University of Colombo

IBMBB

Institute of Biochemistry, Molecular Biology and Biotechnology

Sixth Annual Scientific Sessions

3rd May 2013

Programme and Abstracts

Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

PROGRAMME

Inauguration

9.30 h Lighting of the Traditional Oil Lamp

9.40 h Welcome address by Director IBMBB

9.50 h Address by Vice Chancellor- University of Colombo

10.00 h Institute lecture by Professor Sarath Wimalabandara Kotagama

"Developments in Molecular Biology – Its Implications on Conservation"

11.00 h Vote of Thanks

11.05 h Reception

11.30 h Free papers and Poster presentations

Message from the Acting Director/ IBMBB

I take great pleasure in writing a few words to mark the occasion of the 6th 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 9th Anniversary. IBMBB, the only state Institute in Sri Lanka dedicated to postgraduate training and research in Molecular Life Sciences was established with a soft loan from the 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 several other MPhil and PhD students have registered at the IBMBB, all of them supported either by the SAREC Grant or by other competitive research funds secured by academics of the IBMBB. There have been several firsts in Sri Lanka among its MPhils and PhDs. These include 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. Amongst the MPhil/PhD students who have completed are three academic staff members from the University of Jaffna and one academic staff member from the Eastern University. In addition to the students registered at the IBMBB, several other MPhil/PhD students registered at other Higher Educational Institutes also carried out all or part of their research work at the IBMBB. Two probationary lecturers, one from the Faculty of Medicine, University of Kelaniya and the other from the Faculty of Medical Sciences, University of Sri Jayawardenapura are among such students. Thus the IBMBB is providing the higher educational sector with much needed academics with extensive research training.

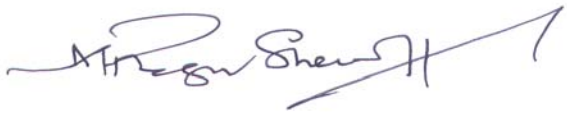
IBMBB commenced two MSc programmes in 2005, one in Molecular Life Sciences and the other in Cellular and Molecular Immunology. Both MSc courses boast a more than 80% completion rate and to date 68 students have graduated. In 2012, an MSc programme in Bioinformatics commenced jointly with University of Colombo School of Computing.

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. In 2009 and 2010 IBMBB did not hold Annual Scientific Sessions as it organized an International Conference in September 2009 to mark IBMBB's 5th Anniversary. IBMBB

students have won several International Awards and its Founder Director, former Director, other permanent academic staff and four other currently serving Board Members are Presidential Research Award winners.

High point of the 6th Annual Scientific Sessions will be the Institute Lecture on **"Developments in Molecular Biology/ Its Implications on Conservation"** to be delivered by Professor Sarath Wimalabandara Kotagama, Professor of Environmental Science, University of Colombo. There will be 27 research presentations including a few from students/researchers from other Higher Educational 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.

I take this opportunity to thank Prof. T.R. Ariyaratne , Acting Vice Chancellor, University of Colombo for his support and gracing the inauguration, Prof. Sarath Wimalabandara Kotagama for accepting the Institute 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 you to Prof. Eric Karunanayake, Prof. Kamani Tennekoon, Prof. Ira Thabrew, Prof. R.L.C. Wijesundara, Dr. Nilanthi Dasanayake, Dr. Shiroma Handunetti and Dr. O.V.D.S.J. Weerasena for reviewing abstracts, Dr. O.V.D.S.J. Weerasena and Ms. Sudeshini Hewage for editing the abstract book, Kanchana and Sashika for designing the abstract book and coordinating printing, Anoma and Abeysinghe for coordinating sponsorships and Champika and Nadeesha for secretarial assistance.



Vidyajyothi Professor Rezvi Sheriff

MD, FRCP, FRCPE, FRACP, FCCP, FNASSL, FSLCGP, FIMA

Senior Professor of Medicine

Acting Director / IBMBB

Oral Presentations

Session I - Human growth and development

11.30 -11.45 h (Abstract 01): 1737 A/G, 2992 C/T & 3238A/G polymorphisms in *H19* gene in a Sri Lankan cohort of mother-baby pairs: Effect of maternal and newborn genotype on birth size
Hewage AS, Jayanthiny P, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundere APDeS

11.45 -12.00 h (Abstract 02): Screening of a cohort of Sri Lankan children with short stature for codon 72 mutation of the *GHRH-R* gene
Navarathne B, Hewage S, De Silva S, Ginihagama D, Jayasinghe H, De Silva KSH, Tennekoon K H

Session II - Filarial Molecular Biology / Bioinformatics /anti filaricidal medicinal plants

12.00 - 12.15 h (Abstract 03): *In silico* characterization of Bax Inhibitor 1 like domain containing Endoplasmic Reticulum transmembrane protein of filarial parasite *Setaria digitata*
Mendis VEN, Senathilake KS, Samarakoon SR, Karunanayake EH

12.15 - 12.30 h (Abstract 04): Sub-cellular localization of filarial parasites-specific protein in *Pichia pastoris*
Rodrigo WWP, Dassanayake RS, Karunanayake EH, Weerasena OVDSJ

12.30 - 12.45 (Abstract 05): Evaluation of antifilarial activity of "Wild Ginger" against adult bovine *Setaria digitata*
Senathilake KS, Samarakoon SR, Karunanayake EH, Tennekoon KH, de Silva ED

Session III - Plant Molecular Biology / Metabolomics

13.30 - 13.45 h (Abstract 06): Quantification of Major Metabolites of the Sri Lankan Tea Germplasm
Jeganathan B, Punyasiri PAN, Kottawa-Arachchi JD, Ranatunga MAB,
Abeysinghe ISB, Gunasekare MTK, Bandara BMR

13.45 - 14.00 h (Abstract 07): Microsatellite based approach towards identification of blister blight resistant and susceptible tea (*Camellia sinensis* L. O. Kuntze) cultivars
Karunarathna KHT, Mewan KM, Weerasena OVDSJ Abeysinghe ISB

14.00 - 14.15 h (Abstract 08): Recent outbreaks of stem canker of tea [*Camellia sinensis* (L.) O kuntze] caused by *Fusarium solani* in Sri Lanka
Pradeepa NHL , Weerasena OVDSJ , Liyanarachchi CJ , Wijesundera RLC , Abeysinghe ISB

14.15 - 14.30 h (Abstract 09): '*Candidatus* Phytoplasma asteris' (group 16SrI) associated with diseases in papaya in Sri Lanka
Kumari WGSM, Abeysighe S and Dickinson M

Session IV - Anticancer Medicinal Plants

14.30 - 14.45 h (Abstract 10): Cytotoxic and apoptotic effects of *Lumnitzera littorea* leaves on human hepatocellular carcinoma (HepG2) cells
Chanthirika S, Samarakoon SR, Tennekoon KH, Thabrew MI, Ediriweera PMK

14.45 - 15.00 h (Abstract 11): Cytotoxic effect of *Mangifera zeylanica* bark in human breast cancer cell line MCF-7
Ediriweera PMK, Tennekoon KH, Samarakoon SR, Thabrew I, de Silva ED

15.00 - 15.15 h (Abstract 12): Evaluation of the cytotoxic activity of *Flueggea leucopyrus* (Willd) fractions on Human Breast cancer (MCF 7) cells
Mendis AS, Ediriweera PMK, Samarakoon SR, ThabrewMI

Session V - Tropical diseases / Immunology

15.15 - 15.30 h (Abstract 13): Laboratory confirmation of Leptospirosis: Comparison between microscopic agglutination test and IgM rapid test Leptocheck WB
Niloofa MJR, Fernando GTG, Fernando TRGN, Wolttan T, De Silva NL, Rodrigo C, Wickremesinghe H, Dikmadugoda N, Premawansa G, Karunanayake L, De Silva HJ, Premawansa S, Rajapakse S, Handunetti SM

15.30 - 15.45 h (Abstract 14): Low serum nitrite and anti-oxidant capacity in severe leptospirosis in Sri Lanka

Fernando TRGN, Niloofa MJR, Fernando GTG, De Silva NL, Rodrigo C, Karunanayake L, Wickremesinghe H, Dikmadugoda N, Premawansa G, De Silva HJ, Rajapakse S, Premawansa S, Handunetti SM

15.45 - 16.00 h (Abstract 15) : Determination of serum lipid peroxide and NOx level in severe leptospirosis patients in Sri Lanka

Wolttan T, Fernando GTG, Niloofa MJR, Rodrigo C, Wickremesinghe H, Dikmadugoda N, Karunanayake L, De Silva HJ, Premawansa S, Rajapakse S, Handunetti SM

Poster Presentation

Cancer Genetics/ Cancer Biology/ Anti cancer medicinal plants

PP 01 (Abstract 16): Preliminary screening of *BRCA*2 exon11for mutations in a group of ovarian cancer patients with family history of ovarian/breast cancer
Bandara KVPK, De Silva S, Karunanayake EH, Tennekoon KH, Karunaratne K

PP 02 (Abstract 17): Ankyrin repeat domine 30 A (NY-BR-1) and Mammaglobin-B as markers for detection of micrometastasis of breast cancer

Muraleetharan S, De Silva W H Y D, De Silva K, Jayaseker P, Goonesinghe R, Seneviratne B, Selvakumara R, Pathirana A, Weerasena O V D S J , Handunnetti S

PP 03 (Abstract 18): Development of multiplex RT-PCR for detection of *Bax, Survivin* and *P53* expression in MCF-7 breast cancer cell line
Chanthirika S, Samarakoon SR, Ediriweera PMK, Weerasena OVDSJ

PP 04 (Abstract 19): Evaluation of the cytotoxic and apoptotic enhancing effects of a methanolic extract of *Mangifera zeylanica* bark in human hepatoma (HepG2) cells.
Mendis AS, Samarakoon SR, Thabrew MI

PP 05 (Abstract 20): Studies of Anti-hepatocarcinogenic Activity of *Indigofera aspalathoides* Extracts
Senthi Maaran S, de Silva ED, Thabrew I, Ediriweera PMK, Samarakoon SR, Tennekoon KH

Plant Molecular Biology/ Plant Pathology

PP 06 (Abstract 21): Preliminary screening of imported seed potatoes for *Ralstonia solanacearum*
Perera AAU, Perera WGS Weerasena OVDSJ, Dasanayaka PN

PP 07 (Abstract 22): Weedy rice (*Oryza sativa f. spontanea*) population diversity found in rice fields in Kurunagala District, Sri Lanka

Karunarathna KDK, Weerakoon SR, Weerasena OVDSJ , Somarathna S

PP 08 (Abstract 23): Molecular Identification of a Sri Lankan *Bacillus thuringiensis* isolate Bt.AB125

Alahakoon RAWMRSU, Weerasena OVDSJ , Samarasekera RR

PP 09(Abstract 24): Development of Barcodes for a Sri Lankan Medicinal Plant *Plectranthus hadiensis*

Amarasinghe APPR, Siriwardhane DAS, Samarasekera RR, Weerasena OVDSJ

PP 10 (Abstract 25): Identification of cellulase producing *Beauveria felina* from decaying tea roots

Bharathy S, Pradeepa NHL, Weerasena OVDSJ

Immunology/ Plants with immunostimulatory effects

PP 11 (Abstract 26): Immunostimulatory activity of combined hot water extract of *Coriandrum sativum* L. and *Coscinum fenstratum* G. in rats

Edward D, Kothalawala SD, Handunnetti SM, Ratnasooriya WD, Premakumara GAS

PP 12 (Abstract 27): Involvement of serum reactive nitrogen species and reactive oxygen species in dengue patients in Sri Lanka

Mapalagamage MS, Handunnetti SM, Premawansa G, Loeb M, De Silva AD, Premawansa S

Abstract No: 01

1737 A/G, 2992 C/T & 3238A/G polymorphisms in *H19* gene in a Sri Lankan cohort of mother-baby pairs: Effect of maternal and newborn genotype on birth size

Hewage AS[1], Jayanthiny P[1], Tennekoon KH[1], Karunanayake EH[1], Kumarasiri JM[2], Wijesundere APDeS[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Castle Street Hospital for Women, Colombo 8

Human *H19*, a maternally expressed gene codes for a regulatory RNA implicated in fetal growth. In a cohort of 60 mother, father, newborn trios we previously described that maternal TT genotype of *H19* 2992C/T polymorphism is associated with a higher birth weight. We have also described *H19* 1737A/G genotype frequencies in 164 healthy mother-baby pairs. Here we report the effect of maternal and newborn *H19* 1737A/G, 2992C/T and 3238 A/G genotypes on birth size in a cohort of 196 healthy mother-baby pairs (inclusive of those for whom findings were previously reported). All newborns were full term. Maternal peripheral venous blood was collected before delivery and cord blood at delivery. PCR/RFLP and sequencing were used for genotyping. The commonest allele and genotype respectively were G and GG for *H19* 1737A/G, C and CC for 2992C/T and A and AA for 3238A/G polymorphisms. Maternal *H19* 2992TT genotype was associated with a significantly higher birth weight compared to CC genotype (one way ANOVA $p<0.05$; birth weight (mean$\pm$SD) 3.17$\pm$0.41, 2.92$\pm$0.47, 2.90$\pm$0.46 kg in TT, CT and CC genotypes respectively), further confirming our previous results. Birth weight was affected to a lesser extent by newborn genotype for *H19* 1737A/G polymorphism (one way ANOVA $p>0.05$, post test for linear trend $p=0.022$). Other birth indices were similar between genotypes for both *H19* 1737A/G and 2992C/T polymorphisms. None of the birth indices were affected by *H19* 3238A/G genotype. Thus some of the *H19* polymorphisms appear to modulate birth weight in healthy full term Sri Lankan newborns.

Supported by NRC Grant 05-28 and Sida/Secretariat for Research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of MPhil/PhD studies of ASH.

Abstract No: 02

Screening of a cohort of Sri Lankan children with short stature for codon 72 mutation of the *GHRH-R* gene

Navarathne B[1], Hewage S[1], De Silva S[1], Ginihagama D[1], Jayasinghe H[1], De Silva KSH[2], Tennekoon K H[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,[2]Department of Paediatrics, Faculty of Medicine, University of Colombo and Lady Ridgeway Childrens Hospital, Colombo

Growth hormone (GH) deficiency leads to short stature**.** A mutation in codon 72 of *GHRH-R* (growth hormone releasing hormone receptor) has been reported as a common genetic disorder found among GH deficient children in South Asian countries. We have previously reported this codon 72 mutation of *GHRH-R* in a Muslim boy with short stature when we screened 13 children. To date we have screened a total of 63 children with clinically and biochemically confirmed GH deficiency for *GHRH-R* codon 72 mutation by direct sequencing. DNA was extracted from peripheral venous blood samples collected, amplified by polymerase chain reaction using target specific primers and sequenced using MegaBACE automated DNA sequencer. Nucleotide sequences obtained were subjected to Bioinformatic analysis. In addition to the previously reported case, 4 more patients were found to carry the same mutation, two of them brothers of Tamil ethnicity and the remaining two unrelated Sinhala patients. This nonsense mutation of codon 72 on *GHRH-R* does not seem to be a common mutation among Sri Lankan children with GH deficiency. This is in contrast to reports for other South Asian/ Asian populations.

Supported by National Science Foundation, Sri Lanka

Abstract No: 03

In silico characterization of Bax Inhibitor 1 like domain containing Endoplasmic Reticulum transmembrane protein of filarial parasite *Setaria digitata*

Mendis VEN, Senathilake KS, Samarakoon SR, Karunanayake EH

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

Lymphatic filariasis, a severely disfiguring infectious disease affecting more than 120 million people worldwide is mainly caused by the filarial parasite *Wucheraria bancrofti*. This study was undertaken to characterize novel proteins of filarial parasites using cattle parasite *Setaria digitata* as a model organism. A cDNA library of *S.digitata* was constructed using lambda ZAP vector. *In-vivo* excised clones were randomly selected and subjected to DNA sequencing. Bioinformatics analysis of the sequences revealed a complete coding sequence which encoded a protein of 245 amino acids. The deduced protein (Predicted Mw = 27695.5 Da, theoretical pI = 9.06) was shown to have six prominent transmembrane helices. The protein had 83% identity to uncharacterized protein family UPF0005 containing protein of *Brugia malayi*, Bax Inhibitor- 1 of *Loa loa* and testis enhanced transcript of *Wuchereria bancrofti.* The conserved domain search revealed the presence of Bax Inhibitor -1 domain. The protein was predicted to be involved in transport and binding and it was said to be localized in the Endoplasmic Reticulum membrane. Automated modelling was done to obtain the tertiary structure of the protein by remote homology modelling, as suitable templates with resolved structures were unavailable. The tertiary structure was obtained as two separate models depending on the availability of templates. This *in-silico* characterization is bound to be useful in further characterization of the protein and in obtaining the complete 3D structure. Advanced studies and wet lab protein characterization is needed to make further predictions as to its suitability as a potential drug target.

This work was supported by the IBMBB, University of Colombo and Sida /Secretariat for research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of the M.Sc studies of VENM.

Abstract No: 04

Sub-cellular localization of filarial parasites-specific protein in *Pichia pastoris*

Rodrigo WWP[1,2], Dassanayake RS[2], Karunanayake EH[1], Weerasena OVDSJ[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo[2]Department of Chemistry, University of Colombo.

Setaria digitata is an animal filarial parasite living in the peritoneal cavity of cattle, goat, sheep etc and can cause fatal diseases to these hosts. Sometimes they can also infect humans as well indicating gradual adaptation as a human pathogen. We selectively cloned a cDNA from this parasite following bidirectional sequence analysis of randomly picked Expressed Sequence Tag (EST) clones. Coding sequence of this gene was found code for a parasitic nematode specific, functionally and structurally unknown protein and therefore, it was designated as *S. digitata* uncharacterized protein (SDUP). The objective of present work was to identify the sub-cellular localization of SDUP in *Pichia pastoris*. To investigate the sub-cellular localization of SDUG *in vivo*, SDUG-GFP-pPICZA vector construct was engineered and stably expressed in *P. pastoris*. Fluorescence microscopy showed this protein to be localized mainly in the nucleus and partly in cytoplasm. Western blot analyses using sub cellular fractions too indicated the same. Bioinformatics analyses indicated that this protein to have nuclear factor localization-like domain, autophosphorylation–like domain and nuclear localization signal etc. Therefore, taking these outcomes, we hypothesize that this protein may translocate into the nucleus following phosphorylation or vice versa.

This work was supported by National Science Foundation (SIDA/2006/BT/04), SAREC Grant for Molecular Biology and Biotechnology and constitutes part of the PhD studies of WWPR

Abstract No: 05

Evaluation of antifilarial activity of Wild Ginger against adult bovine *Setaria digitata*

Senathilake KS[1], Samarakoon SR[1], Karunanayake EH[1], Tennekoon KHT[1], de Silva ED[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2] Department of Chemistry, University of Colombo

Lymphatic filariasis is caused by infection with the parasitic filarial nematodes *Wuchereria bancrofti* and *Brugia malayi,* transmitted by mosquitoes. The lack of an adulticidal drug poses a challenge to filariasis elimination, hence it is essential to develop an effective antifilarial drug which could either kill or permanently sterilize the adult worms. *In vitro* antifilarial activity of hexane, methanol and hot water extracts of rhizomes of 'Wild Ginger' against adult bovine filarial parasite *Setaria digitata* was investigated. Extracts were prepared by sequential extraction with hexane, chloroform, ethyl acetate and methanol. Hot water extract was prepared separately. Extracts were screened at 10-1000µg/ml concentration for *in vitro* adulticidal activity by worm motility assay. Parasites were washed with sterile culture medium (DMEM with 10% FBS) and transferred to each well in 6-well plates containing 5 ml of medium containing extract (two worms per each well). A simultaneous control was kept by adding culture media without extract. Plates were incubated at 37^{o} C for two days in 5% CO_2. Each experiment was performed in three replicates. Motility was observed daily for 2 days and daily motility score was recorded using an arbitrary score of 3 (highly active), 2 (moderately active), 1 (less active) and 0 (immobile for at least 10 sec). The results were expressed as the average motility score with respect to the exposure time. Finally, immobilized worms were immediately transferred in to fresh media to confirm that none of the immotile worms regained motility. All three extracts showed macrofilaricidal activity. Minimal concentration and time taken to complete immobilization of worms for water, methanol and hexane extracts were 500 µg/ml for 48 h, 500 µg/ml for 24 h and 250µg/ml for 24 h respectively. Immobilized worms did not regain motility after 24 h post incubation period in the fresh medium.

This work was supported by the Ministry of Higher Education research grant and constitutes part of the M. Phil / Ph. D studies of KSS

Abstract No: 06

Quantification of Major Metabolites of the Sri Lankan Tea Germplasm

Jeganathan B[1], Punyasiri PAN[1], Kottawa-Arachchi JD[2], Ranatunga MAB[2],Abeysinghe ISB[2], Gunasekare MTK[3], Bandara BMR[4]

[1]Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo, [2]Tea Research Institute of Sri Lanka, Talawakelle, [3]Council for Agriculture Research Policy, Colombo 7, [4]Department of Chemistry, University of Peradeniya

Tea is the second most popular and economically important beverage in the world. The chemical constituents of tea flush (*Camellia sinensis,* L.) play a major role in defining the quality of the final product. However, limited work has been documented on the quantitative analysis of the variation of a broad range of metabolites in tea cultivars. The present study is the first attempt on a large scale metabolite profiling of the Sri Lankan tea germplasm using high throughput techniques. Tea flush samples of 87 germplasm accessions collected from the *ex situ* field gene bank of the Tea Research Institute of Sri Lanka were freeze-dried and analyzed for total polyphenols (TPP) by the ISO/14502-1 spectrophotometric method. The quantitative analysis of six major metabolites (caffeine, four catechins and theobromine) was performed by the ISO/14502-2 method usingAgilent 1260 Infinity HPLC system. The TPP content varied from 153.08±11.64 to 287.88±6.53 mg/g dry weight (dw). The contents of the six major metabolites varied in the following ambits: caffeine (18.11±0.65 – 44.93±4.56 mg/g dw), epicatechin (EC, 6.08±1.57– 25.50±0.70 mg/g dw), epicatechin gallate (ECg, 14.36±0.28 – 47.42±5.19 mg/g dw), epigallocatechin (EGC, 8.19±3.69 – 54.45±2.10 mg/g dw), (EGCg, 41.45±2.48 – 120.86±8.00 mg/g dw) and theobromine (0.71±0.26 – 8.826±0.56 mg/g dw). The highest contents of EGC and EGCg were observed for the accessions PK2 and TRI3041 whilst the lowest for PLLG2, and TRI2043, respectively. High amounts of EC and ECg were observed in high-quality black tea producing cultivars whereas low-quality tea producing cultivars had low amounts of these catechins.

Authors acknowledge the National Research Council of Sri Lanka for Grant 11-023.

Abstract No: 07

Microsatellite based approach towards identification of blister blight resistant and susceptible tea (*Camellia sinensis* L. O. Kuntze) cultivars

Karunarathna KHT[1], Mewan KM[2], Weerasena OVDSJ[1]and Abeysinghe ISB[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo
[2]Biochemistry Division, Tea Research Institute of Sri Lanka, Talawakelle.

Blister blight disease (BB), caused by *Exobasidium vexans* is one of the most devastating leaf diseases in tea (*Camellia sinensis* L.). Development of resistant cultivars is identified as the most effective and sustainable approach to control the disease. Genetic improvement through conventional means require 20-25 years to release a cultivar, and integration of marker-assisted-selection (MAS) into conventional program could accelerate the efficiency and effectiveness of the process. Therefore aim of this study was to develop a marker for identification of BB resistance trait towards subsequent application in marker-assisted-tea-breeding (MATB). The study population consisted of 300 F_1 individuals, derived from a cross between TRI2023 (BB susceptible) X TRI2043 (BB resistant). Based on the F_1 field assessment data, three individuals extremely susceptible and three extremely resistant to BB were used for the marker development using a set of EST-SSR (expressed sequence tag based simple sequence repeat) primers. Among tested EST-SSR primers, the primer-pair designated as EST-SSR073, generated a distinct band specific to BB resistant individuals. The specificity of the band was confirmed by repeated PCR assays and was further validated using a set of recommended tea cultivars with known BB resistant ratings. The outcome clearly reflected the potential of the band to differentiate BB resistant individuals from susceptible and hence could be useful in early selection of the trait, by a simple PCR assay, which is independent of growth stage and field assessments. Therefore this finding is a fundamental pre-requisite towards MAS/ MATB in Sri Lanka.

This work was supported by National Research Council (Grant No: NRC-09-066) and constitutes part of MPhil/PhD studies of KHTK

Abstract No: 08

Recent outbreaks of stem canker of tea [*Camellia sinensis* (L.) O kuntze] caused by *Fusarium solani* in Sri Lanka

Pradeepa NHL[1,2] , Weerasena OVDSJ[2] , Liyanarachchi CJ[1] , Wijesundera RLC[3] , Abeysinghe ISB[1]

[1]Tea Research Institute of Sri Lanka, [2]IBMBB, University of Colombo, [3]Department of Plant Sciences, University of Colombo

During the period 2008-2013 severe outbreaks of stem cankers with unusual die back symptoms were observed in about 35,000 tea bushes from several tea gardens in the Ratnapura district. The diseased plants also exhibited chlorosis, sudden wilting defoliation and vascular discolouration. Severe infestations resulted in girdling of the collar region leading to death of the tea bushes. A fungus was consistently isolated from 85% of the symptomatic material. The fungus was identified as *Fusarium solani* complex based on colony and conidial morphology. The identification was also confirmed based on sequences of two gene regions: the ribosomal DNA (rDNA) internal transcribed spacer region (ITS1-5.8S-ITS2) and TEF-1α (translation elongation factor 1α gene) sequences. The TEF 1α sequence of one isolate was deposited at GenBank (Accession No. JX467676). The pathogenecity of two randomly selected isolates of *F. solani* was tested on healthy plants of cv TRI 2026 and TRI 2025 (10 plants from each). Lesions developed on all inoculated plants after 06 weeks while control plants remained healthy. Koch's postulates were confirmed by consistently reisolating *F. solani* from inoculated plants. Sensitivity of isolates to fungicides Hexaconazole, Bitertanol, Propiconazole and Tebuconazole was also studied. Inhibition was significantly higher with Tebuconazole than with the other 03 fungicides. *F. solani* has a wide host range. However it has never been reported as a pathogen of tea from Sri Lanka, or elsewhere. The aetiological and epidemiological knowledge gained through this study will help better prediction and management of this pathogen.

This work was supported by the Tea Research Institute of Sri Lanka and constitutes part of MPhil studies of NHLP

Abstract No: 09

Candidatus Phytoplasma asteris' (group 16SrI) associated with diseases in papaya in Sri Lanka

Kumari WGSM[1], Abeysighe S[1] and Dickinson M[2]

[1]Department of Botany, Faculty of Science, University of Ruhuna, [2]School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK

Phytoplasmas are unculturable, cell-wall-less bacteria infecting hundreds of plant species. Many newly emerged phytoplasma diseases have been reported during the last decade worldwide. In Sri Lanka, phytoplasma is reported to be associated with diseases in crops such as coconut, sugarcane, and some horticultural crops. However, its phylogenic positions are not clearly defined. Symptoms of dieback, severe reduction in leaf area associated with vein clearing and enation and leaf mottling were observed causing serious damages in papaya cultivations in Moneragala, Hambantota and Matara districts respectively. Phytoplasma 16SrDNA region was partially amplified using universal primer pairs in direct and nested PCR assays. Nested PCR amplicones were sequenced and revealed that above symptoms are associated with phytoplasma belonging to *Candidatus* Phytoplasma asteris' (group 16SrI) with 99% sequence identity. To our knowledge this is the first report describing phylogenic position of phytoplasmas causing diseases in papaya in Sri Lanka. Similar kinds of symptoms were reported in papaya from other countries infected with phytoplasmas belonging to group 16SrI and 16SrII. However, more samples need to be sequenced in order to confirm that all three symptoms of (i) dieback (ii) severe reduction in leaf area associated with vein clearing and enation (iii) leaf mottling are caused by group 16SrI phytoplasmas in the different locations in Sri Lanka. Confirmation of this result has a significant importance for the papaya industry in Sri Lanka since the phytoplasmas of group 16SrI are known to possess the largest host range including plant and insect vectors.

This work was supported by the Internationalization Grant 2012, University of Ruhuna

Abstract No: 10

Cytotoxic and apoptotic effects of *Lumnitzera littorea* leaves on human hepatocellular carcinoma (HepG2) cells

Chanthirika S[1], Samarakoon SR[1], Tennekoon KH[1], Thabrew MI[1], Ediriweera PMK[1]

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Many natural products from plants have been identified to exert anticancer activity. Mangrove plants in Sri Lanka are a likely source of novel molecules with anti-cancer activity. *Lumnitzera littorea* is a mangrove plant which has not yet been studied for anti-cancer activity. This study was carried out to investigate *in-vitro* cytotoxic and apoptotic effects of *L. littorea* in HepG2 cells. Dried leaves of *L. littorea* were sequentially extracted to cold n-hexane, chloroform, ethyl acetate and methanol. Extracts were preliminarily screened for cytotoxic activity against HepG2 cells using the Sulphorhodamine B (SRB) assay. Subsequently ApoTox-Glo™ triplex assay was used to measure the cytotoxicity, cell viability and activity of caspases 3/7 at 4 h post-incubation with most active (n-hexane) extract. Apoptotic effect of the hexane extract was determined by assessing morphological changes of HepG2 cells and DNA fragmentation analysis.

Hexane (IC_{50}: 83.74 ±7.09 µg/mL) and chloroform (IC_{50}: 118 ±5.09 µg/mL) extracts showed strong cytotoxicity against HepG2 cells while ethyl acetate and methanol extracts were less cytotoxic (IC_{50}>500 µg/mL). ApoTox-Glo™ triplex assay showed a dose-dependent reduction of cell viability (EC_{50} = 105.5 µg/mL), enhancement of cytotoxicity (IC_{50} = 49.46 µg/mL) and a significant induction of caspase 3/7 activity ($p < 0.05$) at a dose of 100 µg/mL. Morphological changes of HepG2 cells, DNA fragmentation and activation of caspase 3/7 further confirmed the apoptotic effect of hexane extract. Further investigation of hexane and chloroform extracts may result in identification of new anti-carcinogenic components from *L. littorea* leaves.

This study was supported by the Ministry of Higher Education research grant. ApoTox-Glo™ Triplex Assay kit was provided by Promega Corporation.

Abstract No: 11

Cytotoxic effect of *Mangifera zeylanica* bark in human breast cancer cell line MCF-7

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Breast cancer accounts for nearly a quarter of cancers in Sri Lankan women. Long term use of currently available adjuvant therapy leads to undesirable side effects for oestrogen receptor positive breast cancer, whereas treatment options for triple negative breast cancer are extremely limited. Thus there is a need to identify alternative drugs effective against breast cancer.

Mangifera zeylanica (family: Anacardiaceae), "Etamba" is endemic to Sri Lanka and has been incorporated in some traditional medicines prescribed for cancer. We investigated possible cytotoxic effects of *M. zeylanica* in oestrogen receptor positive MCF-7 breast cancer cell line. Finely powdered dried *M. zeylanica* bark was subjected to cold extraction using hexane, chloroform, ethyl acetate and methanol sequentially. Resulted extracts were screened for cytotoxic activity using Sulphorhodamine B (SRB) assay. The most active extract was further tested for cytotoxicity, cell viability and activity of caspases 3/7 using ApoTox-Glo™ triplex assay after incubating for 4 h with MCF-7 cells. This extract was then subjected to further separation using the Kupchan's solvent-solvent partition scheme. All resulting fractions were screened for cytotoxicity by SRB assay after incubating MCF-7 cells (24 h) with different concentrations.

Of the four extracts resulted from the cold extraction only hexane extract was found to be cytotoxic to MCF-7 cells. It showed highest cytotoxicity ($IC_{50:}$ 87.64 µg/ml) with a dose dependent decrease in viability and an increase in cytotoxicity with no caspase 3/7 activation after 4 h incubation (viability $IC_{50:}$ 263.8 µg/ml, cytotoxicity $IC_{50:}$ 102.6 µg/ml). Cytotoxicity was only contained in the chloroform fraction resulted from the Kupchan's solvent-solvent partition scheme (IC_{50}: 56.37 µg/ml). Chromatographic studies are in progress to isolate the cytotoxic compound/s responsible for cytotoxicity.

This work was supported by National Research Council of Sri Lanka (Grant No: 11-018) and constitutes part of the PhD studies of EPMK. ApoTox-Glo™ triplex assay kit was provided by Promega Corporation.

Abstract No: 12

Evaluation of the cytotoxic activity of *Flueggea leucopyrus* (Willd) fractions on Human Breast cancer (MCF 7) cells

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Cancer is reported to be the second largest cause of mortality in the world. Plant compounds have played a significant role in cancer therapy. Among plant based formulations that are currently being used for cancer therapy by the traditional medical practitioners of Sri Lanka there is an aqueous decoction prepared from aerial plants of *Flugeggea leucopyrus* (Willd). This plant known in Sri Lanka as Katupila (Family Phyllanthaceae), is a medicinal plant that also grows in regions of India, Myanmar, and Pakistan. Standardized aqueous and ethanolic extract of aerial parts of this plant have been reported to exert cytotoxic and anti-proliferative effects on human hepatoma cells (HepG2), human endometrial cells (AN3CA) and breast cancer cells (MCF-7). The present study was carried out to further investigate cytotoxic effects of fractions of *F.leucopyrus* obtained by subjecting finely powdered aerial parts of this plant to sequential soxhlet extraction using hexane, chloroform, ethyl acetate and methanol. Fractions so obtained were tested for cytotoxic activity against MCF-7 cells by evaluating effects on cell survival and overall activity using Sulphorhodamine B (SRB) and 3-(4,5-dimethylthiazol-2yl)-2, 5-biphenyl tetrazolium bromide (MTT) assays respectively. Most active fraction was the chloroform fraction. This was subjected to further separation following the Kupchan's partition scheme. On testing all the resulting fractions, the chloroform fraction again demonstrated the highest cytotoxicity to MCF-7 cells when evaluated by the SRB and MTT assay, with IC_{50} values of 96.03 µg/ml and 183.56 µg/ml respectively.

This work was supported by funds provided by the National Science Foundation (NSF) and IBMBB.

Abstract No: 13

Laboratory confirmation of Leptospirosis: Comparison between microscopic agglutination test and IgM rapid test-Leptocheck WB

Niloofa MJR[1], Fernando GTG[1], Fernando TRGN[1], Wolttan T[1], De Silva NL[1], Rodrigo C[2], Wickremesinghe H[3], Dikmadugoda N[3], Premawansa G[4], Karunanayake L[5], Wickremesinghe AR[6] , De Silva HJ[6], Premawansa S[7], Rajapakse S[2], Handunetti SM[1]

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Leptospirosis is most often diagnosed on clinical grounds, and confirmed by laboratory testing by the microscopic agglutination test (MAT). There are also rapid immunodiagnostic tests which detect IgM antibodies against *Leptospira*. Objective of this study was to compare the laboratory diagnoses by MAT and IgM based rapid immunochromatographic test [Leptocheck-WB test (LCT)] and to evaluate its clinical applicability. Clinically suspected leptospirosis patients (n=455) were recruited from three hospitals (Base Hospital Homagama, National Hospital Sri Lanka, Colombo and Colombo North Teaching Hospital, Ragama) during the period June 2012 to February 2013. All patients were screened with MAT and LCT. A MAT titer of ≥400, a 4-fold rise in MAT titer or seroconversion to >200 and isolation of *Leptospira* organisms from blood stream were considered criteria for laboratory confirmed cases of leptospirosis as per WHO guidelines and compared with IgM Leptocheck test positivity. Confirmed leptospirosis patients were identified from Colombo (43.3%) and Gampaha (10.8%) districts. Positivity of LCT, MAT (acute serum) and MAT (paired sera) was 51.2%, 30.9% and 35.8% respectively. The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of LCT with reference to MAT confirmation were 92.8%, 75%, 81.5%, 68.3% and 94.7% respectively. There was a good agreement between LCT and MAT (κ=0.319). There were significant correlation between MAT titer and LCT positivity (r=0.571, p<0.0001). LCT positive patients had 6.8±3.3 (mean±SD) days of illness and became positive by day 3 of illness. The observed high sensitivity, specificity, ease of use in obtaining results rapidly and no specific skills required for performance, make LCT a suitable test for early diagnosis of leptosirosis.

This work was financially supported by NSF grant NSF/RG/2011/HS/19 and constitutes part of MPhil/PhD studies of MJRN.

Abstract No: 14

Low serum nitrite level and anti-oxidant capacity in severe leptospirosis in Sri Lanka

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Our previous studies have shown high serum nitrite levels in confirmed leptospirosis patients compared to healthy controls. Objective of this study was to determine the serum nitrite levels and antioxidant capacity (AOC) in confirmed leptospirosis patients characterized as having severe and mild disease. Laboratory confirmed, severe and mild leptospirosis patients (SL, n=40 and ML, n=33 respectively) and non-leptospirosis fever (NLF) patients (n=36) were selected. Serum nitrite levels were tested using Griess assay and compared with healthy controls (n=22). Serum nitrite levels were corrected for renal function using the ratio nitrite/Creatinine. Corrected serum NO_2^- levels of SL (0.062 ± 0.02 μM) were significantly lower compared to ML (0.23 ± 0.28 μM; *P<0.01*) at the acute stage whereas it was comparable at the convalescent stage. Within the group of SL patients, the high serum NO_2^- levels observed at the acute stage decreased significantly at the convalescent stage (0.53 ± 0.27 μM; *P=0.013*) and was comparable to that of healthy controls (1.41 ± 0.61 μM). Serum AOC levels were determined by ABTS decolourization method, expressed as serum Trolox equivalent antioxidant capacity (TEAC) and corrected using total serum protein content. Corrected serum AOC levels were significantly lower (15.76 ± 5.32 μM) in SL patients compared to healthy controls (25.05 ± 12.48 μM; *P<0.01*) whereas the corrected serum AOC levels of ML patients were in the same range as the healthy controls. These data indicate that the low bioavailability of nitric oxide and depletion of serum antioxidants are factors contributing to severe leptospirosis. Further it highlights their potential to be developed as biomarkers for severe leptospirosis.

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Abstract No: 15

Determination of serum lipid peroxide and NOx level in severe leptospirosis patients in Sri Lanka

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Biomarkers of oxidative stress such as lipid peroxide (LP) and total serum nitrate and nitrite (NO_x) levels in serum were tested for early categorization of severe leptospirosis patients. FOX-2 assay and modified Griess assay were used for the measurement of serum LP level and NO_x levels. Clinically suspected 141 cases of leptospirosis were recruited, 44 were confirmed by microscopic agglutinating test (MAT) and further categorized as severe (n = 25) and mild (n = 19) patients based on clinical features. Age-gender matched non leptospirosis fever (NLF) patients (n = 21) and healthy individuals (n = 19) were included as controls. LP levels of severe (3.33 ± 0.46 μM), mild (3.24 ± 0.47 μM) and NLF (3.20 ± 0.33 μM) were not significantly different among patient groups although, these values were significantly high ($p < 0.001$) compared to healthy controls (1.25 ± 0.10 μM). Uncorrected NO_x level of severe patients was significantly high (8.41± 0.89 μM) compared to mild patients (4.59 ± 0.47 μM; $p = 0.001$), NLF (5.84 ± 0.59 μM; $p = 0.021$) and healthy controls (4.30 ± 0.30 μM; $p < 0.001$). However, when serum NO_x levels were corrected for renal function using NOx/creatinine ratios, the corrected NO_x levels were significantly low in severe patients (0.014 ± 0.002 μM/μM/l) compared to mild (0.039 ± 0.01 μM/μM/l; $p = 0.029$), NLF (0.040 ± 0.006 μM/μM/l; $p = 0.001$) and healthy controls (0.046 ± 0.005 μM/μM/l; $p < 0.001$). Thus, corrected serum NO_x level may be used as an oxidative stress biomarker and prognostic indicator for severe leptospirosis; the LP level is not specific for severe leptospirosis.

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

Abstract No: 16

Preliminary screening of *BRCA*2 exon11for mutations in a group of ovarian cancer patients with family history of ovarian/breast cancer

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Patients with ovarian cancer have a poor prognosis with less than 20% chance of 5 year survival when diagnosed at stage 3 of the disease. Enumerated risk factors for ovarian cancer include carrying a known pathological mutation of either *BRCA*1 or *BRCA*2 genes. Pathological mutations in relation to ovarian cancer are known to cluster in the exon 11 of *BRCA2* gene. Identification of the mutation profile of *BRCA* genes in Sri Lankan population is likely to provide a valuable diagnostic tool for early detection of some ovarian cancers. In this preliminary study of familial ovarian cancer, mutations or variants of the exon 11 of the *BRCA*2 gene were assessed. After obtaining informed written consent, peripheral venous blood was obtained from five ovarian cancer patients with a family history of ovarian or breast cancer in first degree relatives at National Cancer Institute Maharagama. Genomic DNA was extracted with Wizard® Genomic DNA Purification Kit. Exon 11 of the *BRCA*2 gene was amplified using 9 sets of primers. Entire exon 11 was sequenced with MegaBACE 1000 DNA sequencer. Bioinformatics analysis of the sequencing results was performed with Bio Edit software, BLAST program & SNP database of NCBI and BIC database. Three variants with unknown significance were identified namely 3624 A>G, 4791 A>G and 6741 G>C. One participant is probably a heterogeneous mutant, with a novel mutation 2716 A>G with amino acid change Asn>Asp. Further research is necessary to confirm the latter finding and to assess its biological significance.

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Abstract No: 17

Ankyrin repeat domine 30 A (NY-BR-1) and Mammaglobin-B as markers for detection of micrometastasis of breast cancer

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Breast cancer is the commonest malignancy in women. Although most patients will be cured of their disease with surgery and chemotherapy, a considerable proportion of patients develop metastatic disease later. Metastatic disease has a survival rate less than 20%. Axillary lymph node (ALN) is a marker of metastatic disease. Routine histopathological staining methods used to examine lymph nodes is time consuming and less sensitive than RT-PCR. Objective of this study was to establish an RT-PCR based multimarker assay to detect micrometastasis in breast cancer. Two breast cancer specific markers, NY-BR-1 and MG-B and an epithelial marker, CK-19 were selected for this study. Five ALN samples (half nodes) were obtained and total RNA was extracted. cDNA was synthesized and PCR was carried out. The other halves of ALNs were sent for histopathological examination. Positive control lymph node was positive for all three markers by RT-PCR. Negative controls (peripheral blood samples) were negative for all three markers. Thus, the present study has established a multi-marker RT-PCR for the detection of micrometastasis of breast cancer. Out of five ALN samples tested, one ALN was positive for MG-B and CK-19. CK-19 was expressed in three other ALNs. All five ALNs were negative for NY-BR-1 and this might be due to its stage specificity. Thus, RT-PCR has detected micrometastasis of breast cancer in one out of five ALNs tested. Histopathological results were negative for all half ALN samples tested and only one of the CK-19 positive patient was positive for metastasis by histopathology. Therefore further study should be carried out with large number of samples.

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

Abstract No: 18

Development of multiplex RT-PCR for detection of *Bax, Survivin* and *p53* expression in MCF-7 breast cancer cell line

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The use of multiplex Reverse Transcription Polymerase Chain Reaction (mRT-PCR) is becoming increasingly important cost effective and fast alternative procedure to study molecular mechanism of anti-cancer drugs. An objective of this study was to design and optimize a mRT-PCR to evaluate the expression of *Survivin*, *Bax* and *p53* in MCF-7 cells following Pacilitaxel treatment. The designed primers for mRT-PCR using MP primer (2.0) software were successfully optimized for annealing temperature (56 °C), concentrations of primers (0.15 µM of each), magnesium chloride (5 mM), dNTPs (500 µM), green GoTaq® (Promega) reaction buffer (1.5 **X**) and Taq DNA polymerase (2.5 U). Apoptotic and cytotoxic effects of Pacilitaxel were determined by assessing morphological changes in MCF-7 cells, DNA fragmentation analysis and Sulphorhodamine B assay respectively. Consequently optimized mRT-PCR was used to study the gene expression in Pacilitaxel treated MCF-7 cells. Quantification of mPCR gel band intensities using BIO RAD quantity one software revealed that there were no significant regulations of expressions of these genes ($p > 0.05$) in response to Pacilitaxel treatments (0.01, 1 and 5 µg/mL) for 4 or 24 h post incubations. The results obtained from singleplex real-time PCR with SYBR® green dye showed, Pacilitaxel treated sample (4 h post incubation with 5 µg/mL) had *Bax*: 0.88; *Survivin*: 0.65 and *p53*: 1.08 fold changes in target mRNA compared to the control. This optimized mRT-PCR will be useful to study the expression of *Bax, Survivin*, and *p53* in human cancer cell lines following treatment with anti-cancer drugs.

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

Abstract No: 19

Evaluation of the cytotoxic and apoptotic enhancing effects of a methanolic extract of *Mangifera zeylanica* bark in human hepatoma (HepG2) cells.

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Mangifera zeylanica (etamba) of the family Anacardiaceae, is an endemic species to Sri Lanka. Present investigation was focused on evaluating cytotoxic and apoptotic effects of a methanolic extract of *M. zeylanica* bark in HepG2 cells. Cytotoxicity was assessed by evaluating effects on cell survival and overall activity using Sulphorhodamine B (SRB) and 3-(4,5-dimethylthiazol-2yl)-2, 5-biphenyl tetrazolium bromide (MTT) assays respectively. Results demonstrated dosē dependant cytotoxicity of the crude extract to HepG2 cells. Microscopic observations of cell morphology and DNA fragmentation of HepG2 cells treated with methanolic extract portrayed the general characteristics of apoptosis such as loss of cell volume, cell shrinkage, nuclear condensation and smeared laddering pattern on agarose gel electrophoresis. Analysis of effects of the methanolic extract on expression of apoptosis related genes (*p53, Bax and Survivin*) was carried out by RT-PCR, multiplex and real time PCR. RT-PCR and multiplex PCR showed a significant ($p<0.05$) up regulation in p53, Bax and Survivin at a dose of 100µg/ml. Real time PCR confirmed that p53 increases by 5 fold and Bax and Survivin by 2.8 and 2.6 fold respectively.The overall results confirmed that *M. zeylanica* crude extract has the ability to induce cytotoxicity and apoptosis in HepG2 cells in a time and dose dependent manner. The upregulation of p53 and Bax suggest that cytotoxic effects of the crude extract in HepG2 cells maybe mediated through apoptosis. According to published reports upregulation of p53 is generally accompanied by a downregulation of Survivin expression. Hence why Survivin expression was upregulated by the *M. zeylanica* crude extract in the present study needs further evaluation.

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

Abstract No: 20

Studies of Anti-hepatocarcinogenic Activity of *Indigofera aspalathoides* Extracts

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Indigofera aspalathoides (family: Fabaceae) is a shrub mostly found in Northern Sri Lanka and South India and is used in the traditional medical systems of India and Sri Lanka to treat many ailments including cancer. A flavonoid with potential anticancer properties has been reported from this plant growing in India. However, no scientific investigations of the anticancer properties of this plant growing in Sri Lanka have been reported. These observations provide the rationale for the current investigation. The objective of the project was to investigate the *in vitro* anticancer activity of the organic extracts of *I. aspalathoides* growing in Sri Lanka against the human hepatoma (HepG2) cell line and to isolate and characterize any active compound(s) by spectroscopic methods. Finely powdered, freeze-dried aerial plant material of *I. aspalathoides* was subjected to sequential extraction using hexane, chloroform, ethyl acetate and methanol. These extracts were tested for anticancer activity on the HepG2 cells using Sulforhodamine B (SRB) colorimetric assay. Activity was demonstrated by chloroform fraction. Subsequently solvent-solvent partitioning was done on this active fraction using Kupchan's scheme. The partitioned fractions were then screened for cytotoxicity by SRB assay after incubating HepG2 cells for 24 hours with different concentrations of each fraction. The original chloroform extract showed cytotoxicity with an IC_{50} value of 58 μg/ml. Of the fractions resulted from Kupchan's scheme, the chloroform fraction showed the best cytotoxicity value of IC_{50} 28.8 μg/ml. Further chromatographic studies to isolate the active compound(s) are in progress. This is the first report that show *I. aspalathoides* extracts giving cytotoxic activity against HepG2 cells.

This work was supported by Department of Chemistry, Faculty of Science UOC, and Institute of Biochemistry, Molecular Biology and Biotechnology, UOC.

Abstract No: 21

Preliminary screening of imported seed potatoes for *Ralstonia solanacearum*

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Ralstonia solanacearum is the causative organism of brown rot of potato and is considered in Sri Lanka as a quarantine pest. Therefore imported seed potatoes are visually observed at the entry point and samples are collected by National Plant Quarantine Service, Katunayake for laboratory tests. When presence of *R. solanacearum* is suspected, triphenyl tetrazolium chloride (TZC) medium is used for isolation, as the bacterium grows on this medium as fluidal, irregularly round, white colonies with red-centers. Out of 1400 metric tons of seed potatoes imported during October 2011 to February 2012, majority (80%) were from Netherland, while 12% from USA, 4% from France, and 4% from Germany. Eighty one seed potato samples of 37 different varieties imported during the above mentioned period were selected for the study. Slices taken from both stem end and the eyes of ten tubers per sample were cultured on potato dextrose agar after surface sterilization and incubated at 30 ºC for 3 days to isolate bacteria. Bacterial isolates were screened by using potassium hydroxide test to distinguish Gram negative bacteria. Then the bacterial isolates were cultured on TZC medium to distinguish *R. solanacearum*-like bacteria from other bacteria. *R. solanacearum*-like bacteria were found from 6 out of 8 (75%), 1 out of 2 (50%), 1 out of 11 (9.09%) and 5 out of 60 (8.3%) samples imported from USA, Germany, France and Netherland respectively. The isolation of *R. solanacearum*-like bacteria from 75% and 50% of samples imported from USA and Germany highlights the importance of adoption of stringent quarantine measures when importing seed potatoes from high risk regions to avoid invasions of quarantine-significant pathogens into Sri Lanka.

This work was supported by National Research Council (Grant No. 11-099) and constitutes part of MPhil studies of AAUP

Abstract No: 22

Assessment of morphological diversity of Weedy rice (*Oryza sativa f. spontanea*) bio-types found in rice fields in Kurunagala District, Sri Lanka

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Weedy rice (*Oryza sativa f. spontanea*) has become a major threat to rice cultivation in Sri Lanka. Therefore it is of vital importance to control the weedy rice to enhance the quality and quantity of economical yield of rice. Present research focuses to determine the level of morphological and genetic diversity of weedy rice population found in rice fields in Kurunagala District. Weedy rice bio-types were collected from Kurunagala Districts representing five different locations. All seeds collected from Kurunagala District were subjected to dormancy breaking procedures. Seeds of each bio-type were sown in plastic trays in a plant house at Open University of Sri Lanka. A total of five replicates of each bio-type were planted in black polythene bags with paddy soils. Each of these replicate was arranged in Complete Randomized Design (CRD). Agro-morphological characterization (Using thirty six morphological characters) of weedy rice genotypes and cultivated rice varieties was carried out. The observations and measures/counts were made in accordance with the characterization catalogue published by Plant Genetic Resources Centre, Sri Lanka. Morphological data sets obtained for Weedy rice bio-types of Kurunagala District were subjected to Classification and Regression Tree analysis (CART) using SPSS PC, Ver. 16.0. According to the results, three (03) important characters (Awning after Full Heading, Seedling Height and Panicle Type) split the samples into four (04) child nodes representing the redundancy of the data set. Further, three different weedy rice bio-types were identified in Kurunagala district. All collected bio-types will be subjected to molecular study for confirmation of this result.

This work was supported by National Science Foundation (Grant No. RG/2011/BT/06)and constitutes part of MPhil studies of KDKK.

Abstract No: 23

Molecular Identification of a Sri Lankan *Bacillus thuringiensis* isolate *Bt.*AB125

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Bacillus thuringiensis Berliner *(Bt)* is a facultative anaerobic spore forming gram positive bacterium. It is best known for its production of proteinaceous parasporal inclusions, which are toxic to insect larvae. *Bacillus thuringiensis Bt.*AB125 strain isolated from Sri Lanka was identified for its insecticidal activity against selected lepidopteran pests at laboratory and field levels by previous studies. Recently, the gyrB gene has proven useful at distinguishing between members of the *B. cereus* sensu lato group. Therefore, the objective of this study was to verify the sub species of *Bt.*AB125 strain by gyrB gene sequencing. Amplification of gyrB gene by nested PCR was performed for the *Bt.*AB125 isolate and purified amplicon was sequenced using Big dye terminator cycle sequencing kit and Applied biosystem 3500 dx genetic analyzer according to the manufacturer's instructions. Sequence was searched over the GenBank database at NCBI using BLAST. The result showed that gyrB gene sequence of *Bt*.AB125 has highest similarity to *Bt.* sub spp. *jegathesan*

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Abstract No: 24

Development of Barcodes for a Sri Lankan Medicinal Plant *Plectranthus hadiensis*

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Iriweriya (*Plectranthus hadiensis* (family Lamiaceae)) is known for its medicinal value including *in vitro* cytotoxic activity. *Plectranthus hadiensis* is substituted by other plants of the same genus in herbal medicinal industry due to morphological similarity. Therefore, the objective of this study was to develop barcodes to discriminate Sri Lankan *P.hadiensis*. After morphologically identifying *P. hadiensis* the chemical profiles were developed for its shoots and roots using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). TLC profiles of hexane, ethylacetate and methanol extracts of *P. hadiensis* shoots and roots showed a better separation of their compounds with a solvent system of hexane: ethyl acetate: methanol (51: 48: 1 v/v). HPLC profiles from methanol extracts of shoot and root of *P.hadiensis* resulted nine and eight major peaks respectively. As DNA barcodes, three chloroplast DNA regions, known as *matK, rbcL* and *psbA-trnH* were amplified and sequenced. The results suggested that DNA barcodes have the potential to identify *P. hadiensis.* Out of the three barcode regions selected the *matK* and *psbA-trnH* were found to be the most suitable regions to distinguish *P. hadiensis*. The *rbcL* region was ambiguous in assigning *P. hadiensis* into the correct family or genus, whereas *matK* and *psbA-trnH* accurately placed this plant in the genus *Plectranthus*. The barcode sequences resulted in this study were deposited in GenBank nucleotide database. Chemical profiles and barcode sequences revealed in this study could serve as barcodes for *P. hadiensis* contributing to the conservation and the quality control of *P. hadiensis* herbal formulations.

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Abstract No: 25

Identification of cellulase producing *Beauveria felina* from decaying tea roots

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Fungi are the main natural agents of cellulose degradation as fungi produce cellulases. Cellulases have been used for several years in food processing, feed preparation, paper and pulp industries and textile production. Even though there are many reports on fungi producing cellulases, only a few species have proved higher activities for commercial success. The aims of this study were the isolation, identification and screening of fungal cultures for cellulase production. Wood decaying fungi were isolated from decaying tea roots using induced sporulation method and they were identified based on their colony and conidial characteristics. Majority of the incitants were identified as *Aspergillus* and *Penicillium* spp. (known cellulase producers) while few decaying roots resulted in a fungus resembling *Beauveria* spp. The fungus was grown in Czapek Dox broth and cellulase activity of fungal filtrate was screened using filter paper assay. The screening test revealed that the fungus has significantly higher cellulase activity compared to *Trichoderma harzianum* (known cellulase producer). The amplification of Internal Transcribed Spacer (ITS) region of the fungal rDNA was carried out using fungal specific ITS 1F and ITS 4R primers and the resultant sequence was searched over the Genbank data base using BLASTn. The sequence showed 95 % similarity with the ITS region of *Beauveria felina* (FJ973064.1). According to both morphological features and the ITS DNA sequence the fungus was identified as *Beauveria felina*.

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Abstract No: 26

Immunostimulatory activity of combined hot water extract of *Coriandrum sativum* L. and *Coscinum fenstratum* G. in rats

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The combined hot water extract (HWE) of *Coriandrum sativum* L. (family: Umbelliferae) and *Coscinium fenestratum* G. (family: Menispermaceae) has been used as a combination to treat inflammatory diseases in Sri Lanka. A preliminary study conducted in 2011 had shown the *in vivo* anti-inflammatory activity. The aim of present study was to i) compare immunostimulatory activity of two doses (½ human equivalent dose (½ HED) and HED) to select the optimum dose, ii) investigate immunostimulatory activity of the optimum dose by assessing IgM and IgG antibodies and iii) determine anti-inflammatory activities, by assessing inhibition of rat peritoneal cell infiltration, production of nitric oxide (NO) and reactive oxygen species (O_2^-). Of the two doses (½ HED - 70.65 mg/kg and HED - 141.3 mg/kg), HED resulted in a significantly increased antibody response on day 14 compared to control and ½ HED ($p=0.012$; $p=0.047$ respectively) as measured by sheep red blood cell haemoagglutination and thus, HED was selected as the optimum dose. In the *in vivo* peritoneal cell infiltration assay, HED specifically increased macrophage migration (200.1±20.99 %; $p< 0.001$). HED resulted in significant inhibition of NO and O_2^- production by rat peritoneal cells (77.5±0.7 %; $p<0.001$ and 26.9±2.5 %; $p=0.002$ respectively). Anti-BSA IgM antibodies in HED treated group increased significantly on day 28-35 ($p=0.001$ and $p<0.001$ respectively) as measured by ELISA. In conclusion, this study shows that the HWE of *C. sativum* and *C. fenestratum* possess potent anti-inflammatory activity by inhibiting NO and O_2^- production and enhanced immunostimulatory activity by increasing macrophage migration and inducing IgM production. Therefore, this study scientifically validates the therapeutic use of this combination in Sri Lankan traditional medicine to obtain immunostimulatory effects.

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Abstract No: 27

Involvement of serum reactive nitrogen species and reactive oxygen species in dengue patients in Sri Lanka

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The aim of this study was to assess the serum reactive nitrogen species (RNS) and reactive oxygen species (ROS) levels in dengue patients and to determine whether these levels can be used as prognostic markers for disease severity in dengue infections. Two blood samples (on admission and on discharge) were collected from clinically suspected dengue patients admitted to North Colombo Teaching Hospital (n=75). Dengue infection was confirmed by RT-PCR and IgM ELISA. Thirty dengue fever patients (DF) and twenty dengue hemorrhagic fever patients (DHF) were selected. Age-gender matched healthy control (HC) sera (n=31) were also obtained. Griess and modified Griess assays were done to assess serum nitrite and NOx levels respectively to determine the serum RNS levels. The values were corrected with serum creatinine to normalize for renal function. Serum ROS was assessed using ABTS decolorization assay to determine antioxidant capacity (AOC) and values were corrected by total serum protein content. Corrected serum NOx levels in DHF patients on admission (Mean ± SD, 5.12 ± 2.21 μM/mg) were significantly lower than DF-on admission sera and Healthy controls (7.77 ± 5.17, $p = 0.03$; 6.97 ±3.07, $p = 0.024$ respectively). corrected serum AOC levels in DHF patients on admission (7.67±1.07 μM/mg) were significantly higher compared to other categories (DF on admission, 6.46 ± 0.79 μM/mg, HC, 6.52 ± 0.80 μM/mg; $p < 0001$ and DHF on discharge, 6.53 ± 1.03 μM/mg, $p = 0.004$). These changes may occur due to plasma leakage or higher serum ROS triggering more production of antioxidants. Thus, the assessment of serum NOx and AOC at an early stage have a potential to be used as prognostic indicators for severe dengue infection.

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