzed by MAT. Anti-leptospiral antibodies for at least one pathogenic serovar was found in 177 of the participants enrolled for the study. The predominantly reacting serogroups in this study were Pyrogenes (63/300), Grippotyphosa (39/300), Autumnalis (39/300), Australis (37/300) and Hebdomadis (34/300). Fourteen out of 177 (7.9%) participants tested positive for the presence of pathogenic leptospiral antibodies were reported to have had the disease previously whereas 92.1% (n=163) of them had no record of being previously infected with the disease. This could be due to under diagnosis of the disease, due to high number of asymptomatic cases or due to the participant having a mild form the disease. As a result the number of cases of leptospirosis in the Colombo District can be higher than what is reported in hospitals.

This work was supported by University Research Grant (AP/3/2/2014/RG/14) *and constitutes a part of MPhil studies of KB.*

Abstract No: OP11

Contribution of different types of antioxidants to dengue hemorrhagic fever patients in Sri Lanka

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Oxidative stress arises due to the imbalance between pro-oxidants and anti-oxidants is considered to be one of the postulated contributory factors leading to the pathogenesis of severe dengue infection. Serum antioxidant levels give an indirect measurement of oxidative stress and levels of pro-oxidants present in the body. The main objective was to assess different serum antioxidant types and to seek their association with disease severity of dengue infection. Confirm dengue fever (DF, n=60) and dengue haemorrhagic fever (DHF, n=48) patients were selected from Colombo North Teaching Hospital (CNTH), Ragama. Blood samples (~8 hours fasting) were collected at different stages of disease; admission (A), critical (C) and discharge (D) leading to 5 different disease stages of DF-A, DF-D, DHF-A, DHF-C and DHF-D. Disease was confirmed by rapid detection assay of NS1 antigen and by IgM levels detected and measured using quantifying ELISA kit. ABTS decolorization assay was used to measure antioxidant capacity (AOC). Serum bilirubin, uric acid and albumin levels were also measured using commercially available kits. Antioxidant capacity in DHF-A was significantly lower compared to DF-A ($p=0.027$) and highest AOC was recorded in HC which is significantly higher compared to all patients categories ($p<0.001$). Serum bilirubin in DHF-A was significantly higher than DFA ($p=0.019$) and this may be due to liver involvement. Serum albumin and uric acid levels in all patient categories and HC were similar. Results of multiple regression analysis showed no association between AOC with bilirubin, uric acid and albumin (Durbin-watson factor = 1.560, $p=0.128$). Serum AOC was inversely related to the levels of serum pro-oxidants. Therefore, low levels of serum AOC indicate high levels of serum pro-oxidants in severe dengue infection and may be considered as a marker to differentiate DHF from DF.

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Abstract No: OP12

Screening of selected Sri Lankan cancer patients for somatic mutation of *TP53* gene

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Many drugs used in the treatment of cancers are genotoxic. *TP53* need to be active to respond to such treatment. Thus, before deciding the treatment for the patient, mutation analysis of *TP53* gene is very important. *TP53* is inactivated by somatic mutations in most human cancers. Somatic *TP53* mutation analysis is a better prognostic method than immunohistochemistry. As it is expensive to screen the entire *TP53* gene, regions which are highly prone for mutations can be analyzed to detect most of the mutations. There are no previous studies reported on somatic mutations in the *TP53* gene in Sri Lankan cancer patients. Thus the objective of the current research is to establish the *TP53* mutation spectrum in Sri Lankan cancer patients. DNA was extracted from fresh tissues of thirteen cancer patients. PCR amplification of exonic region of *TP53* was carried out. Purification of PCR amplified products was done, followed by sequencing using Big Dye Terminator v3.1 kit and 3500 Dx genetic analyzer. Bioedit software was used to screen the sequence variants and the mutations. Results showed two pathogenic mutations: one in exon 5 (Codon 175 - CGC>CAC) and the other in exon 8 (Codon 282 - CGG>TGG). A possibly pathogenic mutation in intron 3 (11251-11266 16bp deletion) in 12 patients and several non-pathogenic mutations (intron 2: one mutation in 9 patients, intron 6: one mutation in one patient, intron 7: two mutations in 4 patients) were detected. Further studies are in progress to study a larger number of patients to establish the *TP53* mutation spectrum in Sri Lankan cancer patients.

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Abstract No: OP13

Identification of AFLP marker/s to differentiate herbicide resistant Sri Lankan rice (*Oryza sativa*) varieties

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Introducing herbicide resistant (HR) rice in a cropping program ensures better management of herbicide based control of rice weeds. Development of molecular markers for the purpose of identification of herbicide resistance provides easy selection of HR lines/varieties in rice breeding programs. Present study was conducted to select Amplified Fragment Length Polymorphism (AFLP) marker/s to differentiate resistant and susceptible Sri Lankan rice varieties to broad-spectrum herbicide, glyphosate. Glyphosate (0.5 g/L) was applied to 25 different rice varieties by following complete randomized design with three replicates and glyphosate resistance was evaluated. After application of herbicide, plants which showed ≥50% resistance were considered as resistant to glyphosate. In the field experiment twelve varieties (Bg359, At362, Bw364, Ld365, Bg366, Bg369, Bg403, Bg379-2, Bg403, *Kaluheenati*, *Pachchaperumal* and *Kurulu thuda*) showed high resistance (≥50%) to glyphosate and four varieties were susceptible (Bg300, Bg304, H4 and *Rathal*). AFLP analysis was carried out using 16 fluorescently labeled AFLP primer combinations and fragments were separated by capillary electrophoresis. Fragments were analyzed using Genemapper® v4.1 software and sized according to Liz 600 size stranded. Fragments were compared between resistant and susceptible varieties. The 97 bp fragment of E12M34 (TGT AAA ACG ACG GCC AGT GAC TGC GTA CCA ATT CAG GAT GAG TCC TGA GTA AAA C) was found to be a specific marker for glyphosate resistant rice varieties. The 78 bp fragment of E12M31 (TGT AAA ACG ACG GCC AGT GAC TGC GTA CCA ATT CAG GAT GAG TCC TGA GTA AAA A) was identified as a specific marker for glyphosate susceptible rice varieties. However, these markers require further validation using more rice varieties with known glyphosate tolerance.

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Abstract No: OP14

Screening of novel chitinolytic *Bacillus thuringiensis* strains isolated from Sri Lankan soil

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Bacillus thuringiensis (Bt) is an insecticidal bacterium, marketed worldwide for control of many important insect pests of insect orders, Lepidoptera, Coleoptera and Diptera. It has been reported that chitinases are widely distributed in *Bt* strains and some of the chitinase producing strains can enhance the insecticidal activity of *Bt* . The objective of the present study is to investigate the chitinase producing *Bt* strains. Sixteen *Bt* strains, isolated from soil; AB6, AB7, AB8, AB10, AB11, AB12, AB13, AB14, AB15, AB16, AB17, AB19, AB20, AB21, AB22 and AB23 were grown in Luria Bertani broth. Overnight grown cultures were inoculated on colloidal chitin agar using toothpick heads and incubated at room temperature. The hydrolysis zone was measured 5 days after incubation. *Bt* strains produced hydrolysis zones over 0.5 cm were subjected to secondary screening; disk dipping method, where autoclaved paper disks were dipped in the bacterial cultures and placed on colloidal chitin agar plates. The plates were incubated at room temperature for one week. Hydrolysis zone and colony diameter was measured and the chitinolytic index was calculated. The experiment was done in triplicates. From the 16 *Bt* strains screened, all strains exhibited chitinase activity for primary screening by producing hydrolysis zones over 0.5 cm except AB21. Chitinolytic index obtained from the disk dipping method revealed that *Bt* strain AB15 exhibited the highest chitinolytic index of 1.571 followed by *Bt* strains AB20, AB10, AB6, AB19, AB22, AB13, AB8, AB23, AB17, AB7, AB11, AB12, AB14 and AB16. This study demonstrated that high chitinase activity (chitinolytic index > 1.0) was shown by 15 *Bt* strains. This chitinase activity could enhance the insecticidal activity of those *Bt* strains against insect pests.

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Abstract No: OP15

Effect of Pinacidil on the expression of SUR2A in rat embryonic heart-derived H9c2 cells

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Cardiac K_{ATP} channels are composed of Kir6.2 (pore forming subunit) and SUR2A (regulatory subunit). SUR2A is an atypical ATP binding protein and it is an integral part of heart K_{ATP} channels. Recent studies suggest, increased SUR2A expression alone can induce cardioprotection under stress conditions. At present, it is yet unknown whether long-term changes in K_{ATP} channels activity would have any effect on SUR2A levels in cardiomyocytes. The current study explores the effects of ion channel opener (100 μM Pinacidil) on the expression of SUR2A subunit in rat embryonic heart-derived H9c2 cells. Quantification of protein concentration was performed by Bradford assay and western blotting technique was used to investigate the expression of SUR2A in H9c2 cells. Western blotting results showed no significant difference in expression of SUR2A in H9c2 cells with Pinacidil (100 μM) exposure for 24 h ($p>0.05$; $n=3$). Moreover, loading control (Pan-Actin) also showed no significant difference between Pinacidil (100 μM) treated and untreated H9c2 cells ($p>0.05$; $n=3$). In conclusion, current study confirms that long term channel activity of cardiac K_{ATP} channels (using 100 μM Pinacidil) has no influence on the expression of cardioprotective regulatory subunit SUR2A in H9c2 cells.

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Abstract No: OP16

Sequence variants in the promoter region of *GH1* gene in a cohort of growth hormone deficient (GHD) children in Sri Lanka

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Alterations in the *GH1* gene lead to growth hormone deficiency (GHD). We previously reported two pathogenic and one possibly pathogenic mutations following sequencing of the exonic and intronic regions of the *GH1* gene in children with GHD. Expression of *GH1* gene is regulated by proximal promoter and locus control regions. The promoter region of *GH1* gene is highly polymorphic. To screen for genetic alterations in the promoter region, a subset of 34 GHD children negative for pathogenic mutations in the exonic and intronic region of the *GH1* gene were selected. 731 bp upstream to the transcription start site was directly sequenced and ten genetic variants were observed. All these variants were accumulated within 500 bp from the transcription start site. One of these was novel and the location is c.-394G>A. The remaining nine were reported variants, rs1811081, rs2011732, rs2005171, rs71651677, rs11568828, rs2005172, rs11568827, rs6171 and rs695. Of these, rs2005171, rs2005172, and rs6171 variants reported to show altered gene expression were observed in homozygous state (prevalence rate: 11.77%, 14.71% and 11.77% respectively). Out of these variants, rs2005172 was also observed in adults of normal height (prevalence rate: 23.53%) in a homozygous manner, while rs2005171 and rs6171 were not detected. Normal height individuals studied carried several other variants seen in GHD children, and one of them also carried four variants (rs2005170, rs61761357, rs71640267 and rs41299067) not observed among GHD children. Thus rs2005171 and rs6171 are likely to be at least partly responsible for growth hormone deficiency leading to short stature in the cohort studied.

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Abstract No: OP17

Draft Genome Sequence of *Lactobacillus jensenii* Strain MD IIE-70(2)

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Lactobacillus jensenii predominates in the microflora of a healthy human vagina and plays an important role in protecting the host from urogenital infections. This report describes a draft genome sequence of *L. jensenii* strain MD IIE-70(2), selected for its superior probiotic properties compared to other *Lactobacillus* strains tested. The mapping of 1,403,599 reads generated by two sequencing runs on the Ion Torrent PGM using a 314 chip onto the available draft genome sequences revealed the highest level of similarity to *L. jensenii* strain 1153. The assembly of reads performed by Ion Torrent Assembler and CLC Genomics Workbench de novo assembly programs. The total size of the assembly (1,759,880 bases) and G+C content (34.3%) are in perfect agreement with figures for the genomes of other strains of this species. Data annotation was conducted by using the NCBI GenBank annotation pipeline and RAST server. The latter reported 1,754 coding sequences and 60 RNAs. Compared with other genomes of this species, the differences found were mainly attributed to prophage-related genes, transposases, and insertion sequence (IS) elements. Focus on genes supporting probiotic action revealed the presence of five genes encoding putative adhesins, genes encoding pyruvate oxidase that is responsible for H_2O_2 production and genes responsible for resistance to β-lactam antibiotics and fluoroquinolones, beneficial when combining probiotic treatment with antibiotic therapy. A combination of the recent advancement in genetic engineering techniques and the data reported in this study may also assist in the construction of novel beneficial probiotic strains with improved properties.

Abstract No: PP1

Screening of selected natural compounds for anti-cancer activity regulated via induced differentiation on cancer stem cells (NT2)

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Cancer stem cells (CSC), a subset of cells that possesses stem cell characteristics cause implications such as therapeutic resistance to conventional anti-cancer therapies and relapse of cancer. Hence novel approaches such as differentiation therapy that ceases CSC proliferation by inducing them into a more differentiated phenotype that can undergo apoptosis or normal cell cycle holds the key to eliminate cancer. The present study was carried out to evaluate the potential of selected compounds (curcumin, gedunin, govaniadine, and 3-O-α-L-arabinosyloleanolic) to induce differentiation in CSC model NTERA-2 cl. D1 (NT2) cells. Anti-CSC activity of the compounds were evaluated using Sulforhodamine B (SRB) assay and ability to induce differentiation in NT2 was evaluated by non-adherent cell culturing method followed by analysis of morphological changes and gene expression levels of two key CSC markers SOX2, OCT4 and neuronal differentiation marker NEUROD1 using real time PCR. Results were further confirmed using immunocytochemistry analysis. All trans retinoic acid (ATRA) was used as the positive control as it is known to induce differentiation in NT2 cells. All the compounds used showed strong dose and time depended anti-CSCs activity (IC50 values ranging from 1.45 µg/mL – 14.59 µg/mL at 24 h, 1.25 µg/mL - 8.49 µg/mL at 48 h and 0.61 µg/mL – 6.55 µg/mL at 72 h). Induced differentiation was identified morphologically in govaniadine treated NT2 cells and confirmed by down regulation of SOX2 expression (fold change 0.68) and expression of NEUROD1 by real time PCR and immunocytochemistry analysis. Thus, the results revealed that govaniadine 1.5 µM treatment was able to induce differentiation in NT2 cells. The result suggests for the first time that govaniadine, a novel natural compound extracted from Corydalis govaniana Wall roots can induce differentiation in NT2 CSCs which will be useful in eliminating drug resistant CSCs.

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Abstract No: PP2

Evaluation of the effects of proanthocyanidins extracted from the bark of *Thespesia populnea* (L.) Sol.ex Correa, on cell survival and apoptosis of human hepatoma (HepG2) cells

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Thespesia populnea Sol.ex Correa (Gansurya), belongs to the family Malvaceae and is commonly found in Asian countries including Sri Lanka. Proanthocyanidin fractions such as aqueous soluble proanthocyanidin (AQSPA) and ethyl acetate soluble proanthocyanidin (EASPA) isolated from the bark of *T. populnea* have been reported to mediate cytotoxic activity in MCF-7 breast cancer cells. The main aim of the present investigation was to determine whether proanthocyanidins from *T. populnea*, can also protect against the proliferation of human hepatoma (HepG2) cells. The effects of proanthocyanidinson HepG2 cells were determined by evaluating their cytotoxicity, using the Sulphorhodamine assay, as well as their ability to modulate apoptosis by microscopic observation of morphological changes, expression of some selected genes associated with apoptosis (*Bax*, *Survivin* and *p53*), Caspase 3/7 activity and DNA fragmentation. The proanthocyanidin fractions exerted time and dose-dependent cytotoxicity with IC_{50} values ranging from 128 - 203.9 µg/mL at 24 h, 109.5 - 139.2µg/mL at 48 h and 41.77 - 45.64 µg/mL at 72 h. Induction of apoptosis in HepG2 cells was indicated bymorphological changes, activation of caspase 3/7, DNA laddering and significant increases ($p<0.05$) in the expression levels of *Bax* and *p53* in a dose dependent manner. The overall results confirmed that the proanthocyanidins from the bark of *T. populnea* has the potential to mediate cytotoxicity and induce apoptosis in HepG2 cells in a time and dose dependent manner. This is the first study to demonstrate that apoptosis is a mechanism by which proanthocyanidins extracted from *T. populnea* mediate anti-proliferative effects.

This work was supported by the IBMBB and constitutes a part of the MSc studies of LT.

Abstract No: PP3

Immunochemical characterization of venom of medically important ants in Sri Lanka and determination of venom cross-reactivity with Western ant species

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The number of reported cases of anaphylaxis in response to insect stings is on the increase. Fatalities have also been reported in certain cases, bringing into focus the medically important subject of Hymenoptera venom allergy (HVA). In Sri Lanka, the ant species responsible include *Odontomachus simillimus* (Smith, 1858), *Diacamma rugosum* (Forel, 1911), *Tetraponera rufonigra* (Jerdon, 1851) and *Solenopsis geminata* (Fabricius, 1804). The aim of this study was to obtain crude venom profiles for the four species of ants, identify possible allergenic venom components and determine cross-reactivity of patient serum with commercially available whole body extract (WBE) of the Western fire ant species, *Solenopsis invicta* (Buren, 1972). The ant species responsible for causing anaphylaxis were identified using accepted taxonomic keys, appropriate body length and weight measurements were also made. Crude venom components were separated under denaturing conditions using SDS-PAGE. The approximate molecular weights were estimated by calculating the respective R_f values and possible allergenic components identified with reference to the available literature. Sera was collected from patients that developed severe allergic reactions following ant stings (n=12) and the venom specific-IgE levels were quantified through the Phadia ImmunoCap method using the commercially available WBE of *S. invicta*. A total of 10 - 32 different crude venom proteins were obtained for the four species and 3 - 7 possible allergenic venom components were identified. Eight out of 12 patients showed positive results for the ImmunoCap test (IgE concentration >0.35 KU_A/L), indicating cross-reactivity with WBE of *S. invicta*. These preliminary findings on venom cross-reactivity indicate potential for development of assays to diagnose patients with ant venom allergy and to establish primary immunotherapy protocols for patient desensitization in Sri Lanka.

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Abstract No: PP4

A pilot study on selected point mutations of *CYP21A2* gene among a cohort of Sri Lankan children with Congenital Adrenal Hyperplasia

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Congenital Adrenal Hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency of one of the five enzymes required for synthesis of cortisol hormone in the adrenal cortex. As steroid 21-hydroxylase deficiency (21-OHD) is the most frequent, CAH in most contexts refers to 21-OHD. Depending on 21-hydroxylase enzyme activity, CAH patients are categorized into salt wasting (SW), simple virilizing (SV) and nonclassic (NC) types. 21-hydroxylase enzyme is encoded by the gene *CYP21A2* belonging to the family of genes called CYP. This study was performed to identify the prevalence of selected point mutations I2G, Q318X and R356W in *CYP21A2* gene among a cohort of Sri Lankan children with SW type of CAH. Blood samples were obtained and genomic DNA was extracted. Extracted DNA was quantified and subjected to PCR amplification using two pairs of primers to obtain amplicons containing the selected point mutations. Purified PCR products were quantified and subjected to SNaPshot SNP analysis using three SNaPshot primers specific for each mutation. Allele frequency of Q318X mutation was calculated to be 50%. Allele frequencies of I2G and R356W mutations could not be calculated as the presence of I2G could be detected only when the specific primer was used individually and the presence of R356W could not be detected with any of the optimized conditions. In order to get a proper estimation of allele frequencies, more samples need to be analyzed. A method such as direct sequencing may be applied to detect the presence of I2G and R356W mutations.

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Abstract No: PP5

A molecular phylogenetic analysis of six unknown amphibian specimens of the genus *Pseudophilautus* in Sri Lanka

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Pseudophilautus is a genus of shrub frogs which are endemic to Sri Lanka and to Western Ghats of South Western India belonging to the family of Rhacophoridae. It is important to understand the evolutionary relationship of these amphibians since many of these species are already extinct while a few of them have been rediscovered by researchers. This study was designed to perform a molecular phylogenetic analysis of selected six frog specimens in the genus *Pseudophilautus,* isolated from the Peak Wilderness, central hills of Sri Lanka. DNA was extracted from ethanol preserved muscle tissue samples. A segment of 16S rRNA was amplified by PCR using specific primers. Sequencing was carried out for both forward and reverse strands and the molecular phylogenetic analysis was performed using MEGA 6 software. Maximum likelihood analysis of these six specimens placed them in a monophyletic group along with *Pseudophilautus bambaradeniyai*. The pairwise genetic distance was 0% between *P. bambaradeniyai* and the three specimens, P.705, P.724 and P.712. However it shows 0.3% pairwise distance between P.715, P.727, P.726 and *P. bambaradeniyai* and 0.5% pairwise distance among P.715, P.727 and P.726 specimens. Despite showing considerably low pairwise genetic distances, these separate species show different morphological characters between themselves. This may be due to various reasons, especially geographical barriers existing among the natural habitats of these species. Further, an ongoing study on P.726 specimen was proved to be morphologically similar to extinct species *Pseudophilautus zal*. Therefore, the identification of P.726 may lead to the rediscovery of *P. zal*.

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Abstract No: PP6

Biological signatures across seven dominant human cancers in Sri Lanka

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Cancer is a disease in which abnormal cells acquire the ability to divide without control and invade other tissues. These attributes arise due to genetic and epigenetic alterations. This research was focused on identifying the relationships of prominent seven common cancers in Sri Lanka by focusing on genetic inter cancer heterogeneity and cross cancer similarity. Breast, colon, oesophagus, lung, ovary, prostate and uterine cancers were considered. Objectives of this study were to understand signatures and interrelationships among cancers and establish unique and common gene patterns of cancer types. ICGC data was used to develop the signatures with genes which were highly mutated in patients. Data mining techniques and biological networks were used to understand the interrelationships among cancer genes. Hallmarks of cancers, therapeutic targets and genetic interaction networks were used to improve the signature effectiveness. R package, WEKA tool, Gene MANIA web portal and NGC data portal and were used to develop the signatures. Common tumor suppressor and oncogene signature, common gene signature and individual cancer type signatures were developed with 34 genes. Cancer signatures were improved by combining non cancer genes, targetable signaling pathways and metastasis. Association rules, gene cluster parameters and literature evidence were used for signature validation. According to the results the colon cancer signature and the uterine cancer signature can be concluded as strong signatures. Breast cancer and prostate cancer have high gene variations reduced the effectiveness of the signatures. Furthermore, research can be enhanced by introducing proteomics and metabolomics details.

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Abstract No: PP7

Mutational analysis of B cell activating factor receptor (BAFF-R) and inducible costimulator (ICOS) in patients with common variable immunodeficiency (CVID) in Sri Lanka

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Common variable immunodeficiency (CVID) is the most widespread symptomatic primary immunodeficiency in adults. A number of distinct genetic defects including mutations in the inducible co-stimulator (ICOS) and B-cell activating factor receptor (BAFF-R) have been identified in some patients with CVID. The BAFF-R is important for the development and survival of B lymphocytes and the ICOS is important for the development of regulatory T cells. The aim of this study was to identify the possible BAFF-R and ICOS mutations in patients who have been clinically diagnosed with CVID. Blood samples of 12 patients and 5 controls were obtained from a previous study with the approval of the Ethics review committee of the Medical Research Institute. DNA was extracted and the exon regions were amplified by PCR with specific primers. The PCR products were sequenced and the analysis was performed by Geneious, Bioedit softwares. Two mutations were identified from the sequence analysis of the. BAFF-R gene. The heterozygous rs77874543 missense mutation in the gene encoding for BAFF-R results in the amino acid changes Proline to Arginine in codon 21 which is located at the extracellular domain of BAFF-R, was recently reported to impair BAFF-R's multimerization contributing to CVID. The other rs7290134 3' UTR mutation is silent. Two polymorphisms were identified from the sequence analysis of ICOS gene. The rs10172036 mutation is a variant in the introns and has no effect on the change of amino acid sequence. The other mutation is rs10183087 in the 3' UTR, has not been previously reported in CVID patients, but may affect mRNA stability and degradation as well as nuclear export. According to the literature, the identified mutation rs10183087 has been shown to affect chronic lymphocyte leukemia and graft transplantation. We conclude that further investigations with increased number of patients and controls are necessary for confirmation of these findings.

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Abstract No: PP8

DNA barcoding and latex protease activity of Sri Lankan mountain papaya

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Sri Lankan mountain papaya is known as highland papaya or mountain papaya due to its morphological similarity to papaya and ecological preference to high altitudes. It is a sparsely branched herbaceous tree which is habituated in Nuwara-Eliya and Badulla districts. Species of mountain papaya have been researched in other countries mainly due to the cold resistance and Papaya Ringspot Virus (PRSV) resistant traits while latex is reported to have a stronger proteolytic effect. Different synonyms are widely used to describe this plant and the taxonomy of Sri Lankan mountain papaya has not been explained clearly. Therefore DNA barcoding was carried out to confirm the taxonomy of the plant. Three chloroplast DNA barcoding regions *matK, rbcL* and *trnH-psbA* were amplified by PCR and sequenced. The *matK* and *trnH-psbA* were found to be the most suitable regions to distinguish Sri Lankan mountain papaya as *Vasconcellea cundinamarcensis* of genus *Vasconcellea* in family Caricaceae. The barcode sequences resulted from *matK* and *psbA-trnH* regions were made available in GenBank database at NCBI and the phylogenetic trees were constructed using Mega 6 software. Phylogenetic tree clearly clustered Sri Lankan mountain papaya with other *Vasconcellea cundinamarcensis* sequences at GenBank. Sri Lankan mountain papaya was morphologically characterized and the voucher specimens were deposited at the national herbarium, Peradeniya, Sri Lanka. Latex protein analysis of Sri Lankan mountain papaya was conducted using standard proteolytic activity assay. Proteolytic activity assay revealed a higher proteolytic activity of *Vasconcellea cundinamarcensis* than homolog *Carica papaya*.

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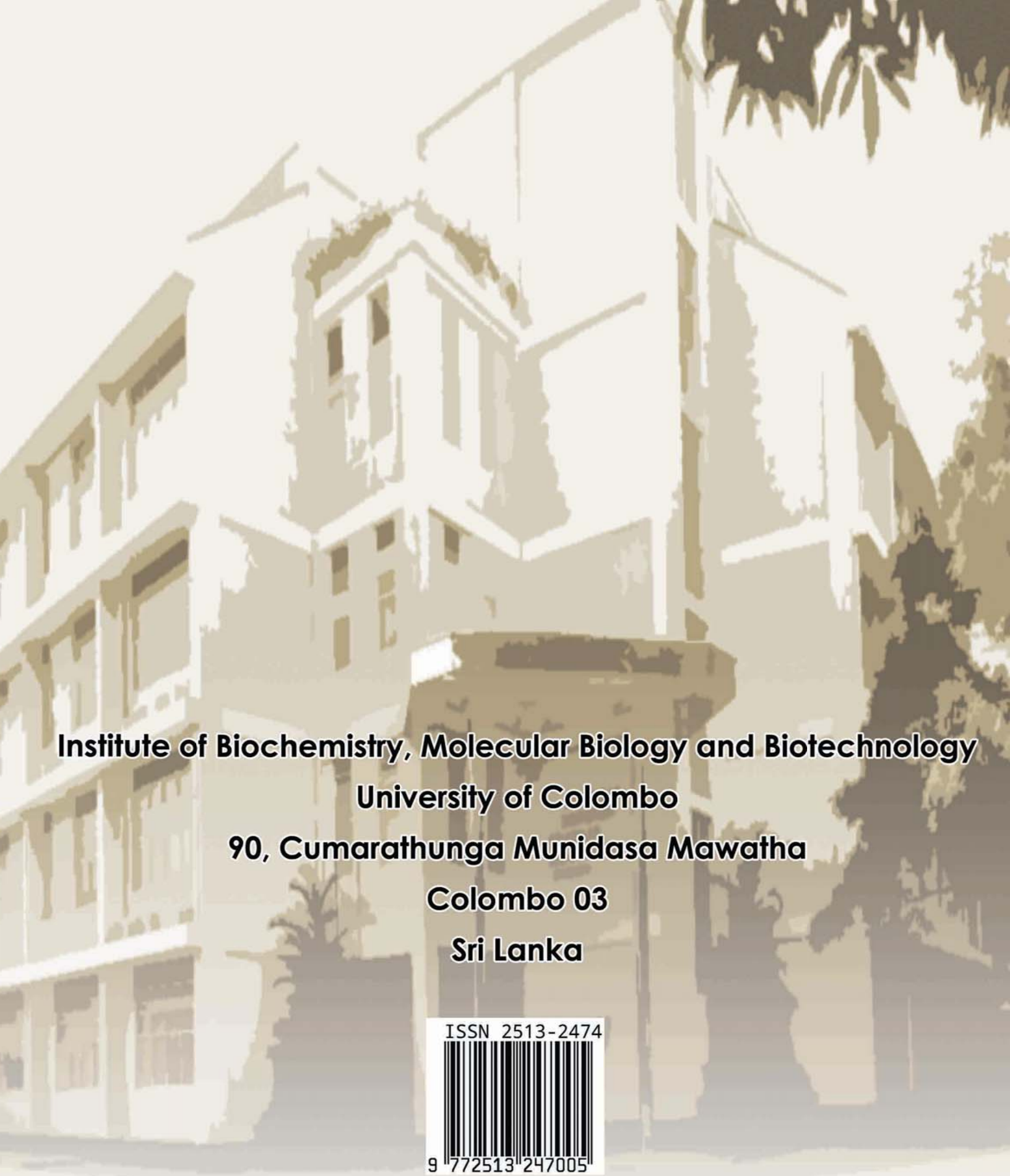

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