

*University of Colombo*

**IBMBB**

Institute of Biochemistry, Molecular Biology and Biotechnology



## **Seventh Annual Scientific Sessions**

**21<sup>st</sup> May 2015**

### **Programme and Abstracts**

Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

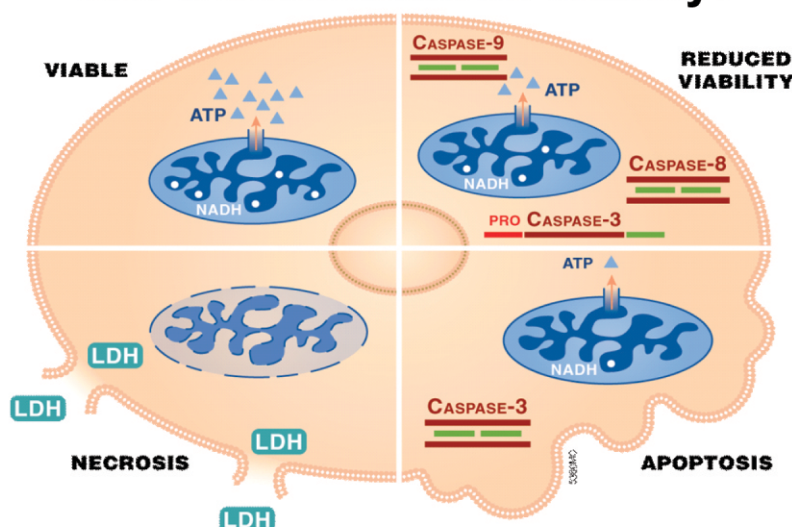


# What happened to my cells?

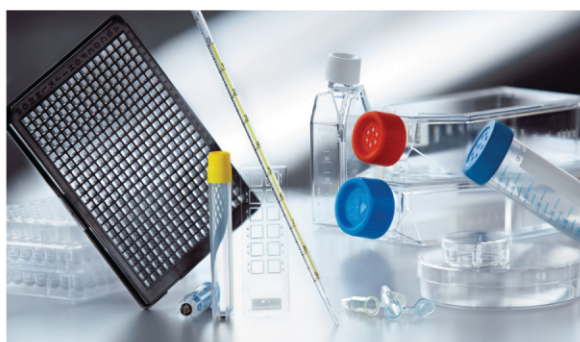
Did they **live**?  
Did they **die**?  
What **pathway**?

Was it **Necrosis**?  
Was it **Apoptosis**?  
**GSH/GSSG** ratio  
change?

Was it **Mitochondrial toxicity**?



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# **Institute of Biochemistry, Molecular Biology and Biotechnology**

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## **Our Vision**

**" To be an  
International Centre of Excellence  
in  
Molecular Life Sciences "**



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## **PROGRAMME**

|                 |                                                                                                                                                                                                    |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 8.30 h          | Registration                                                                                                                                                                                       |
| 9.00 h          | Inauguration                                                                                                                                                                                       |
| 9.05 h          | Lighting of the traditional oil lamp                                                                                                                                                               |
| 9.10 h          | Welcome address by Director IBMBB                                                                                                                                                                  |
| 9.20 h          | Address by Chairman-University Grants Commission                                                                                                                                                   |
| 9.30 h          | Address by Vice Chancellor- University of Colombo                                                                                                                                                  |
| 9.40 - 10.30 h  | Professor Stanley Wijesundera Memorial lecture by<br>Senior Professor Kamani Tennekoon - “From the mitochondrial<br>Eve to contemporary Sri Lankan women: A glimpse into the<br>genetic landscape” |
| 10.30 - 10.35 h | Vote of Thanks                                                                                                                                                                                     |
| 10.35 - 11.00 h | Tea                                                                                                                                                                                                |
| 11.00 - 12.30 h | Free papers – Oral presentations Session I                                                                                                                                                         |
| 12.30 - 13.30 h | Lunch                                                                                                                                                                                              |
| 13.30 - 15.00 h | Free papers – Oral presentations Session II                                                                                                                                                        |
| 15.00 - 15.45 h | Viewing Poster presentations & Tea                                                                                                                                                                 |
| 15.45 - 17.00 h | Free papers – Oral presentations Session III                                                                                                                                                       |



## **Message from the Director/ IBMBB**

It is my pleasure to send this message to mark the occasion of the 7<sup>th</sup> 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 11<sup>th</sup> 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 with financial support from Swedish Government in 2004. This was the culmination of a SAREC supported research programme led by its Founder Director, Professor Eric H Karunanayake. 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. Both MSc courses have a >80% completion rate and to date 76 students have graduated. IBMBB has begun a third MSc programme in Bioinformatics and the first batch of students commenced their studies in 2012.

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 six Annual Scientific Sessions starting from 2006 and two International conferences in 2009 and 2014 to mark the 5<sup>th</sup> and 10<sup>th</sup> anniversaries.

High point of the 7<sup>th</sup> Annual Scientific Sessions will be the Professor Stanley Wijesundera Memorial lecture on “From the mitochondrial Eve to contemporary Sri Lankan women:

A glimpse into the genetic landscape” to be delivered by Professor Kamani Tennekoon, Senior Professor of Molecular Life Sciences. Prof. Tennekoon is the principle researcher of several programmes being conducted at IBMBB. This year there will be seventeen oral presentations and fourteen poster presentations including a nine 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 Institutes, Health Sector and Agricultural Sector is clearly evident. The presentations will be in different research areas including research on Medicinal plants, Molecular Biology, Biotechnology, Nanotechnology, Immunological aspects & Infectious diseases.

I take this opportunity to thank Prof. Mohan de Silva, Chairman, University Grants Commission, (UGC), Prof PSM Gunaratne, Secretary, UGC, Dr Priyantha Premakumara and Prof. Lalitha Mendis, Acting- Vice-Chancellor, University of Colombo for their support and gracing the inauguration, Prof Kamani Tennekoon 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 of abstracts received; Prof. Eric Karunanayake, Prof. Ira Thabrew, all the Academic & Academic support staff members of IBMBB, and external reviewers. I would like to thank the chairpersons of the three sessions of oral presentations, Prof Eric Karunanayake, Prof Ira Thabrew, Prof Sunil Premawansa, Dr Rajiva De Silva, Dr Jagath weerasena and Dr Prasanna Galhena. I specially thank Mr Kanchana Senanayake, Mr Sashika Niranjana and Mr Nipuna Ratnayake for designing the abstract book and coordinating printing, Ms Anoma Jayasoma for coordinating sponsorships, Ms Thanuja Athapaththu for arrangements on refreshments, Ms Sumali Champika and Ms Nadeesha Jayawardena for secretarial assistance and Ms Jayamini Harasgama for editorial help & for support in compiling the abstract book.



Dr Shiroma Handunnetti  
Director / IBMBB  
Chairperson-Organizing Committee

## Oral Communications

### Session I - Medicinal Plants

**Chairpersons:** *Prof Eric karunanayake & Prof Ira Thabrew*

**OP1: 11.00 – 11.15 h** - Enhanced *in vitro* anti-cancer efficacy of doxorubicin loaded chitosan-alginate nanoparticles on breast cancer (MCF-7) cells

Katuwavila KANP, Perera ADLC, Samarakoon SR, Karunaratne V. Karunaratne DN

**OP2: 11.15 – 11.30 h** - Isolation of quercetin from the bark of *Mangifera zeylanica* and its cytotoxicity against MCF-7 breast cancer cells.

Ediriweera PMK, Tennekoon KH, Samarakoon SR, Thabrew I, de Silva ED, Adhikari A, Choudhary MI

**OP3: 11.30 – 11.45 h** - Isolation of lupeol an anti-carcinogenic compound from the leaves of *Connarus championii*

Jayarathna DDP, Tennekoon KH, Samarakoon SR, Thabrew I, Karunanayake EH, de Silva ED, Adhikari A

**OP4: 11.45 – 12.00 h** - A comparison of the cytotoxic effects of *Vernonia zeylanica* (L.) Less fractions on three breast cancer phenotypes

Mendis AS, Thabrew MI, Samarakoon S. Tennekoon KH

**OP5: 2.00 – 12.15 h** - *In vitro* anti-inflammatory activity of methanol/dichloromethane extract of *Vernonia zeylanica* (L.) Less

Rukshala BAD, Handunnetti SM, de Silva ED

**OP6: 12.15 – 12.30 h** - Evaluation of anti inflammatory activity of *Alpinia calcarata* leaf extracts: An *in vitro* study

Chandhrakanthan M, Kathirgamanathar S, Handunnetti SM, Premakumara GAS

## **Session II – Immunology & Infectious Diseases**

**Chairpersons: Prof Sunil Premawansa & Dr Rajiva De Silva**

**OP7: 13.30 – 13.45 h** - *In vitro* effects of selected anti-wolbachial antibiotics against filarial parasite *Setaria digitata*

Senathilake NHKS, Samarakoon SR, Karunanayake EH, Tennekoon KH

**OP8: 13.45 – 14.00 h** - Do antibodies play a role in ROS production by HL 60-derived neutrophils against *Leptospira*?

Fernando TRGN, Niloofa R, Karunanayake L, de Silva HJ, Rajapakse S, Premawansa S, Handunnetti SM

**OP9: 14.00 – 14.15 h** - TNF- $\alpha$  and IL-2 levels in dengue patients - changes with clinical profiles

Senaratne UTN, Noordeen F, Carr J

**OP10: 14.15 – 14.30 h** - Dengue NS1 antigen vs. dengue virus nucleic acid: Is the former a better marker in the latter stages of severe dengue?

Sirisena PDNN, Noordeen F, Fernando LK

**OP11: 14.30 – 14.45 h** - Role of antibody response in disease severity of Leptospirosis

Niloofa R, Fernando TRGN, Karunanayake L, de Silva HJ, Premawansa S, Rajapakse S, Handunnetti SM

**OP12: 14.45 – 15.00 h** - Passive transfer and decay of maternally transferred IgG antibodies against measles, mumps and rubella in infants in Colombo district, Sri Lanka

Nadhikala M, Pathirana PPSL, Peiris S, Handunnetti SM, Galagoda GCS



### **Session III – Molecular Biology & Biotechnology**

**Chairpersons:** *Dr Jagath Weerasena & Dr Prasanna Galhena*

**OP13: 15.45 – 16.00 h** - Studies towards the development of transgenic rice expressing ferritin

Rodrigo WWP, Achala HHK, Bandara KGWW, Rathnayaka NRMKND, Withana WTGSL, Chandrasekharan NV, Senaratne SG

**OP14: 16.00 – 16.15 h** - Molecular screening of some selected water bodies in Sri Lanka for Microcystin producing genotypes

Piyathilaka MAPC, Pathmalal MM, Tennekoon KH, De Silva BGDNK

**OP15: 16.15 – 16.30 h** - Haematological changes and DNA damage elicited by hexavalent Cr toxicity in the Asian common toad, *Duttaphrynus melanostictus*

Fernando VAK, Dangalle CD, Perera IC, Weerasena OVDSJ, Premawansa S, Handunnetti S, Wijesinghe MR

**OP16: 16.30 – 16.45 h** - Isolation, identification and molecular characterization of *Bacillus thuringiensis* serovar *graciosensis* from Sri Lankan soil

Baragamaarachchi RY, Weerasena OVDSJ, Samarasekera RR

**OP 17: 16.45-17.00 h** - DNA barcoding of a novel amphibian species from the Central highlands of Sri Lanka

Wickramasinghe N, Tennekoon KH, Samarakoon SR, Bandara IN, Vidanapathirana DR, Wickramasinghe LJM

## Poster presentations

### Immunology & Infectious Diseases

**PP1** - Immunochemical characterization of venom of *Apis dorsata* Fabricius (*Bambara*) in Sri Lanka (Hymenoptera; Apidae)

Gunasekara DLPE, De Silva NR, Handunnetti SM, Dias RKS, Witharana EWRA, Dasanayake WMDK, Premawansa S

**PP2** - Genetics of common variable immunodeficiency (CVID): Role of transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI)

Sathkumara SMHD, De Silva NR, Handunnetti SM, De Silva AD

**PP3** - Adjuvant effect of combined hot water extract of *Coriandrum sativum* L. and *Coscinium fenestratum* on rat antibody response against *Leptospira* antigens

Siriwardene AR, Handunnetti SM, Karunanayake L, Premawansa S

**PP4** - Coagulation disorders in patients with leptospirosis

Threeswaran R, Gooneratne L, Handunnetti SM, Rajapakse S

### Molecular Biology & Biotechnology

**PP5** - Screening of a cohort of young onset Breast cancer patients for c.2175\_2176insA mutation in the *BRCA2* gene.

Jayasekara BMLP, De Silva WS, Tennekoon KH, De Silva K, Dassanayake A

**PP6** - Occurrence of *Bacillus subtilis* in fermented traditional rice

Kotelawala JT, Samarasekara JRR, Weeresaena OVDSJ, Divisekera WMDD

**PP7** - SSR Markers revealed genetic diversity of some finger millet accessions collected from six districts of Sri Lanka

Wakista PW, Dasanayaka PN, Illeperuma RJ, Perera SACN

## Medicinal Plants

**PP8** - Possible potentiation of anti-proliferative effect of tamoxifen in MCF-7 breast cancer cells by a bioactive fraction of mangrove plant *Scyphiphora hydrophyllacea*: A pilot study  
De Silva OKC, Samarakoon SR, Tennekoon KH, Fernando EHN

**PP9** - Cytotoxic effects of hopenone-I isolated from leaves of the mangrove plant *Scyphiphora hydrophyllacea* on breast cancer (MCF-7) cells.  
Fernando EHN, Samarakoon SR, de Silva ED, Tennekoon KH, Thabrew MI, Ediriweera PMK, Wijayabandara MDLO, Adhikari A, Choudhary I

**PP10** - Isolation of two kaempferol glycosides from the leaves of *Olax zeylanica*  
Wijayabandara MDLO, Wijayabandara MDJ, de Silva ED, Choudhary MI, Adhikari A, Fernando EHN, Samarakoon SR

## Bioinformatics

**PP11** - Molecular docking study of selected natural compounds for cancer stem cells targeted anti-cancer activity  
Karthika M, Samarakoon SR, Tennekoon KH, Siriwardana A

**PP12** - Analysis of the occurrence of cis-regulatory elements in the first introns of human genes  
Perera JARP, Tirimanne TLS, Weerasinghe RA, Wijesinghe CR

**PP13** - *In silico* analysis of missense mutations of human growth hormone gene (*GHI*):  
The impact on protein structure, dynamics and stability  
Samarasinghe SR, Tennekoon KH, Valverde JR, De Silva S

**PP14** - *In silico* characterization of a motif in SWIB domain of MDM2 like protein of cattle filarial parasite *Setaria digitata*  
Vahinipriya M, Karunanayake EH, Tennekoon KH, Samarakoon SR

## **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. This year Professor Stanley Wijesundera Memorial Lecture will be delivered by Professor Kamani Tennekoon, Senior Professor of Molecular Life Sciences, IBMBB, University of Colombo, Director, IBMBB from 2007 to 2012 and former Professor of Physiology, Faculty of Medicine, University of Colombo.

## ***Professor Stanley Wijesundera Memorial Lecture***

### **From the mitochondrial Eve to contemporary Sri Lankan women: A glimpse into the genetic landscape**

***Kamani H. Tennekoon***

Senior Professor of Molecular Life Sciences, IBMBB, University of Colombo

Currently available genetic evidence confirms the emergence of the anatomically modern human in Africa. The oldest mitochondrial genome dating back to more than 100,000 years ago is considered to have evolved in Africa. “Daughters” of this “mitochondrial Eve” have transmitted the mitochondrial (mt) genome to the present day men and women inhabiting the planet earth. Along the way the mtDNA accumulated mutations which signify branches or haplogroups of the mitochondrial tree. These have become continental specific. All non African mitochondrial haplogroups arose from sibling haplogroups M and N which have their root in L3 or “out of Africa” macrohaplogroup. How these populated the Indian subcontinent is debatable. A southern coastal route from East Africa as well as an inland movement is suggested with evidence for and against both. Recorded history of Sri Lanka gives some clues to human migration to and from Sri Lanka, while archeological evidence confirms the presence of prehistoric settlements. Analysis of the mt DNA of contemporary men and women can tell us where our maternal ancestors came from and the genetic relatedness between ethnic, cultural and religious groups. Our studies clearly demonstrate that Vedda individuals have a maternal genetic lineage different from other Sri Lankan ethnic groups supporting the notion that they descend from the oldest inhabitants of the country. Interestingly, Sri Lankan Tamils are closer to Sinhalese and furthest from Indian Tamils genetically, despite differences in the culture, religion and language between Sinhalese and Tamils and similarities between Sri Lankan and Indian Tamils. Malays and Muslims cluster closer to each other. South Asian (Indian) mt DNA haplogroups are the commonest among Sri Lankans supporting historical evidence of migrations from India, or more or less simultaneous colonization of both Sri Lanka and India through the beach comber route. A considerable presence of West Eurasian haplogroups particularly among the Sinhalese, Sri Lankan Tamils and Vedda, the ethnic groups with a history of longer settlements in the country suggest early migration of women carrying these haplogroups into the country. Predominance of Indian mt DNA haplogroups among Malays and Muslims support the notion of their largely male migration and marriage to local women.



**Abstract No: OP1**

**Enhanced *in vitro* anti-cancer efficacy of doxorubicin loaded chitosan-alginate nanoparticles on breast cancer (MCF-7) cells**

Katuwavila KANP<sup>1,3</sup>, Perera ADLC<sup>1,2</sup>, Samarakoon SR<sup>4</sup>, Karunaratne V<sup>2,3</sup> Karunaratne DN<sup>1,2</sup>

<sup>1</sup>Post Graduate Institute of Science, University of Peradeniya, Peradeniya, <sup>2</sup>Dept. of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya, <sup>3</sup>Sri Lanka Institute of Nanotechnology, Mahenwatta, Pitipana, Homagama, <sup>4</sup>Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

The anthracycline antibiotic doxorubicin (DOX) is a drug used in the treatment of different cancers including breast cancer. The clinical usage of doxorubicin has limitations since it is associated with increased risk of cardiotoxicity with high administrated dosage. We hypothesise that toxic side effects could be minimized by maximized efficiency achieved by encapsulating DOX in nanoparticles. The purpose of the present study was to assess and compare *in vitro* anti-cancer effects of DOX loaded chitosan-alginate nanoparticles on MCF-7 cells with free DOX. Cytotoxicity of DOX loaded chitosan-alginate (DCANP) against MCF-7 cells was compared with free DOX using sulphorhodamine (B) (SRB) assay for 24, 48 and 72 h post incubations. The induction of apoptosis in MCF-7 cells by DOX loaded nanoparticle and free DOX was evaluated by detecting the morphological changes by light and fluorescence microscopy. *In vitro* cellular uptake of DCANP was compared with that of the free DOX using fluorescence microscopy for 30 min, 2 and 24 h. Results of the cytotoxicity assay demonstrated that the DCANP enhanced cytotoxicity time and dose-dependently (IC<sub>50</sub> values were 7.66, 2.19 and 0.04 µg/mL for 24, 48 and 72 h respectively) on MCF-7 cells whereas free DOX had an optimized, dose-dependent, higher cytotoxicity even at 24 h post incubation (IC<sub>50</sub> value was 1.08 µg/mL). Morphological changes of MCF-7 cells observed under light microscopy and detection apoptosis under fluorescence microscopy supported the results of *in vitro* cytotoxicity. Overall results indicated that DCANP enhanced the efficacy of the DOX while releasing the DOX slowly from the nanoparticles with reported pro-apoptotic effects of DOX.

*This work was supported by HETC QIG window 3 Grant to the University of Peradeniya.*

**Abstract No: OP2**

**Isolation of quercetin from the bark of *Mangifera zeylanica* and its cytotoxicity against MCF-7 breast cancer cells**

Ediriweera PMK<sup>1</sup>, Tennekoon KH<sup>1</sup>, Samarakoon SR<sup>1</sup>, Thabrew I<sup>1</sup>, de Silva ED<sup>2</sup>, Adhikari A<sup>3</sup>, M. Iqbal Choudhary<sup>1</sup>

<sup>1</sup>Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,  
<sup>2</sup>Department of Chemistry, Faculty of Science, University of Colombo, <sup>3</sup>H. E. J 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. Quercetin is considered as one of the most abundant flavonoids present in plants with various biological activities including anti-cancer properties. The present study was aimed to isolate active cytotoxic compounds from the chloroform extract of the bark of *M. zeylanica* by bioactivity guided isolation. Preliminary screening of extracts of *M. zeylanica* separated by sequential extraction confirmed that the chloroform extract contained cytotoxic effects to breast 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. The structure of active compounds were elucidated by means of combination of <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, 2D-NMR (COSY, HSQC, HMBC) and ESI-MS techniques and one of these was confirmed to be quercetin. Isolated quercetin exhibited cytotoxicity against MCF-7 breast cancer cells with the IC<sub>50</sub> value of 19.1 µg/mL (24 h).

*This work was supported by the National Research Council of Sri Lanka (Grant No 11-018).*

**Abstract No: OP3**

**Isolation of lupeol an anti-carcinogenic compound from the leaves of *Connarus championii***

Jayarathna DDP<sup>1</sup>, Tennekoon KH<sup>1</sup>, Samarakoon SR<sup>1</sup>, Thabrew I<sup>1</sup>, Karunanayake EH<sup>1</sup>, de Silva ED<sup>2</sup>, Adhikari A<sup>3</sup>

<sup>1</sup>Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, <sup>2</sup>Department of Chemistry, Faculty of Science, University of Colombo, <sup>3</sup>H. E. J Research Institute of Chemistry, University of Karachi, Pakistan

Potential anti-carcinogenic properties of a Sri Lankan endemic plant *Connarus championii* (Family: Connaraceae) was evaluated using phenotypically different breast cancer cell lines [MCF-7 (ER positive) and triple negative MDAMB-231 (ER, PR, and HER2/neu negative)] and normal breast epithelial cell line (MCF-10A). Leaves were air-dried and subjected to sequential extraction with hexane, chloroform, ethyl acetate and methanol. Cells were exposed to plant extracts (25 - 400 µg/mL) for 24 h and cytotoxicity was assessed using Sulforhodamine B assay. Morphological changes were observed in ethidium bromide/acridine orange stained cells. Hexane extract was cytotoxic to both MCF-7 (IC<sub>50</sub>:72.05 µg/mL) and MDAMB-231 (IC<sub>50</sub>:38.90 µg/mL) cancer cells, but less toxic to normal breast epithelial cells (IC<sub>50</sub>:151.25 µg/mL). Chloroform extract was cytotoxic to MDAMB-231 (IC<sub>50</sub>:29.83 µg/mL) cancer cells and to normal breast epithelial cells (IC<sub>50</sub>:20.37 µg/mL). Ethidium bromide/acridine orange staining confirmed morphological evidence of apoptosis following treatment with the hexane and chloroform extract in the cell lines to which these extracts were cytotoxic. Hexane crude extract which was cytotoxic to both cancer cell lines but not to normal cells was subjected to normal phase column chromatography and active fractions were obtained by bioactivity guided fractionation. Reverse phase column chromatography was used to isolate possible cytotoxic compounds. MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, <sup>1</sup>H-<sup>1</sup>H COSY, NOESY, HMBC, HSQC data of the isolated compound matched unambiguously with lupeol, a reported cytotoxic compound. This is the first report of lupeol being isolated from *Connarus championii*.

*This work was supported by Ministry of Higher Education Grant for Drug Leads from Medicinal Plants. DDPJ received an overseas training fellowship from the National Science Foundation.*

**Abstract No: OP4**

**A comparison of the cytotoxic effects of *Vernonia zeylanica* (L.) Less fractions on three breast cancer phenotypes**

Mendis AS, Thabrew MI, Samarakoon S, Tennekoon KH

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

*Vernonia zeylanica* (L.) Less is an endemic plant commonly known as Pupula (Sin.), (Family Compositae). *V. amygdalina*, another species which belongs to the same family, has been shown to inhibit proliferation of MCF-7 cells. The present study was carried out to investigate cytotoxic effects of fractions of *V. zeylanica* obtained by sequential extraction of powdered aerial parts of this plant using Hexane, Chloroform (CHCl<sub>3</sub>), Ethyl acetate (EtAc) and Methanol in (a) three breast cancer cell lines MCF-7 (*ER positive / PR positive, Her2 negative*), SKBr-3 (*ER negative, PR negative, Her2 positive*), and MDA-MB-231 (*Triple negative*), and (b) MCF-10A breast cell line. Sulphorhodamine (SRB) and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays were carried out to identify cytotoxic fractions. CHCl<sub>3</sub> and EtAc fractions exerted dose-dependent cytotoxicity to all three breast cancer cell lines. The CHCl<sub>3</sub> fraction exerted cytotoxicity with an IC<sub>50</sub> of 66.29 for SKBr-3, 66.33 for MDA-MB-231 and 83.69 µg/ml for MCF-7 respectively, and MCF-10A: 493.4 µg/ml, when evaluated by SRB assay. MTT assay IC<sub>50</sub> values were 74.49, 85.49 and 99.13 µg/ml for SKBr-3, MDA-MB-231 and MCF-7 respectively. The IC<sub>50</sub> values of SRB assay for EtAc fraction were 32.37, 99.94, 99.65, 508.4 µg/ml for SKBr-3, MDA-MB-231, MCF-7 and MCF-10A respectively. MTT assay IC<sub>50</sub> values were 38.68, 106.15, 133.5 µg/ml for SKBr-3, MDA-MB-231 and MCF-7 respectively. Both assays demonstrate selective cytotoxicity to specific breast cancer phenotypes. Overall findings of the present study indicate that the CHCl<sub>3</sub> and EtAc fractions exerted greater cytotoxicity in the Her-2 positive phenotype than in the Her-2 negative phenotype.

*This work was supported by Ministry of Higher Education Grant for Drug Leads from Medicinal Plants.*

**Abstract No: OP5**

***In vitro* anti-inflammatory activity of methanol/dichloromethane extract of *Vernonia zeylanica* (L.) Less**

Rukshala BAD<sup>1</sup>, Handunnetti SM<sup>1</sup>, de Silva ED<sup>2</sup>

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<sup>2</sup>Department of Chemistry, University of Colombo

*Vernonia zeylanica* (L.) Less (Family: Asteraceae) is a medicinal plant used for boils, bone fractures, eczema and asthma in folklore. Anti-nociceptive and anti-bacterial activities have been reported previously. Present study was aimed to investigate the anti-inflammatory activity by assessing methanol/dichloromethane extract (MDE) of *V. zeylanica* for *in vitro* inhibition of nitric oxide (NO) and superoxide production. NO inhibitory activity of 7.8-250 µg/ml MDE was tested using lipopolysaccharide (LPS)-stimulated (1 µg/mL) mouse macrophages RAW264.7 and carrageenan-induced (5 mg/Kg) rat peritoneal cells (RPC). NMMA (1mM) was used as the positive control and showed 78-70.1% inhibition of NO production. Griess method was used to quantify the NO production. IC<sub>50</sub> of MDE for NO inhibitory activity was 105 µg/ml with RAW cells, 80 µg/ml for RPC and these activities were dose-dependent ( $r=0.998$  and  $r=0.915$ ;  $p<0.05$ ). Quantitative NBT assay was carried out to determine superoxide inhibitory activity of RAW cells stimulated *in vitro* with PMA. DPI (10 µM) was used as the positive control and showed 61% superoxide inhibitory activity. MDE (at 500 µg/ml) showed 55.3% of superoxide inhibitory activity in RAW cells. These results demonstrated potent *in vitro* anti-inflammatory activity of MDE of *V. zeylanica* reflected by high NO and superoxide inhibitory activities. Thus, further purification is pursued to isolate bioactive compounds from *V. zeylanica*.

*This work was supported by Ministry of Higher Education Grant for Drug Leads from Medicinal Plants.*

**Abstract No: OP6**

**Evaluation of anti-inflammatory activity of *Alpinia calcarata* leaf extracts: An *in vitro* study**

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*Alpinia calcarata* Roscoe is widely used in Sri Lankan traditional medicine as a remedy for a variety of inflammatory diseases. Recent reports claimed possible advantageous effect of herbal formulations containing *A. calcarata* as a major ingredient in treating rheumatoid arthritis conditions. This study aims to evaluate the nitric oxide (NO) production inhibition and membrane stabilization potential of *A. calcarata* leaf extracts using *in vitro* assays. *A. calcarata* leaves were collected from Western province of Sri Lanka and extracted sequentially by hot and cold methods using organic solvents (Petroleum ether, Dichloromethane, Ethyl acetate, Methanol and Water). RAW 264.7 murine macrophages were stimulated by bacterial lipopolysaccharides and treated with 6 different doses (250, 125, 62.5, 31.25, 15.62 and 7.81 µg/mL) of leaf extracts which were selected as a non-toxic range based on MTT assay, to evaluate the potency on inhibition of NO production. Membrane stabilization properties were investigated by heat-induced hemolysis assay of RBC's from freshly collected healthy human blood. Extracts of *A. calcarata* leaves showed a significant reduction in NO production and heat-induced hemolysis compared to controls ( $P<0.05$ ). Leaf extracted by hot extraction methods showed prominent inhibition than the cold extracts. The highest inhibition of NO production and heat-induced hemolysis was observed for petroleum ether extracts. At 250 µg/mL concentration of the extract, a marked inhibition ( $78.65\pm2.24\%$ ) of NO production was observed. At 100 µg/mL, the hot petroleum ether extract inhibited hemolysis ( $72.33\pm5.56\%$ ) in close range to the standard drug, aspirin ( $82.10\pm4.67\%$ ) at 100 µg/mL. In conclusion, *A. calcarata* leaf extracts showed a significant inhibition of NO production and hemolysis *in vitro* and have a potential therapeutic effect on rheumatoid arthritis by (a) inhibiting production of inflammatory mediators and (b) stabilizing immune cell membranes.

*This work was supported by National Research Council of Sri Lanka (NRC Grant: 12-081).*



**Abstract No: OP7**

***In vitro* effects of selected anti-wolbachial antibiotics against filarial parasite *Setaria digitata***

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Tetracycline and doxycycline are effective in eliminating essential endosymbiotic *Wolbachia* species from *Wolbachia* dependent filariae causing death of adult female filarial worms. Tetracycline treatment of the *Wolbachia*-free nematodes was reported to have no effect on the worm viability. *Setaria digitata* is an experimental model for screening anti-filarial drugs. However to date, reports on *Wolbachia* dependence of *S. digitata* have been contradictory. In the present study effects of tetracycline and doxycycline on worm motility and cytotoxicity of *S. digitata* were investigated *in vitro*. Both drugs were tested in triplicates at doses of 10 to 1000 µg/mL by worm motility assay. Motility was observed relative to solvent control for 12, 24 and 48 h. Immobilized worms were incubated in fresh media for 24 h to confirm death. After the incubation period, adult female worms were dissected to release intra uterine developmental stages and microfilariae. Cell viability of both adult and microfilariae was assessed by MTT cell viability assay and apoptotic features were visualized by ethidium bromide / acridine orange staining. Results indicated that doxycycline is more effective in inhibiting adult worm motility (IC<sub>50</sub> of 100 µg/mL for 24 hours) while tetracycline was less effective (IC<sub>50</sub> of 500 µg/mL). Result of the MTT cell viability assay and ethidium bromide / acridine orange staining confirmed the cell death in treated adult worms and the results were consistent with the worm motility assay results. However, both drugs at the doses used did not induce cell death in completely developed microfilariae.

*This work was supported by Ministry of Higher Education Grant for Drug Leads from Medicinal Plants.*

## Abstract No: OP8

### **Do antibodies play a role in ROS production by HL 60-derived neutrophils against *Leptospira*?**

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NADPH oxidase in professional phagocytes catalyses the respiratory burst and produces superoxide free radicals which generates microbicidal reactive oxygen species (ROS) such as O<sub>2</sub><sup>-</sup>, OH<sup>-</sup> and H<sub>2</sub>O<sub>2</sub>. Knowledge about ROS production by human neutrophils in response to *Leptospira* is limited. Therefore, the aim of this study was to evaluate the role of antibodies in ROS production by neutrophils interacted with *Leptospira*. HL-60 cells were treated with dimethyl sulfoxide to differentiate in to mature neutrophils. *Leptospira biflexa* Serovar Patoc 1 were grown in Ellinghausen-McCullough-Johnson-Harris media containing 10% rabbit sera. Intracellular ROS production of neutrophils interacted with *L. biflexa* in the presence of sera from leptospirosis patients, was assessed using quantitative nitroblue tetrazolium (NBT) assay. IgM and IgG levels of laboratory confirmed, clinically categorized severe and mild leptospirosis patients (SL and ML; n=30/group) were compared with the ROS levels produced by HL-60 derived neutrophils in the presence of respective sera. Although IgM and IgG levels were significantly higher in SL patients (p<0.01), neutrophils incubated with these sera showed a significantly low production of ROS compared to than that by sera of ML patients (p=0.012). Same assays performed with pathogenic *L. interrogans* Serovar Pyrogenes showed that there was a significantly higher ROS production in the presence of both SL and ML sera (p<0.001). These results suggest that ROS production by neutrophils may not be dependent on opsonization of both saprophytic and pathogenic *Leptospira* by IgM or IgG. Further, high ROS production in neutrophils interacted with *L. interrogans* over *L. biflexa* could be due to the ability of pathogenic strains to induce a higher innate immune response.

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**Abstract No: OP9**

**TNF- $\alpha$  and IL-2 levels in dengue patients: Changes with clinical profiles**

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Dengue infection is endemic to tropical and subtropical regions of the world including Sri Lanka. The objective of the present study was to assess TNF- $\alpha$  and IL-2 responses in patients with Dengue infection with varying clinical severity. A commercially available cytokine ELISA was used to detect TNF- $\alpha$  and IL-2 in 67 confirmed dengue patients (by RT-PCR and serology), 15 healthy controls and 6 blood culture positive typhoid patients. The 67 dengue patients consisted of 24 dengue fever (DF) patients and 43 dengue haemorrhagic fever (DHF) patients. Clinical data was collected with a help of a CDC style questionnaire. A statistical test with P-value <0.05 was considered significant in mean comparison, or in correlation analysis. No significant elevation in TNF- $\alpha$  levels was noted between DF and DHF patients (mean of DF=51.8 pg/ml, mean of DHF=96.2 pg/ml; p=0.5) but a significant variation was observed in the IL-2 levels between DF and DHF (mean of DF=59.7 pg/ml, mean of DHF=166.9 pg/ml; p=0.02). No significant rise in TNF- $\alpha$  and IL-2 levels was noted with an increase in HCT (>45%), thrombocytopenia, leucopenia and PCR positivity. The mean TNF- $\alpha$  and IL-2 values of DF and DHF were higher than that of healthy controls. A positive correlation was observed between the IL-2 and SGOT/SGPT levels (r=0.31 and 0.28 respectively). A significant difference was noted in the IL-2 concentrations between DHF and typhoid patients (p=0.005). Moreover, a significant correlation was noted with the disease severity within different grades of DHF (r=0.380; p=0.002). TNF- $\alpha$  levels did not show a significant rise with disease severity whereas IL-2 showed a positive correlation with different grades of DHF (r=0.38; p=0.02). Higher levels of IL-2 at the early stages of symptomatic dengue infection might be used as a prognostic marker for the progression to DHF.

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**Abstract No: OP10**

**Dengue NS1 antigen vs. dengue virus nucleic acid: Is the former a better marker in the latter stages of severe dengue?**

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Laboratory diagnosis of dengue infections is done by detecting different viral and immune markers in patients with a clinical diagnosis of dengue. A definitive diagnosis is useful in the timely management of dengue infected symptomatic patients. Choice of an assay to diagnose dengue infections depends on the longevity of viraemia. Blood samples were collected from 91 patients from Gampaha and Negombo hospitals with a clinical diagnosis of dengue fever (DF) or dengue haemorrhagic fever (DHF), categorized based on progressive accumulation of fluid in pleural or peritoneal cavities on serial ultra sound scanning. Samples were taken from patients who had fever for a minimum of 3 days to a maximum of 9 days. The sera were tested for dengue NS1 antigen using an ELISA. Reverse transcription polymerase chain reaction (RT-PCR) was performed using primers targeting the DENV capsid gene. Patients with DF had 67% PCR positivity on capsid gene whereas 50% NS1 positivity on fever day 3. Patients with DHF had 100% PCR positivity whereas 83% NS1 positivity on fever day 4. However, in the DHF group from fever day 5, PCR sensitivity declined, but NS1 positivity remained high even in the latter stages. There was no significant difference in NS1 positivity and leucopenia in both DF and DHF patients whereas platelet counts of the DF and DHF patients showed significant drop in platelet counts ( $p < 0.0001$ ). NS1 is useful to detect DENV infection even in the latter stages of DHF. Further, investigations are in progress to evaluate NS1 as a marker to predict the severity of DF and DHF.

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**Abstract No: OP11**

**Role of antibody response in disease severity of Leptospirosis**

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Clinical manifestations of leptospirosis range from mild flu like illness to complicated presentations involving multiple organs. The humoral immune response in severe and mild leptospirosis (SL & ML) patients was compared to assess the role of antibody response in disease severity. Sera from 100 each of laboratory confirmed, SL and ML patients and healthy controls (n=100) were tested for Immunoglobulin M and G (IgM & IgG), using an in-house enzyme linked immune-sorbent assay (ELISA) which detects antibodies against a mixture of antigens from five serovars (*L. interrogans pyrogenes*, *sejroe*, *australis*, *canicola* and *icterohaemorrhagiae*) which are the most prevalent in Sri Lanka. Total anti-leptospiral IgM antibody prevalence in both ML and SL patients was 96%. In contrast, anti-leptospiral IgG prevalence was 35% and 76% respectively. The mean anti-leptospiral IgM level of ML and SL patients were significantly higher than that of control group (Mean OD<sub>450</sub> value at 1:100 dilution were 0.778, 0.770 and 0.163 respectively; ANOVA,  $P<0.001$ ). However, the mean anti-leptospiral IgG levels of SL patients were significantly higher than that of ML patients and control group (0.610, 0.358 and 0.216 respectively; ANOVA,  $P<0.001$ ). Further, IgG levels of ML sera were significantly higher compared to the control group (ANOVA,  $P<0.001$ ). The same pattern was observed when IgM and IgG titers were analysed. Age (Mean 40.8 years; ANOVA,  $P>0.05$ ), gender (chi-square test,  $P>0.05$ ) and days of illness (Mean 7.1 days; t test,  $P>0.05$ ) were comparable among the groups. These results suggest that high anti-leptospiral IgG may be associated with the severity of disease in leptospirosis.

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**Abstract No: OP12**

**Passive transfer and decay of maternally transferred IgG antibodies against measles, mumps and rubella in infants in Colombo district, Sri Lanka**

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The degree of transfer of maternally derived IgG antibodies against measles, mumps, and rubella (MMR) infections in Sri Lankan infants was studied by ELISA using 200 serum samples obtained from 100 pairs of mothers and their newborns and, the decay of these antibodies was studied using 280 samples obtained from infants of 6 to 12 months of age, in order to determine the optimal time for vaccination. The ratio of IgG concentrations of mother-infant pairs was 0.71:1 indicating that antibody levels of the infants exceeded the antibody levels of mothers reflecting an efficient transfer of maternal antibodies to newborns. The percentage of infants who were sero-positive against measles, mumps and rubella were 96.5%, 88.5% and 98.5% respectively. In the 8 months old infants recruited, 91.6% were antibody negative for measles and mumps virus, and 98% of infants were sero-negative against rubella. There were significant negative correlations between the age of infants and antibody levels against MMR ( $r = -0.413$ ,  $-0.307$  and  $-0.417$  respectively;  $p < 0.0001$ ). The mean measles antibody titers were 195 and 62 mIU/ml respectively, at 7 and 9 months of life. The mean mumps antibody titers were 56 and 26.4 U/ml at 7 and 10 months respectively. The mean rubella antibody titers were 7 and 1.4 IU/ml at 7 and 11 months respectively. One hundred percent sero-negativity was observed for MMR by completion of 9, 10 and 11 months respectively. Concentrations of passively acquired antibodies decreased rapidly within 6 to 9 months of life and a higher proportion of infants were sero-negative by end of 8 months. Thus, advancing MMR vaccination from current practice of completion of 12 months of age could reduce the number of susceptible subjects in infancy, help in preventing infections and thereby eliminate these infections.

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**Abstract No: OP13**

**Studies towards the development of transgenic rice expressing ferritin**

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Iron deficiency, a widespread nutritional disorder, is a serious cause of anemia worldwide. Fortification of rice, a dominant food staple with iron is a long term solution to the above problem, where genetic engineering plays an effective role. This study aims at increasing overall iron content in rice seeds by introducing Soybean (*Glycine max* (L). Merr) Ferritin gene into rice (*Oryza sativa* ssp *indica*) seed under the control of the rice seed specific globulin (*Glb*) promoter. Total RNA from soybean cotyledons were reverse transcribed and amplified by PCR using Ferritin gene specific primers. Amplified product (783 bp) was purified and cloned into pCR<sup>®</sup>2.1-TOPO vector (pCR<sup>®</sup>2.1-TOPO-*ferr*). Genomic DNA isolated from rice variety Bg 94-1 was PCR amplified by *Glb* promoter specific primers. Amplified product (1024 bp; globulin promoter) was cloned into pGEM<sup>®</sup>-T Easy vector. Recombinants were screened and sequenced for confirmation. To construct pCAMBIA1391Z-*Glb* recombinant plasmid, the promoter region was cleaved with *Pst*I and *Bam*HI restriction enzymes and cloned into pCAMBIA1391z vector. Recombinants were screened by restriction digestion and colony PCR, and were confirmed by sequencing. The ferritin gene was cleaved and cloned in pCAMBIA1391Z-*Glb* recombinant vector to construct pCAMBIA1391Z-*Glb-ferr* fusion cassette. Recombinants were identified, isolated and were transformed into competent *Agrobacterium* cells. Transformed colonies were confirmed by colony PCR. A rice plant regeneration protocol was optimized to incorporate the transgene into rice plant. Rice seed derived calli were transformed with *Agrobacterium* carrying the transgene. Currently work is underway to regenerate genetically modified rice plants.

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**Abstract No: OP14**

**Molecular screening of some selected water bodies in Sri Lanka for Microcystin producing genotypes**

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Dry zone reservoirs in Sri Lanka are presently characterized by blooms of cyanobacteria with temporal appearance of cyanotoxins. Selected dry zone fresh water bodies in Sri Lanka were screened for the presence of cyanobacterial genotypes which could produce Microcystins (MC) using Polymerase Chain Reaction (PCR). Ten dry zone water bodies were selected namely, Kurunegala lake, Giradurukotte reservoir, Rathkinda lake, Ulhitiya lake, Kondawatuwana reservoir, Jayanthi lake, Hot water spring 1 (HWS 1), Hot water spring 2 (HWS 2), Kantale lake and Unnichchi reservoir. According to morphological identification, *Microcystis* spp. was the dominant cyanobacteria in all reservoirs except in HWS 1 and HWS 2, where the *Oscillatoria* spp. was dominant. Total MC content in raw water ranged from 0.00 ppb to 3.98 ppb respect to the Enzyme Linked Immuno Sorbent Assay (ELISA). Rathkinda lake, HWS 1 and HWS 2 reflected absence of MCs while the highest value was recorded in Jayanthiwewa (3.98 ppb). PCR was carried out to amplify *mcy E* gene (230-250 bp), a gene involved in MC biosynthesis. Presence of MC producing genotypes was proven by positive PCR bands for the reservoirs with MCs. Even though MCs were not detected in HWS 1 and HWS 2 by ELISA, PCR reflected the presence of MC producing genotype. Absence of MCs in Rathkinda lake was proven by the negative results for PCR. Hence it can be concluded that, molecular screening could reflect the actual ability of MC production even when the toxin is not detected by ELISA at a given moment.

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**Abstract No: OP15**

**Haematological changes and DNA damage elicited by hexavalent Cr toxicity in the Asian common toad, *Duttaphrynus melanostictus***

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Amphibians have the potential to function as a sentinel group to indicate the status of heavy metal pollution in freshwater ecosystems. Asian Common toad (*Duttaphrynus melanostictus*) was exposed to a series of environmentally relevant Cr<sup>6+</sup> concentrations (0.002 to 0.2 mg/L), to monitor haematological alterations and DNA damage using acute (96 hrs) and chronic (28 days) exposure trials. Results of chronic exposure reveal significant reductions in MCV and neutrophil percentages ( $p < 0.05$ ) and although not significant, reductions in total RBC counts and PCV, MCHC and MCH indices. Similarly, RBC morphometry, i.e. cell length and nuclear width, were significantly altered at 0.02 mg/L. Acute exposure however, only resulted in significant changes in RBC morphometry, which was evident even at the lowest level of 0.002 mg/L. DNA damage in RBCs assessed using the comet assay and measured in terms of the tail length and tail DNA percentage were significantly high in those exposed to 0.015 mg/L and above, compared to a negative control ( $p < 0.05$ ). Significant dose – dependent trends were evident for alterations in some haematological parameters and as well as for DNA damage. This study has shown that exposure to field levels of Cr<sup>6+</sup> has the potential to induce alterations in haematological parameters and also to elicit DNA damage in the studied species. These findings whilst demonstrating the potential of the amphibian model to monitor heavy metal pollution, suggests that field levels of heavy metals are detrimental to this species and possibly therefore to other aquatic fauna. This leads us to suggest that it is critical to consider toxicity to aquatic fauna when establishing safe limits for the discharge of aquatic pollutants.

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**Abstract No: OP16**

**Isolation, identification and molecular characterization of *Bacillus thuringiensis* serovar *graciosensis* from Sri Lankan soil**

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*Bacillus thuringiensis* (*Bt*) serovar *graciosensis* is the 66<sup>th</sup> serovar of *Bt*, originally isolated in Azores from soil. We report the characterization of Sri Lankan *Bt* isolates, isolated from soil of North central (*Bt* AB6, *Bt* AB7), North western (*Bt* AB10) and Western provinces (*Bt* AB16) of Sri Lanka as *Bt* serovar *graciosensis*. Isolation was done by acetate heat treatment method and identified by culturing in chromogenic *Bacillus* agar. Typical *Bt* colonies appeared in blue and blue/green colour. Coomassie blue staining was carried out to detect the presence of parasporal crystals; a unique property of *Bt*, which distinguish it from other *Bacilli*. *Cry* gene analysis done by PCR under optimized conditions showed the presence of *cry1* gene in all four isolates, *cry9* and *cry10* genes in *Bt* AB6, *Bt* AB7 and *Bt* AB10 isolates. *GyrB* gene sequencing was performed for serovar identification. Amplification was carried out with specific primers by nested PCR and the purified amplicon was sequenced using Big dye terminator cycle sequencing kit according to the manufacturer's instructions. Sequences were searched over the GenBank database at NCBI using BLAST. The results showed that *gyrB* gene sequence of *Bt* AB6, *Bt* AB7, *Bt* AB10 and *Bt* AB 16 have highest similarity to *Bt* serovar *graciosensis*. Presence of lepidopteran toxic *cry1* and *cry9* genes, as well as dipteran toxic *cry10* gene may indicate specific toxicity of *Bt* serovar *graciosensis* against lepidopteran and dipteran pests. Insecticidal bioassays should be carried out in order to confirm the activity for these insects, as there is no available literature regarding the inherent toxicity of *Bt* serovar *graciosensis*.

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**Abstract No: OP17**

**DNA barcoding of *Pseudophilautus dilmah* the newly described amphibian species from the Central Highlands of Sri Lanka**

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In this study, DNA barcoding technique was used in the discovery of *Pseudophilautus dilmah* the newly described species of amphibian in the genus *Pseudophilautus* from the Central Highlands. As a result of field exploration on amphibians in Sri Lanka a potentially novel species of *Pseudophilautus* from Loolcondera Estate, Nuwara-Eliya district was sampled. The phylogenetic relationships were determined using the mitochondrial 16S ribosomal RNA region and showed that this species was genetically distinct from other members of *Pseudophilautus*. Whole cellular DNA was extracted from ethanol preserved muscle tissue sample. Amplifications of the 16S ribosomal RNA region were carried out using forward and reverse primers designed for that region. Purified PCR products were sequenced and, molecular phylogenetic analysis was performed using MEGA 6. Maximum likelihood tree was rooted with *Raorchestes charius*. Maximum likelihood analysis of the 16S rRNA fragment placed the newly described species in a monophyletic group along with *P. schmarda* and *P. hankeni*. Pairwise genetic distance between *P. dilmah* and *P. schmarda* was 1.9% whereas it was 1.6% with *P. hankeni*. There was a 10% divergence in the 16S rRNA gene between *P. dilmah* and *P. bambaradeniyai*, the morphologically most closely related species. Although 3% genetic distance is a good indication for species level divergence it is reported that interspecies genetic distances could vary from 1% to 10% and allopatric species are known with less than 3% differences. Overall results showed that the newly described species, *Pseudophilautus dilmah* is genetically distinct from other members of *Pseudophilautus*.

*This work was funded by the Dilmah Conservation, Sri Lanka.*

**Abstract No: PP1**

**Immunochemical characterization of venom of *Apis dorsata* Fabricius (*Bambara*) in Sri Lanka (Hymenoptera; Apidae)**

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The increasing frequency of reported anaphylaxis in patients due to *Apis dorsata* stings and lack of immunochemical knowledge of the venom of *A. dorsata* in Sri Lanka essentially demand the immunochemical characterization of its venom. This preliminary study aims to characterize the venom profile of *A. dorsata* workers and determine cross reactivity of the serum IgE in patients who were stung by *A. dorsata*, with *A. mellifera* (Western honey bee) venom. A novel method, electrical stimulation of worker bees, was carried out for the first time in Sri Lanka enabling the extraction of venom without killing the bees and with fewer contaminations. Eighteen out of 21 protein bands resulted from SDS-PAGE were analogous to molecular weights of proteins identified for *A. mellifera* venom. Western blot with 16 patients' sera (9 anaphylaxis, 7 mild anaphylaxis) resulted in 3 bands, a doublet of 30 kDa and 31 kDa, possibly phospholipase A<sub>2</sub> identified with all patients' sera and a 3<sup>rd</sup> band of 40 kDa, possibly hyaluronidase identified in 4 patients who had severe reactions. Similar Western blot pattern was seen with patients' sera using *A. mellifera* venom. Intensity of resulting bands increased with the severity of allergic status in the patient. Phadia immunocap resulted in geometric mean value of 2.465\*/4.884 KU<sub>A</sub>/L of venom specific IgE level in 16 patients. High levels of venom specific IgE in the patients' sera were shown in more severe patients. This data suggest the presence of IgE against *A. dorsata* that cross react with *A. mellifera* venom components which indicate the possibility of using *A. mellifera* venom for immunotherapy for protection against *A. dorsata* stings and use of Phadia immunocap as an *in vitro* diagnostic method for *A. dorsata* bee venom allergy in Sri Lanka.

*This work was supported by IBMBB, University of Colombo and research grant No 46/2014 from the Medical Research Institute and constitutes part of the MSc study of DLPEG.*



**Abstract No: PP2****Genetics of common variable immunodeficiency (CVID): Role of transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI)**

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Common variable immunodeficiency (CVID) is the commonest clinically manifested primary immunodeficiency, which represents a heterogeneous group of hypogammaglobulinemias of largely unknown molecular defects. The hallmark of the disease is the elevated susceptibility to recurrent infections of respiratory and gastrointestinal tract, mainly due to encapsulated bacteria. Although the primary cause of CVID is still not known, defects in TACI encoded by TNFRSF13B gene are more frequently found to be disease associated in about 10% of CVID patients, but may require additional immunological or genetic defects for complete expression of the phenotype, as unaffected heterozygotes have also been described. In this preliminary study, 9 clinically diagnosed CVID patients and 5 healthy controls were screened for TACI associated genomic mutations. The coding sequence of TACI gene was amplified using five exon specific primer sets. PCR products were purified and were subsequently subjected to downstream automated Sanger sequencing. Sequencing electrophorograms were compared against the TACI reference sequence. Pathogenicity of each variant was assessed using bioinformatics tools such as PolyPhen-2. Total of 7 sequence variants were identified, among which one was a novel synonymous SNP (NG\_007281.1:g.36585G>C) and another was a mis-sense mutation which has led to an amino acid substitution at position 251 of TACI protein (P251L). Interestingly, P251L was found in both patients and control subjects at a similar frequency which may suggest possible disease predisposing role rather than disease causing. Further studies with large number of participants are needed as such studies will disclose the additional genetic and environmental factors that act in concert with simple heterozygous alterations in TNFRSF13B to give rise to antibody deficiencies in humans.

*This work was supported by IBMBB and Medical Research Institute (Grant number 40/2013) and constitutes part of the MSc studies of SMHDS.*

**Abstract No: PP3**

**Adjuvant effect of combined hot water extract of *Coriandrum sativum* L. and *Coscinium fenestratum* on rat antibody response against *Leptospira* antigens**

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Leptospirosis is an infectious disease caused by pathogenic bacteria of the genus *Leptospira*. Detectable antibody titers appear only after 7-10 days of infection therefore, more sensitive laboratory tests are required for confirmation of Leptospirosis. Previous studies have shown that combined hot water extract (CHWE) of *Coriandrum sativum* L. and *Coscinium fenestratum* G. have significant immunostimulatory effect in rats and have a boosting effect on antigen specific IgG antibody levels in rats. The aim of this study was to determine the adjuvant effect of CHWE in eliciting an antibody response against *Leptospira* antigens. Three groups of rats, negative control, positive control and test group were immunized against *L. biflexa* antigens on day 1 and 15. Negative control and test group were orally treated with water and CHWE of *C. sativum* L. and *C. fenestratum* G., where as Freund's adjuvant was used as a positive control. Rat anti-leptospiral antibodies were detected using ELISA and microscopic agglutination test (MAT). On day 7, mean MAT titer of the CHWE group was high (but not significant) compared to both control groups ( $p=0.213$  &  $p=0.183$ ) and by day 21, MAT titers of both control groups increased significantly ( $p=0.038$  &  $p=0.042$ ). In contrast, rat anti-leptospiral IgM antibodies in CHWE group, detected by ELISA, increased from day 7 to 21 and were significantly higher compared to control ( $p=0.024$ ,  $p=0.015$ ,  $p=0.010$ ; ANOVA). High MAT titers in the CHWE group by day 7, reflects an early antibody response against *Leptospira* surface antigens which are functionally important. Further, the significantly higher IgM response in the CHWE group as detected by ELISA supports the adjuvant effect of the CHWE.

*This work was supported by IBMBB, University of Colombo and constitutes part of the MSc studies of ARS.*

## **Abstract No: PP4**

### **Coagulation disorders in patients with leptospirosis**

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The aim of this study was to evaluate conventional coagulation and rotational thromboelastometry (ROTEM) parameters in leptospirosis patients. This study included 20 serologically confirmed leptospirosis patients (14 severe, 6 mild), 6 patients with dengue and 5 with bacterial sepsis, admitted to National Hospital Sri Lanka, and 6 healthy individuals. Prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen, D-dimer, platelet count and ROTEM were performed in all. ANOVA post-hoc comparison using Bonferroni was applied to all parameters. PT and aPTT were prolonged in >80% of leptospirosis patients. TT was not significantly prolonged in leptospirosis compared with other three groups. Fibrinogen was significantly elevated in severe leptospirosis ( $p=0.001$ ) and sepsis ( $p=0.001$ ) compared with healthy controls and dengue. 30% of leptospirosis had thrombocytopenia (17% in mild and 36% in severe), while 100% with dengue were thrombocytopenic and none with sepsis. D-dimer was elevated in 80% of leptospirosis and (100%) sepsis patients but not in dengue. No significant difference in inTEM clotting time (CT), and exTEM CT was found in leptospirosis when compared to other 3 groups. inTEM Clot Formation Time (CFT) and exTEM CFT in dengue were significantly high compared to severe ( $p=0.001$ ) and mild ( $p=0.005$ ) leptospirosis. inTEM Maximum Clot Firmness (MCF) ( $p=0.001$ ), exTEM MCF ( $p=0.001$ ) and fibTEM MCF ( $p=0.003$ ) were significantly lower in dengue than in leptospirosis. Only 1 patient with leptospirosis had bleeding manifestations. These results indicate that abnormalities in conventional coagulation parameters occur in leptospirosis. However, unlike in dengue, ROTEM parameters in leptospirosis were not significantly altered compared to sepsis and healthy controls. Patterns of conventional and ROTEM assays indicate that different mechanisms may be involved in the disturbance of coagulation abnormalities in leptospirosis and dengue.

*This work was supported by IBMBB, University of Colombo and NRC Grant No 12-077 and constitutes a part of the MSc study of RT*

**Abstract No: PP5**

**Screening of a cohort of young onset Breast cancer patients for c.2175\_2176insA mutation in the *BRCA2* gene.**

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Breast cancer is an extremely complex disease and is the most frequently diagnosed cancer in women worldwide. About half of these cases occur in economically developing countries. In Sri Lanka, it accounts for 26% of all cancers in women. Genetic predisposition for familial breast cancer accounts for approximately 5–10% of all breast cancers. A combination of *BRCA1* and *BRCA2* gene mutations appears to be responsible for about 15-20% of these cases. It has been shown that ethnically different populations exhibit different germ-line mutation spectra for these genes. Even though, the contribution of mutations in these two genes to Sri Lankan familial breast families has been explored before, the contribution of these mutations to young onset breast cancer patients remains relatively unexplored. Previous studies have shown a pathogenic novel mutation c.2175\_2176insA to be present among 4 Sinhalese women out of 95 patients with breast cancer. Three of these patients were in the age group of 35-47. This research explored the prevalence of this particular mutation in 16 Sri Lankan young onset familial breast cancer patients by screening the initial segment of *BRCA2*, exon 11. DNA extracted from patients' blood was subjected to PCR amplification and to direct sequencing. Through result analysis, it was revealed that none of the samples carried this mutation. However, the presence of a previously reported silent polymorphism c.2229T>C was detected in 3 patients of the same cohort. Analysis of further samples is necessary to refute or confirm that c.2175\_2176insA is a common pathogenic mutation among Sri Lankan young familial breast cancer patients.

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**Abstract No: PP6**

**Occurrence of *Bacillus subtilis* in fermented traditional rice**

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Traditionally, cooked rice left to ferment overnight and, along with added coconut milk and other condiments, is consumed for breakfast, which is called *Diyabath*. *Diyabath* has healing properties for common stomach ailments such as gastritis and diarrhoea, which are caused by pathogenic microorganisms. The objective of this study was to isolate and identify probiotic character displaying micro-organisms associated with the fermentation process of traditional rice varieties. Rice samples used in this study were obtained from the Rice Research Institute of Sri Lanka, Bathalagoda. The traditional rice varieties used were “Madathawaalu”, “Pachchaperumaal”, “Suwandel”, “Suduheenati” and “Kuruluthuda”. Serial dilutions were made from overnight fermented rice and cultured on a selective medium of deMan, Rogosa and Sharpe agar. Then further selections were made based on their morphological and probiotic potential tests such as Gram’s and Endospore staining, motility and catalase tests, acid, bile, pH and salt tolerance. Four bacterial colonies identified from above screening were grown separately and DNA was extracted. PCR amplification was carried out using 16S ribosomal RNA gene specific primers 27F(5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R(5’-TACGGYTACCTTGTTACGACTT-3’). The PCR products were then subjected to a nested PCR using primers 27F and WLAB2R (5’- TCGAATTAAACCACATGCTCCA-3’). The PCR fragments were bi-directionally sequenced and sequences were searched over the GenBank database using BLAST. The four bacterial isolates were identified as *Bacillus subtilis*.

*This work was supported by the IBMBB and constitutes part of the MSc studies of JTK.*

**Abstract No: PP7**

**SSR Markers revealed genetic diversity of some finger millet accessions collected from six districts of Sri Lanka**

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Finger millet (*Eleusine coracana* L.) is an important food crop widely cultivated in the arid and semi-arid regions of the world including Sri Lanka. The grains contain higher protein, fat and minerals than rice, corn and sorghum. Germplasm identification and characterization is an important link between conservation and utilization of plant genetic resources. The present study was conducted to reveal genetic diversity of twenty three finger millet germplasm accessions collected from six districts of Sri Lanka using 10 (SSR) primer markers. Out of these primers, eight primers (UGEP 05, UGEP 10, UGEP 15, UGEP 68, UGEP 78, UGEP 102, UGEP 106 and UGEP 110) were polymorphic and other two were monomorphic (UGEP 03 and UGEP 31). Calculation of gene diversity and Polymorphic Information Content (PIC), estimation of genetic distances and cluster analysis were performed using Power Marker version 3.25. Shared alleles were calculated and cluster analysis was performed with Neighbor joining method and TreeView software (version 1.6) to construct the tree diagram. Gene diversity varied from 0.0832 (with primer UGEP 78) to 0.7297 (with primer UGEP 15). PIC also varied from 0.0797 to 0.6862 with same primers (UGEP 78 and UGEP 15) respectively. Frequency based shared allele distance matrix exhibited distances between finger millet germplasm accessions ranging from 0.100 to 0.700. The highest distances were observed between accession Kurakkan 5 and Galbora Kurakkan (collected from Kurunagala Hambantota districts); Kurakkan 8 (collected from Kurunegala district) and Galbora Kurakkan; Kurakkan 8 and Thummas Kurakkan (collected from Anuradhapura district). Dendrogram constructed on the basis of SSR polymorphism revealed the pattern of genetic relatedness among 23 finger millet germplasm accessions grouping them into 2 main clusters (except 3 accessions). The identified genotypes could be used in breeding and conservation programmes of finger millet crop.

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**Abstract No: PP8**

**Possible potentiation of anti-proliferative effect of tamoxifen in MCF-7 breast cancer cells by a bioactive fraction of mangrove plant *Scyphiphora hydrophyllacea*: A pilot study**

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Tamoxifen is a nonsteroidal antiestrogen used as a chemopreventive agent and as an adjuvant therapy for estrogen receptor positive breast cancer. Combination treatment of tamoxifen is a novel approach that is used to enhance the effectiveness and to reduce its side effects. In the present study, a subfraction isolated from the anti-carcinogenic hexane leaf extract of a mangrove plant *Scyphiphora hydrophyllacea* was used in combination with tamoxifen. Cytotoxic effects of tamoxifen and subtraction alone or in combination on MCF-7 cells were evaluated by Sulphorhodamine B assay 24 h post-incubation. Median drug effect analysis method was used to analyze the combined effect. Apoptosis was assessed by morphological observations using light and fluorescence microscopy. Results showed a significant cytotoxic effect of tamoxifen (IC<sub>50</sub>: 3.92 µg/mL) and subfraction (IC<sub>50</sub>: 54.23 µg/mL) on MCF-7 cells. Cell survival was significantly decreased (p<0.05) when tamoxifen (1, 2, 4 and 8 µg/mL) was co-administered with subfraction concentration of 30 µg/mL compared to cells treated with subfraction or tamoxifen alone and the untreated control. Combinational drug analysis showed a synergistic effect when 30 µg/mL of subfraction was combined with either 4 µg/mL or 8 µg/mL of tamoxifen. Apoptosis related morphological changes were prominent in cells treated with the combination of tamoxifen (8 µg/mL) and subfraction (30 µg/mL) than in cells treated with the two drugs individually. Thus it can be concluded that the subfraction of *S. hydrophyllacea* is capable of potentiating the antiproliferative effect of tamoxifen on MCF-7 cells in a dose dependent manner.

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**Abstract No: PP9**

**Cytotoxic effects of hopenone-I isolated from leaves of the mangrove plant *Scyphiphora hydrophyllacea* on breast cancer (MCF-7) cells.**

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*Scyphiphora hydrophyllacea* is a mangrove plant of the Rubiaceae family which is found in Australia, Bangladesh, China, India, Indonesia, Malaysia, Singapore, Sri Lanka and Thailand. The leaves of this plant are used to treat stomach ache and has been reported to possess potent anti-hepatocarcinogenic properties. In order to isolate the constituents responsible for the anti-cancer activity, plant material was sequentially extracted into hexane, chloroform, ethyl acetate and methanol with sonication at room temperature. Cytotoxic activity evaluation of the crude extracts by the MTT assay against breast cancer (MCF-7) cells showed that hexane extract possess the most potent activity. Activity-guided fractionation of the hexane extract by repeated column chromatography on normal phase silica gel yielded a pure crystalline compound which showed moderate cytotoxic activity against MCF-7 cells in the MTT assay (IC<sub>50</sub> at 24 h post incubation = 17.2 µg/mL, IC<sub>50</sub> at 48 h post incubation = 14.09 µg/mL). The isolated compound was identified as hopenone-I based on MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, <sup>1</sup>H-<sup>1</sup>H COSY, NOESY, HMBC, HSQC spectroscopic data which matched unambiguously with that of hopenone-I. This is the first report of hopenone-I from *S. hydrophyllacea*.

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**Abstract No: PP10**

**Isolation of two kaempferol glycosides from the leaves of *Olax zeylanica***

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*Olax zeylanica* is a leafy vegetable which has been used over many generations by Sri Lankans for its medicinal properties including diuretic, anti-cancer and wound healing activities. In order to isolate the pharmacologically active constituents, the plant material was sequentially extracted into hexane, dichloromethane, ethyl acetate and methanol with sonication at room temperature. Screening the above extracts for anti-oxidant activity by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay showed that the methanol extract possess the most potent anti-oxidant activity with an IC<sub>50</sub> of 28.8 µg mL<sup>-1</sup>. Subsequent activity-guided fractionation of the methanolic extract by successive column chromatography applications on normal phase silica gel led to the isolation of two flavonol glycosides, kaempferol-3,7-O-α-L-dirhamnopyranoside and kaempferol-7-O-α-L-rhamnopyranoside for the first time from this plant. Structures of these compounds were established on the basis of MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, HMBC, NOESY experiments. The above compounds have been reported to possess a wide variety of biological activities including immunostimulation, anti-microbial, anti-cancer, anti-inflammatory and anti-diabetic activities. Hence the presence of these compounds in the edible parts of *O. zeylanica* provides successful justification for the ethnomedical use of this plant in the improvement of human health.

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**Abstract No: PP11**

**Molecular docking study of selected natural compounds for cancer stem cells targeted anti-cancer activity**

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Cancer stem cells (CSCs) play significant roles in tumor initiation, relapse, angiogenesis, metastasis and therapy. Collectively Wnt, notch and hedgehog are major pathways that have been linked to the resistance of CSCs to conventional therapy. Most conventional therapies are directed at the rapidly growing tumor mass but not at the slow dividing CSCs. Eliminating CSCs has been suggested as a promising approach to cure cancer. *In-silico* molecular docking simulations were carried out for the binding of 35 selected natural compounds with receptor proteins which are involved in the main signaling pathways of CSCs, such as  $\beta$ -catenin chain A and Smo receptor from the Wnt and hedgehog pathways respectively, using Hex 8.0.0, DOCK6 and AutoDock Vina software. Additionally docking interaction residue analysis, score functions such as Drug score, X score, Per Contact Score (PCS) and Average affinity PCS and drug-likeness study were carried out for the selected compounds. Overall, 11 compounds were identified with good binding energy, interaction, binding affinity and better drug likeness for  $\beta$ -catenin chain A involved in Wnt inhibition. There was no considerable overall binding ability for Smo inhibition. Energy values for Wnt inhibition obtained using AutoDock Vina were as follows, Gedunin (-7.3 kcal/mol), Kaempferol (-6.1 kcal/mol), Methylripariochromene A (-5.3 kcal/mol), Myrigalone G (-5.1 kcal/mol), Catechin (-6.5 kcal/mol), Myricetin (-6.5 kcal/mol), Discretine (-5.6 kcal/mol), Laurolitsine (-5.9 kcal/mol), Myricitrin (-6.3 kcal/mol), Nordicentrine (-6.0 kcal/mol) and Phloretin (-5.4 kcal/mol). These novel Wnt inhibitors need further attention to assess their potential application in CSC targeted therapy.

*This work was supported by the IBMBB and constitutes part of the MSc studies of KM.*

## **Abstract No: PP12**

### **Analysis of the occurrence of cis-regulatory elements in the first introns of human genes**

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Gene expression is primarily regulated through the interaction between transcription factors and their cognate cis-regulatory elements found in the promoter region of a gene. However, research studies have revealed, that the first introns of certain genes also substantially play a role during gene expression. A recent study has shown that the majority of the first introns of human housekeeping and tissue specific genes harbor cis-regulatory elements such as CAAT, GC and TATA boxes. The immediate objectives of this study are to investigate the frequency of occurrence of CAAT, GC and TATA boxes in the first introns of human housekeeping and tissue specific genes and to evaluate the sequence similarity of first introns of closely related species to humans. Appropriate first intron sequences were extracted from the Ensembl genome browser. The knuthmorrispratt algorithm written in perl, was executed to obtain motif counts. Results show, that the motif counts do not directly proportionate to first intron length. Moreover, the average CAAT box count is higher in first introns of tissue specific genes. Further, more than 95% of first intron sequence conservation was observed among closely related species to humans which is a novel finding. Conclusively, this study speculates that the occurrences of motif counts are unlikely to occur by chance in the first introns. It can also be suggested that there may be a correlation between the higher CAAT box counts and tissue specificity. Further, first intron sequences between human and closely related species are very highly conserved during evolution.

*This work was supported by the IBMBB and constitutes part of the MSc studies of JARPP.*

## Abstract No: PP13

### ***In silico* analysis of missense mutations of human growth hormone gene (*GHI*): The impact on protein structure, dynamics and stability**

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Mutations of *GHI* gene results in deficient or biologically inactive human growth hormone (hGH) leading to short stature. Although several missense mutations are reported, basis for their biological effects are not clear. It is necessary to investigate the effects on the protein structure to understand biological consequences of missense mutations. In this study, we performed a comprehensive analysis on 8 missense mutations in *GHI* gene (D112G, C53F, C53S, R77C, R183H, R16C, R16L, C189Y) in order to examine their effects on receptor binding and signal transduction *in silico*. X-ray structures in PDB (1HGU, 1HWG) were used as templates to create homology models of hGH mutants in bound/unbound conformations. We developed a “*Successive relaxations*” approach to create successive models using exclusively experimental information. Models were built using TRITON which includes MODELLER and refined by molecular dynamics refinement protocol. UCSF Chimera was used for pre-processing, visualization and analysis of structures. Validity of our approach was assessed by modeling an experimentally known GH mutant (G120R). G120R and 3 inert mutants (L9P, M14S, V110I) were used as reference controls. Comparing each mutant with the wild type (and putative wt controls), we observed that almost all the mutations decreased the effectiveness of signal transduction although the changes were not large enough to completely inhibit it. Being the first in depth modeling study of hGH mutations our methodology provides plausible explanations in good agreement with experiment in all cases where data was available, thus supporting the applicability of this approach to understanding mutations in *GHI* gene.

*This work was supported by IBMBB, University of Colombo and constitutes the part of the MSc studies of SRS.*



**Abstract No: PP14**

***In silico* characterization of a motif in SWIB domain of MDM2 like protein of cattle filarial parasite *Setaria digitata***

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Human lymphatic filariasis (HLF) is prevalent in tropical and subtropical countries and about 90% of the disease is caused by the parasite *Wuchereria bancrofti*. None of the drugs currently available for HLF can clear adult nematodes that lodge in the lymphatic system. Therefore, potential research strategies are needed to design effective drugs to eradicate HLF globally. In the present study, *Setaria digitata*, an animal filarial nematode closely resembling *W. bancrofti* was studied as a model organism due to difficulties in procuring *W. bancrofti* parasite material. cDNA library of *S. digitata* has been previously constructed. A 422 bp long cDNA clone was sequenced. The ORF of 142 bp encodes a polypeptide of 46 amino acids and shows an amino acid identity of 57% with the senescence associated protein of *Medicago truncatula*. Molecular weight, molecular formula, extinction coefficients, estimated half life, instability index, aliphatic index, sub cellular localization of the protein were predicted using ExPASy proteomics tools. 3D structure of the protein was obtained by homology modeling (SWISS model) using SWIB domain of MDM2 protein of *Xenopus laevis* as the template, which shows 20.59% sequence identity. SWIB domains are ATP-dependent chromatin-remodelling proteins that facilitate transcription activation and are conserved from yeast to humans. They consist of 4 helices capped by two small 3-stranded beta-sheets in duplicate. MDM2 plays an important role in keeping TP53 protein under control. Since TP53 binds to the SWIB domain of MDM2, this study brings new dimensions to the further structural and functional analysis of MDM2 protein of *S. digitata* and its evaluation as a potential new drug target against HLF.

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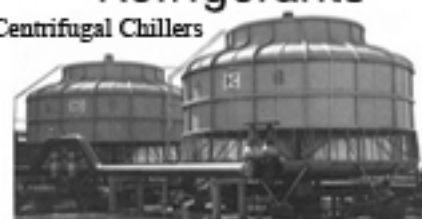
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