Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

Proceedings of the 12th Annual Scientific Sessions of the IBMBB

31st May 2023

Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

Our Vision

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

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Message from the Director – IBMBB

Professor Prasanna Galhena

Professor in Biochemistry and Clinical Chemistry

On behalf of the academics and students at the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo, I would like to extend my warmest welcome to all the delegates and participants for the 12th Annual Scientific Session at IBMBB, University of Colombo.

This is one of the key events at IBMBB, that facilitates an interactive dialog among all the stakeholders in the field of Life Sciences. Annual Scientific Session 2023 primarily focuses on the key issues pertaining to multidisciplinary applied research. Validation of novel molecular diagnostics, exploring novel drug leads, and execution of fundamental applied research are some of the highlights of the Conference.

Annual Scientific Session 2023 will be graced by Prof. Stanley Wijesundara memorial lecture delivered by Prof. Rohan Siriwardana, Consultant Surgeon sharing his experiences in Liver transplantation.

I would like to express my sincere gratitude to the distinguished invited speakers for their presence and contribution to the conference. I also thank all the resource people who have contributed in numerous ways to making the event a success.

Finally, I would like to keep a note of the dedicated team at IBMBB who worked tirelessly in bringing you a productive conference despite all the challenges during this difficult time.

Prof. Prasanna Galhena

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Professor Stanley Wijesundera Memorial Lecture

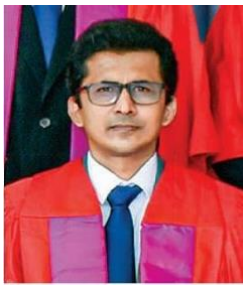
Introduction

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; *Vidyajyothi* Professor Eric Karunanayake, Founder Director, IBMBB & Emeritus Professor of Biochemistry, University of Colombo; Professor Lal Chandrasena, Senior Professor of Biochemistry, University of Kelaniya and Professor E Dilip de Silva, Senior Professor of Organic Chemistry, University of Colombo; Professor Kamani Tennekoon, Senior Professor of Molecular Life Sciences, IBMBB, University of Colombo, Former Director, IBMBB & former Professor of Physiology, Faculty of Medicine, University of Colombo; *Vidyajyothi* Professor Rezvi Sheriff, Senior Professor of Medicine, Kotelawala Defense University, Sri Lanka and Former Senior Professor of Medicine, Faculty of Medicine, University of Colombo; Dr Rajiva de Silva, Consultant Immunologist & President-Allergy and Immunology Society of Sri Lanka; *Vidyajyothi* Professor Harendra de Silva, Emeritus Professor of Paediatrics at the University of Colombo, Honorary Fellow of the Royal College of Paediatrics and Child Health; Senior Professor Sagarika Ekanayake, Professor in Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura; *Vidyajyothi* Professor Vajira H. W. Dissanayake**,** Dean, Faculty of Medicine; Chair and Senior Professor Department of Anatomy, Genetics and Biomedical Informatics University of Colombo.

This year Professor Stanley Wijesundera Memorial Lecture will be delivered by Prof. Rohan Siriwardana, Professor in Gastroenterology and Hepatobiliary Surgery, Faculty of Medicine - University of Kelaniya.

Professor Stanley Wijesundera Memorial Lecture



Liver Transplantation; a dream to a reality

Prof. Rohan Siriwardana

Professor in Gastroenterology and Hepatobiliary Surgery

Faculty of Medicine - University of Kelaniya.

The liver, the largest solid organ in the body, performs a diverse range of functions essential for life. Liver failure leads to suffering and loss of life, and the only method for saving a life once the liver fails is through liver transplantation. This procedure is the ultimate gift of life, but it comes with significant challenges. The first liver transplantation was performed in the United States in 1969, and it took almost half a century for the first liver transplant to be performed in Sri Lanka. The complexity of the surgery, the need for specialized training, and the availability of infrastructure are the primary challenges. However, the sustainability of the surgery relies on the support of many other specialties. The real challenge of liver transplantation is to sustain what has been started and flourish as a team. This talk will explore the journey of liver transplantation over the last 10 years.

Abstract No: OP1

Isolation, purification and activity study of laccase enzyme from *Rigidoporus microporus* isolates

Geethma KS[1], Weerasena OVDSJ[1], Gunaratna LNR[1], Fernando THPS[2]

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

[2]Department of Plant Pathology and Microbiology, Rubber Research Institute of Sri Lanka

Laccase (EC 1.10.3.2 benzendiol: oxygen oxidoreductase) is multicopper oxidase that can catalyze multiple phenolic and non-phenolic compounds by reduction of molecular oxygen to water. Although laccases are widespread among different organisms, from bacterial strains to higher plants, the characteristic properties of fungal laccases have reached more attention in the past decade. Fungal laccases are mainly involved in de-lignification and plant pathogenesis. The white-rot basidiomycete *Rigidoporus microporus* (RM) is considered a significant laccase producer. A total of nine pre-identified RM isolates from different locations in Sri Lanka were screened for laccase production in PDA media with 0.04% guaiacol. Laccase enzyme was expressed in a synthetic liquid media supplemented with guaiacol (a laccase activity inducer). Culture filtrates were harvested, and proteins were precipitated by ammonium sulphate at an 80% saturation level. Laccase production was quantified in different RM isolates using Fast Protein Liquid Chromatography (FPLC). Laccase activity of the isolates were compared using ABTS assay and the decolorization abilities of textile dye (Acid Blue 113). The decolorization percentage of each sample has been evaluated and RM4 showed the highest decolorization percentage (82.16%) at 72 hours. The isolate RM4 that recorded with overall highest laccase activity was purified to homogeneity with size exclusion chromatography on Superose 12HR 10/30 column and the enzyme was further purified using a strong anion exchange chromatography column (HiTrap Q FF). The molecular weight of the purified laccase was approximately 60 kDa as estimated by SDS-PAGE and a native PAGE. Enzyme activity was measured as 857.3 U/L by ABTS assay. Laccase enzyme of RM4 was 66.7-fold purified with a 10.3% yield.

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

Abstract No: OP2

Development of a molecular method to detect adulteration of *Saraca asoca* (Roxb.) Willd. (Ashoka) with Polyalthia longifolia in herbal drug industry

Ravindrakumar S[1], Gunaratna LNR[1], Weerasena OVDSJ[1], Tennakoon TMSG[2]

[1] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo.

[2]Research and Development Center, Link Natural Products (Pvt) Ltd, Malinda, Kapugoda, Sri Lanka

Saraca asoca (Roxb.) Willd is a medicinal plant used to treat many gynecological disorders. However, due to the scarcity of this plant, raw materials of the plant are commonly adulterated with many species and the most commonly used adulterant is *Polyalthia longifolia* (Sonn.) Thwaites. known as False Ashoka. This research focused on developing a cost effective and fast molecular method to detect adulteration of *S. asoca* plant materials with *P.longifolia*. Specific primers were designed from 2 chloroplast genome barcoding regions (matK and rbcL) of *P. longifolia* sequences obtained from the GenBank database and analyzed by using bioinformatics tools. The forward primer for rbcl derived primer was PL-RBCL- 5' ATGGGGCGGGCCCTGGAAAGTC 3' and the reverse primer was PL-RBCL -5' ATACTCCTGAATATGAAACCAAAGATAC 3'. The forward primer for matK derived primer was PL-MATK- 5' GGGTCTCAAATTTCTTTATAGAAGTC 3' and the reverse primer was PL-MATK 5' CTATCAGAATTCGAAAAGTCTCCA 3'. Genomic DNA was extracted from the leaves of both plants using the Phytospin d™ plant genomic DNA extraction kit (Ceygen Biotech). PCR was optimized for specific amplification of each genomic region from *P. longifolia*. PCR was carried out with 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.3 µM forward and reverse primers, 1U of ThermoRead^{TM} Taq Polymerase (CeyGen biotech) and 1x buffer. The PCR program was, the initial denaturation at 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 63°C for 1 minute, extension at 72°C for 1 minute and a final extension at 72°C for 4 minutes. Both primers sets were combined to develop a duplex PCR assay which resulted in 386 bp amplicons for rbcL and 545 bp amplicons for matK respectively. The developed duplex PCR assay could detect *P. longifolia* in DNA extracted from mixed samples of 0.01% adulteration, demonstrating the high specificity and sensitivity of the PCR assay.

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

Abstract No: OP3

Effect of *Exobasidum vexans* Massee on flavonoid production and expression of selected flavonoid biosynthetic enzymes during the early stage of blister blight disease development on tea (*Camellia sinensis* [L.] O. Kuntze)

Anuforo C[1], Punyasiri PAN[1], Weerasena OVDSJ[1], Gunaratna LNR[1], Siriwardana RSGTN[1], Sinniah GD[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo.

[2]Plant Pathology Division, Tea Research Institute of Sri Lanka

The effect of *Exobasidum vexans* infection on flavonoid production and expression of genes of selected flavonoid biosynthetic enzyme dihydroflavonol 4-reductase (DFR), anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR) during the early stage of blister blight development on tea leaves *using* selected cultivars TRI 2043, CY9, TRI2024 and TRI2025 was studied with HPLC for catechin quantification and relative gene expression (Ct ($2^{-\Delta\Delta Ct}$) method) with qPCR. Healthy samples were compared with early stages of disease development for each cultivar and the results revealed that the tested cultivars had lower expression of LAR in the early stage of the infection. Early stage of disease on TRI 2043 recorded high expression of DFR and ANR with relative fold change of 210.05, and 4.38 respectively when compared to that recorded in the healthy leaf sample and later stage of blister blight. CY9 had a higher expression of DFR and ANR in the later stage of blister blight compared to the early stage. High expression of DFR leads to accumulation of leucoanthocyanidins. The general low expression of LAR indicates an overall low content of (-)-Flavan-3-ols, while high expression of ANR lead to an accumulation of (+)-Flavan-3-ols, when the HPLC and gene expression data are compared for ANR, the susceptible cultivar TRI 2024 and TRI 2025 despite having high expression of ANR, had a preferential accumulation of Epigallocatechin gallate (EGCG) (antimicrobial property mainly against *Colletotrichum fructicola* infection and not *Exobasidum vexans* infection). In contrast, there was a significantly higher accumulation of Epicatechins (EC) and Epicatechin gallate (ECG) in the TRI 2043 cultivar (resistant) during the early stage of blister blight than other tested cultivars (using One way ANOVA and Tukey's multiple comparisons post hoc test with significant difference at $p<0.05$). Probably the increased levels of Epicatechins and Epicatechin gallate during early stages of blister disease may have a role in resistance of *Camellia sinensis* to *Exobasidum vexans.*

This work was supported by the Association of Commonwealth University Research Grant FE-2020-239 and constitutes a part of the MSc studies of CA.

Abstract No: OP4

Morphological and molecular characterization of *Leucinodes orbonalis* Guenee (Brinjal Shoot and fruit borer) from selected locations in Sri Lanka

Gunachandra KSR, Suvanthini T[2], Gajapathy K[1]

[1]Department of Zoology, Faculty of Science, University of Jaffna, Jaffna.

[2]Department of Agricultural Biology, Faculty of Agriculture, Kilinochchi.

Brinjal shoot and fruit borer (*Leucinodes orbonalis*) is one of the most detrimental pests affecting brinjal cultivation. Understanding existing morphological and genetic variations based on geography and the host variety are important in developing efficient pest management strategies. The present study was designed to assess the morphological and genetic variations of *L.orbanalis* infesting brinjal varieties; namely, HORDI Lena iri, Thinnaweli purple, Plastic cultivar and Eerku vellai from randomly selected locations in Anuradhapura, Badulla, Jaffna, Kandy, Kilinochchi, Monaragala, Polonnaruwa and Puttalam districts. The morphological variations of all life stages emerging from infested brinjals collected from all locations were studied independently in lab-rearing colonies. The genetic variations of *L.orbanalis* were investigated using DNA sequences obtained for D3 region of 28S rDNA and cytochrome c oxidase subunit I (COX I) genes. There were no morphological variations observed among life stages that emerged from the colonies. In terms of developmental period, the only difference was observed during the transformation of larval to pupal stage, which exhibited 11.98 days at 26-28^{0}C and 6.04 days at 34-36^{0}C. The pest spent significantly lower time in HORDI Lena Iri (1.98±0.35 days) and higher time in Eerku vellai (2.00±0.00 days). This could be because of variations in seed arrangement and flesh texture. The phylogenetic tree constructed using the COX I sequence (681 bp) revealed five unique groups, whereas no variation was observed in the D3 sequences. Jaffna, Chavakacheri, Kilinochchi, and Monaragala populations were identified as four distinct clades. The populations of Bandarawela, Anuradhapura, and Kaithady were grouped together. Among them, Kaithady population from the Plastic cultivar was separated distinctly from the Bandarawela and Anuradhapura populations infesting HORDI Lena iri. Monaragala population showed higher sequence divergence and more distantly related to all other populations. According to the study, molecular variations were noticed among different populations of *L. orbanalis*, which can be helpful to design a breeding programme to develop a resistant brinjal variety.

Abstract No: OP5

A Study on Allelic and Expression Differences of Selected Salt Responsive Genes in At 354 and Bg 352 Rice Varieties

Peiris DPRP[1], Weerasena OVDSJ[1], Gunaratna LNR[1], Kottearchchi NS[2]

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

[2]Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka

Rice is a world famous staple food. In Sri Lanka most of the crop loss is due to biotic and abiotic stress. Salinity is a major cause of abiotic stress . A previous study has identified two salinity responsive candidate genes in Oryza sativa known as Os11g0655900 and Os12g0624200. At 354 is salinity resistant and Bg 352 is salinity susceptible rice variety. According to a previous study based on Next Generation Sequencing (NGS), there are 6 bp and 3 bp insertions in Os11g065900 and Os12g0624200 genes respectively whereas those are absent in Bg 352. Therefore, this study was conducted to observe the expression differences in Os11g0655900 and Os12g0624200 genes under different saline conditions and to confirm the above mutations as detected by NGS, using Sanger sequencing. Expression of those two genes in At 354 and Bg 352 were observed using quantitative Real Time PCR (qPCR) under different salinity levels. Results indicates, the expression of Os11g0655900 gene in At 354 increased after salinity exposure and the highest expression was observed after 5 days. The expression of Os12g0624200 gene in At 354 increased after salinity exposure and the highest expression was observed after two days of exposure. Both genes in Bg 352 showed a reduction in expression than in the control. According to the Sanger sequencing of Os11g0655900 gene it has been shown that At 354 consists of the 6 bp insertion which is absent in Bg 352. Also, 3 bp insertion in At 354 was observed only in the Os120624200 gene as observed by NGS. Therefore, it can be suggested that the mutations observed in At 354 may contribute to salinity responsive increase in expression of above genes and thereby salinity resistance of At 354. However, further research is needed to confirm the findings of this study.

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

Abstract No: OP6

RNAi-mediated yEGFP gene knockdown in *Pichia pastoris*

Dharmarathna CS[1], Gunawardena YINS[3], Dassanayake RS[1†], Shashi Kumar[2], Hettiarachchi C[1]

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

[2]International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi, India

[3]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Ragama

Gene knock-down in living organisms for therapeutic purposes is becoming a widely studied area with the uprising advancements in current technologies. RNA interference (RNAi) is being used to suppress genes related to infectious diseases as well as genetic diseases in humans such as HIV and HPV-related cancers. The present study was conducted as a model study focused on monitoring the knock-down of yEGFP gene in *Pichia pastoris* which has an artificially reconstituted human RNAi pathway. The objective of this study was to test whether the reconstituted human RNAi pathway is functional in *P. pastoris*, using yEGFP as a reporter gene. For this purpose, two genetically modified *P. pastoris*; yEGFP expressing reporter strain, and both yEGFP and RNAi expressing ADT strain were developed. The fluorescence expression of each strain was visualized using the FITC filter in Nikon ECLIPSE N*i*-U upright microscope. Photographed fluorescent intensities exhibited by yEGFP in *P. pastoris* cells were analyzed using Image J software. The wild-type *P. pastoris* was used as the control. The average mean fluorescent intensity of the yEGFP reporter strain was 40.148 ± 13.824. RNAi expressing ADT strain reported 5.969 ± 1.700 mean fluorescent intensity. The control wild-type strain showed 0.031 ± 0.002 average mean fluorescent measurement. Taken together, these results showed low mean fluorescent in ADT strain compared to the yEGFP expressing reporter strain due to the RNAi-mediated knocked down yEGFP gene in ADT strain. In conclusion, a successful knockdown of yEGFP gene expression can be observed in RNAi reconstituted *P. pastoris*. In future aspects, RNAi reconstituted *P. pastoris* has the potential to be used to study the gene knockdown by replacing the yEGFP gene with any gene of interest related to human diseases.

[†]This article is dedicated to the memory of Prof. Ranil Samantha Dassanayake who passed away tragically while this research was being conducted. This is one of his last works.

Abstract No: OP7

Characterization of mealybug species associated with different host plants using morphological and molecular methods

Sathsarani KWI[1], Suvanthini T[1], Gajapathy K[2]

[1]Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna

[2]Department of Zoology, Faculty of Science, University of Jaffna

Mealybugs are significant pests with a wider host range, such as okra, tomato, turkey berry, coffee, brinjal, guava, papaya, pineapple, soursop, cassava, croton, and shoe flower. The aim of the research was to study the variation in mealybug species associated with different host plants. Mealybug samples (n = 180) were collected from selected fields in Kilinochchi, Jaffna, and Monaragala districts. They were morphologically identified based on published taxonomic keys. PCR was performed to confirm the species by amplifying the D3 region of the 28s rDNA. The sequences were analyzed for variation, and the phylogenetic tree was constructed using maximum-likelihood method. The field study revealed that mealybugs exist as a complex associated with ant species such as *Oecophylla smaragdina* and *Technomyrmex albipes*, mealybug destroyers, and sooty mold, which itself caused enormous damage to plants in terms of quantity and quality. Ten mealybug species belonging to seven genera were identified. Those were; *Phenacoccus solenopsis, Phenacoccus solani, Phenacoccus manihoti, Planococcus minor, Planococcus lilacinus, Ferrisia virgate, Coccidohystrix insolita, Paracoccus marginatus, Pseudococcus vibruni,* and *Rastrococcus mangiferae.* Among them, *P. solenopsis* was the most abundant species recorded in okra, tomato, turkey berry, and hibiscus crops, while *F. virgata* was the second most abundant mealybug species recorded in guava, brinjal, and croton crops. *P. solenopsis, F. virgate, P. minor,* and *P. lilacinus* were found in more than one host plant, confirming their preference for multiple host plant species. DNA sequence data were consistent with morphological identification. Phylogenetic tree analysis revealed that one *P. solenopsis* (Accession no. ON787841) was clustered separately from the rest of the same species identified (ON787838, ON787840, and ON787844), which were clustered with sequences from China and the USA. This might be a unique genetic variant belonging to Sri Lanka. This study contributes to understanding the species variation, host preference and other insects associated with mealybug species, which might be important in designing management strategies.

Abstract No: OP8

Association of *PNPLA3* gene variants with non-alcoholic steatohepatitis (NASH) related hepatocellular carcinoma (HCC) patient cohort in Sri Lanka

Samarasinghe SASM[1], Hewage AS[1], Siriwardana RC[2], Tennekoon KH[1], Niriella MA[3], Silva SD[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo.

[2]Department of Surgery, Faculty of Medicine, University of Kelaniya. [3]Department of Medicine, Faculty of Medicine, University of Kelaniya

NASH is the hepatic manifestation of metabolic syndrome. Worldwide, NASH is one of the major causes of HCC. Apart from the environmental factors (unhealthy diet, lack of exercise, and weight gain), genetic variants in the key genes of lipid metabolism can predispose to the development of NASH-related HCC. Three single nucleotide polymorphisms (SNPs) (rs738409, rs2281135, and rs2294918) in the *PNPLA3* gene have been linked with the progression of NASH. The aim of the current study was to analyze the genetic association of NASH-related HCC with respect to three common SNPs in the *PNPLA3* gene in a cohort of Sri Lankan patients. A group of 48 patients with NASH-related HCC and 25 age and gender-matched healthy controls were genotyped. Primer extension-based SNP analysis was used to genotype all three polymorphisms. In the *PNPLA3* gene, the most common genotype and allele were CG (79%) and C (60.4%) for the rs738409 polymorphism, GA (73%) and G allele (59.4%) for the rs2281135 polymorphism and GG (65%) and G (77%) for the rs2294918 polymorphism. Collectively, 77.1% of our study cohort were carriers of all three *PNPLA3* variants. Two out of three tested SNPs, *PNPLA3* rs738409 (Relative risk=1.80, 95% CI: 1.13-2.866; P=0.002) and *PNPLA3* rs2281135 (Relative risk=1.52, 95% CI: 0.988-2.343; P=0.027) showed significant associations with NASH related-HCC. The present study shows *PNPLA3* (rs738409, rs2281135) variants were significant genetic determinants of NASH-related HCC in the Sri Lankan population.

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Abstract No: OP9

Alpha-hederin modulates β-catenin pathway target genes and induces caspase dependent programmed cell death in breast cancer stem cells

Saliu TP [1], Seneviratne NN [1], Faizan M [1], Rajagopalan U[1], Adhikari A[2], Senathilake KS[1], Galhena PB[1], Tennekoon KH[1], Samarakoon SR[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka

[2]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Intriguing evidence demonstrates that breast cancer stem cells (bCSCs) play a vital role in tumor cell proliferation, metastasis, recurrence and chemoresistance in breast cancer. β-catenin, one of the frequently over activated proteins in breast cancer cells help maintaining the bCSCs population in the cancer cell mass by constitutive activation of Wnt/ β-catenin pathway. Interaction of T-cell factor (Tcf) DNA binding proteins and β-catenin protein play a critical role in the activation of proliferative genes in bCSCs in response to upstream Wnt/β-catenin signaling. For the identification of novel and effective Wnt/ β-catenin pathway inhibitors, a library of 100 natural compounds was docked *in silico* against Tcf binding site of β-catenin. Protein ligand complexes with binding energy less than -7 kcal/mol were investigated for protein-ligand binding interactions. Stability of the protein-ligand complexes was studied by performing 100 ns molecular dynamics (MD) simulations. Alpha-hederin (AH) with binding affinity of -8.2 kcal.mol^{-1} having a stable MD profile was studied in vitro for anti-proliferative and apoptotic effects using bCSCs isolated from a triple-negative breast cancer cell line (MDA-MB-231). Further, oral bioavailability and possible toxic effects of AH were predicted using *in silico* tools. AH significantly decreased the viability of bCSCs which was evaluated by WST-1 assay. Apoptosis in bCSCs was induced with treatment of AH which resulted in a potent increase in caspase3/7 activity and nuclear DNA fragmentation. AH downregulated the transcription of Wnt/β-catenin downstream target genes, *CD44* and *Cyclin D1* while inducing the transcription of the tumor suppressor gene *p53*. Results of the drug-likeness study indicated that AH possesses acceptable overall drug-likeness. AH is widely known as an inhibitor of the proliferation of various cancer cells, current *in vitro* study demonstrates the potential anti-cancer activities; anti-proliferative, apoptotic effects and regulation of Wnt/ β-catenin pathway in bCSCs by AH inhibitors.

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Abstract No: OP10

Cytotoxicity of endophytic fungi strains of *Rhizophora mucronata* using human hepatocellular carcinoma (HepG2) cell line

Thusyanthan J, Wickramaratne NS, Rajagopalan U, Senathilake KS, Samarakoon SR, Tennekoon KH, Thabrew MI

Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo

Endophytic fungi have been considered as a potential source of novel anticancer therapeutics since most of the endophytic fungi have not been explored for their chemical constituents. The present study was carried out to screen cytotoxicity of extracts of selected endophytic fungal strains isolated from *Rhizophora mucronata* (a mangrove plant) against human hepatocellular carcinoma cells (HepG2). Five endophytic fungal strains (Rhm/l/A4/1, Rhm/R/A3/0, Rhm/R/A4/0, Rhm/R/A2/0 and Rhm/R/A4/1) were isolated from the leaves of *R. mucronata* and extracted into dichloromethane and methanol to assess cytotoxicity against HepG2 cells by using Sulforhodamine B (SRB) assay. Thymoquinone was used as the positive control (IC_{50} = 6.15 µg/mL). Extract with potent cytotoxicity on HepG2 was used to test on normal liver epithelial cell line (THLE-3) for toxicity and further investigated for its apoptotic effects by (a) analyzing morphological changes of HepG2 and (b) determining caspase 3/7 and lactate dehydrogenase (LDH) activities. Active cytotoxic extract was further investigated for radical scavenging activity [by using 2, 2-diphenyl-1-dipicrylhydrazyl (DPPH) assay] and total phenolic content (TPC). Dichloromethane extract of Rhm/R/A3/0 (DRA3) exhibited a lower cytotoxicity (IC_{50} =110.4 µg/mL) against HepG2 cells after 24 hours of incubation but potent cytotoxicity after 48 and 72 hours of incubation (IC_{50} = 13.3 and 10.5 µg/mL respectively). Further DRA3 showed a lower toxicity (IC_{50} =180.2 µg/mL) against THLE-3 cells compared to HepG2 cells after 48 h exposure. DRA3 showed comparatively less radical scavenging activity (EC_{50} > 100 µg/mL) and increased LDH activity in cell lysate compared to the standard nicotinamide adenine dinucleotide (NADH). Total phenolic content was less than 50 mg/g of gallic acid equivalents. Morphological changes of apoptosis were evident in DRA3 treated HepG2 cells with a significant ($p<0.0001$) time and dose dependent increase in caspase 3/7 activities. Current study provides comprehensive evidence that DRA3 is a potent source of anti-cancer drug leads against hepatocellular carcinoma with less toxic to normal liver cells.

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Abstract No: OP11

Initial validation of analyte-specific fluorescence in-situ hybridization probes targeting MYC, BCL2, and BCL6 gene rearrangements

Karunathialaka ST[1], Kaluarachchi NP[2], Williams HAS[3], Abeygunasekara PH[4], Senathilake NHKS[1], Galhena BP[5]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo
[2] Lanka Hospital Diagnostics, Sri Lanka . [3]Department of Pathology, Faculty of Medicine, University of Kelaniya. [4]Department of Pathology, National Cancer Institute, Maharagama
[5] Department of Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Kelaniya

B-cell lymphomas (BCL) are malignant, heterogeneous group of lymphoproliferative disorders considered as a sub-type of non-Hodgkin lymphomas (NHL). BCLs arise from different stages of the differentiation process of B-cells with a high diversity of malignancy, thus making their diagnosis and management extremely challenging. MYC, BCL2, and BCL6 gene rearrangements are closely associated with BCLs and usually appear as composite rearrangements. Molecular cytogenetic techniques such as karyotype, immunohistochemistry (IHC), and fluorescence in-situ hybridization (FISH) have been employed in detecting vital molecular rearrangements of BCLs. However, the detection of these molecular anomalies depends on the sensitivities of each technique. Interphase FISH has emerged as an effective, highly sensitive, and reproducible molecular cytogenetic technique. FISH assays usually employ non-FDA-approved, analyte-specific reagents (ASR) that should be validated prior to their use in clinical practice. In the present study, an extensive probe colocalization was carried out as part of the validation process. Sequential G to FISH banding was performed using dual fusion probes targeting t(8;14) [MYC/IGH], t(14;18) [IGH/BCL2], and a break-apart probe targeting 3q27 [BCL6 rearrangement] separately on twenty metaphases of peripheral blood samples obtained from healthy individuals. Since a single metaphase consists of two alleles for each chromosome, a total of forty hybridization signals for a single chromosome was accommodated in the analysis. Hybridization signals were captured coordinated with previously karyotyped metaphases. The outcome of the present study, clearly indicated that all three sets of probes accurately hybridized with the intended locations in all forty loci tested for each chromosome, thus reporting 100% probe specificity. The fulfillment of 100% probe specificity is one of the prime prerequisites prior to their use in clinical diagnostics. Therefore, the present study justifies the use of the above analyte-specific FISH probes for the remaining validation process for their clinical diagnostic use.

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Abstract No: OP12

Comparison of uniparental inheritance in a cohort of Adivasi inhabiting in the Ratugala area

Fernando AS[1] , Bandyopadhyay E[2], de la Fuente Castro C[2], Witonsky D[2], Karunanayake E[1], Somadeva R [3], Rai N [4],Tennekoon KH[1], Raghavan M[2], Ranasinghe R[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo [2]Department of Human Genetics, University of Chicago . [3]Postgraduate Institute of Archeology, University of Kelaniya . [4]Birbal Sahni Institute of Palaeosciences, Lucknow, India

Sri Lanka holds the evidence for the oldest skeleton of the anatomically modern human who lived in the South Asian region; known as the 'Balangoda Man'. The ancestors of Sri Lankan Adivasi share morphology with the Balangoda Man, and hence the Adivasis are believed to be the earliest inhabitants of Sri Lanka. In the current study, we compared the maternal and paternal ancestry of Ratugala Adivasi to obtain a better understanding of the uniparental inheritance of the present-day descendants of the early inhabitants of Sri Lanka. DNA was extracted from five Ratugala Adivasi individuals [males (N=4) and females (N=1)] using the Qiagen investigator kit. The whole genome was sequenced through Illumina Novaseq 6000 platform. The mitochondrial genome was extracted using samtools and bcftools upon quality checking. The mitochondrial haplogroup (mt hg) was determined via Haplogrep2 and Y haplogroup (Y hg) through yHaplo software. Out of the study individuals, four were assigned to R30b2a mt hg and one individual to the U7a2 mt haplogroup. The high prevalence of R30b2a mt hg was consistent with the previously published data. When analyzed for Y hg, two individuals reported R2a (M124), one each with R1a1a1b2a2a (Z2125) and H1b1. The individual reported with U7a2 mt hg was assigned to R1a1ab2a2a (Z2125) Y hg and those lineages are associated with near east (pre-Steppe) lineages while the rest of the maternal and paternal lineages are associated with autochthonous early Indian lineages. The geographical derivations of the reported maternal and paternal haplogroups suggest a possible influence by the early Near East and Indian lineages on the genetic constitution of Adivasis ancestors of the studied individuals.

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Abstract No: OP13

Length heteroplasmy analysis in the C-stretch of Mitochondrial DNA hypervariable region I in Sinhalese, Sri Lankan Tamil and Vedda populations in Sri Lanka

Welikala AHJ, Fernando A, Kotelawala JT, Tennekoon KH, Ranasinghe R
Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Numerous migrations from neighboring countries have significantly affected the genetic diversity of Sri Lankan populations since prehistoric times. However, inadequate genetic information on these populations makes it challenging to understand their genetic relatedness and gene flow patterns. Some studies have considered the length heteroplasmy in the C-stretch of mitochondrial DNA (mtDNA) hypervariable region I (HVS-I) (base pairs 16180-16193) as a reliable genetic marker to explore genetic differences among distinct populations. Hence, the present study has focused on analyzing C-stretch variations in three major contemporary populations in Sri Lanka; Sinhalese, Sri Lankan Tamil, and Adivasi (Vedda) populations. Thirteen different South Asian populations were also included in the study together with the Aboriginal populations in Australia and Papua New Guinea to understand possible genetic links of Sri Lankan populations to neighboring nations. Sequences of mtDNA HVS-I were analyzed for C-stretch variations in all study individuals (N= 1352). Nineteen different haplotypes were identified. However, (C_5TC_4) haplotype was the most significant in all populations. In addition, approximately 13% of the study population exhibited C-stretch length heteroplasmy. Out of them, C_{10} and C_{11} variations displayed high frequencies. All study populations displayed relatively low genetic diversities ranging from 0.00 (Adivasis) to 0.64 (Australian and Papua New Guinea aborigines). No significant differences between Sinhalese and Sri Lankan Tamils were observed regarding diversity measures. According to the pair-wise distance matrix (Fst) and Multi-Dimensional scaling (MDS) plot, the Adivasi (Vedda) population clustered separately from other study populations. Sinhalese clustered together with Bangladesh Bengalis and Telegu populations in India while Sri Lankan Tamils with the Jammu-Kashmir populations. The results indicate that C-stretch variations in mtDNA HVS-I region are useful genetic markers to understand the genetic relationships of distinct populations.

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Abstract No: OP14

Species delimitation of blindsnakes by molecular phylogenetic analysis of mitochondrial DNA sequences

Wickramasinghe N[1,2], Wickramasinghe LJM[2], Tennekoon KH[1], Samarakoon SR[1], Vidanapathirana DR[2], Gower DJ[3]

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

[2]Herpetological Foundation of Sri Lanka

[3]Natural History Museum, London

Blindsnakes belong to the infraorder Scolecophidia, comprising 4 families: Leptotyphlopidae, Typhlopidae, Gerrhopilidae and Xenotyphlopidae. Sri Lanka has a known fauna of 10 species of Scolecophidians, all but two are endemic; eight are *Indotyphlops*, and two *Gerrhopilus*, representing two families; Typhlopidae and Gerrhopilidae. No dedicated surveys have been carried out in Sri Lanka on blindsnakes for over 72 years. The current study evaluated the species diversity of Sri Lankan blindsnakes using a single-locus species delimitation method, proposing species limits for the two families: Typhlopidae and Gerrhopilidae. The entire island of Sri Lanka has been considered as the study area in the current study. Field sampling was opportunistic. Whole-genomic DNA was extracted from tissues of 79 specimens; we present here the results obtained for 56 samples (49 Typhlopidae + 7 Gerrhopilidae) of a selected part of a single mitochondrial, Cytochrome b gene (*cyt b* ~ 738 bp amplified), through single-locus species delimitation. Preliminary phylogenetic analyses were run, treating the two families separately, using Maximum Likelihood and Bayesian Inference methods; utilizing IQ tree and MrBayes software respectively. Based on preliminary analyses, five major monophyletic groups were recognized for the entire 56 sequences of *cyt b*. For each of these assembled groups pairwise distances were calculated using MEGA X. Two different tree-based species delimitation methods; Poisson tree processes (PTP: Zang et al., 2013) and Multi-rate poisson tree processes (mPTP: Kapli et al., 2017), were used for each of the major clades. Results obtained via tree-based species delimitation methods and conventional methods were integrated to obtain final conclusions, that indicated three distinct species for Gerrhopilidae, at least 9 distinct species for Typhlopidae, and the probable re-discovery of all but one (*Indotyphlops vaddae*) of the currently known species diversity of the island. These findings will facilitate site-based conservation actions; and updates to the IUCN Red List.

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Abstract No: OP15

In silico prediction and in vitro validation of new anti-aging natural compounds

Hennadige ND, Senathilake NHKS, Samarakoon SR, Galhena BP

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

Aging is an inevitable process where the bodily functions necessary for survival deteriorates. The characteristics of aging are affected by all living organisms. Aging is a complex process influenced by genetic and environmental factors that affect the physiological pathways and is associated with increasing susceptibility to conditions such as neurodegenerative diseases, cancer and heart diseases. In this study, a compound library of 22 natural compounds were screened in silico by molecular docking and molecular dynamics against anti-aging protein drug targets that are SIRT1, SIRT6, MMP9, MMP2, hyaluronidase, collagenase and elastase. High binding affinity was observed in; alpha-hederin (-6.7 kcal/mol) for SIRT1 protein target, epigallocatechin (-9.0 kcal/mol), K-3 (-10.3 kcal/mol) and mangiferin (-8.7 kcal/mol) for SIRT6 protein target, epigallocatechin (-9.3 kcal/mol) and K-3 (-10.3 kcal/mol) for MMP9 protein target, epigallocatechin (-9.3 kcal/mol), K-3 (-10.3 kcal/mol) and DC-3B (-8.6 kcal/mol) for MMP2 protein target, K-3 (-8.2 kcal/mol) and DC-3B (-8.1 kcal/mol) for hyaluronidase protein target, K-3 (-8.2 kcal/mol) and DC-3B (-7.8 kcal/mol) for collagenase protein target, K-3 (-6.8 kcal/mol) and DC-3B (-6.4 kcal/mol) for elastase drug target. The following compounds were analyzed in vitro since there were no findings based on all the protein anti-aging targets selected in this study. In vitro cytotoxic effects on the selected compounds based on the high binding affinity in molecular docking were evaluated by Sulforhodamine B (SRB) assay (Alpha Hederin IC50 13.29 µM, epigallocatechin IC50 19.71 µM, K-3 IC50 28.46 µM, DC-3B IC50 29.35 µM, mangiferin IC50 135.9 µM). The anti-oxidant potential of these compounds were investigated by, α-diphenyl-β-picrylhydrazyl (DPPH) assay and glutathione-s-transferase (GST) activity assay. Epigallocatechin (EC50 of 36.27 ± 2.62 µg/mL) and mangiferin (EC50 of 43.95 ± 3.23 µg/mL) showed relatively high DPPH scavenging activity. Elevated GST activity was observed in epigallocatechin at 1.25 µg/mL (0.1436 ± 0.0054 U/mL), mangiferin at 20 µg/mL (0.1442 ± 0.0031 U/mL) and DC-3B at 5 µg/mL (0.1381 ± 0.0082 U/mL).

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

Abstract No: OP16

Virtual Screening and Molecular Dynamics Based Identification of Bismahanine as a Potential Anti-Aging Compound

Mishal MFM, Senathilake KS, Samarakoon SR

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

Since the middle of the 19th century, human life expectancy has been steadily increasing in many parts of the world, including Sri Lanka. At the age of 60 or older, individuals become more susceptible to chronic illnesses with a rising burden of multimorbidity. Hence, the identification of new anti-aging compounds that might be present in common herbs and edible plants will open up avenues for formulating effective and readily available anti-aging formulations. To identify new anti-aging phytochemicals, a library of 1426 plant secondary metabolites was constructed in-silico based on the previous research work of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB) and by carrying out database and literature searches. The phytochemical library thus constructed was screened using AutoDock Vina (Lamarckian algorithm) in PyRx software. It was screened against validated anti-aging drug targets, mTOR1 and sirtuin-1. Absorption, distribution, metabolism, excretion, and toxicity (ADMETox) parameters were predicted using the FAFdrug4 online server to determine the drug likeliness of identified potential anti-aging compounds. To verify the findings, molecular dynamic simulations were carried out for potential hits in Desmond. Possible other targets of the identified potential anti-aging compounds were predicted by target fishing and a network pharmacology approach using the String database. Based on the binding affinity values, 35 hits were selected for interaction analysis and ADMETox predictions. Out of the 35 compounds, bismahanine from *Murraya koenigii* was predicted to have very high anti-aging potential by eliciting the required binding stability. Molecular dynamics simulations confirmed that the complexes formed by bismahanine with mTOR1 and Sirtuin-1 were stable up to 100 ns in an aqueous environment. Target fishing and network pharmacology analysis provided limited evidence for potential off-target effects of bismahanine. Overall results indicate that bismahanine may be a natural compound with potent anti-aging properties. However, results should be experimentally validated through in-vitro and in-vivo experiments.

Abstract No: OP17

The *in vitro* effect of *Mikania cordata* aqueous leaf extract on wound healing

Vijithsingh NN, Anuradha K, Handunnetti S, Fernando N, Senarath K

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

Traditional and modern wound-healing methods come in a variety of forms, each with its own level of clinical acceptance, efficacy, and limits. Traditional medicines that are used most frequently to treat skin lesions are those which are derived from herbs. The current *in vitro* study focuses to assess the wound healing ability of aqueous extract (AE) of *Mikania cordata*. *M. cordata* is an Asteraceae plant with a vine that grows on trees or on the surface of the soil. *M. cordata* has been used for its antibacterial, anti-inflammatory, and wound-healing properties. In this study, an AE was produced using fresh leaves and EA. hy926 cell line was used to examine the capacity to heal wounds compared to the positive control Allantoin. Cells were treated with a series of concentrations of AE of *M. cordata* ranging from 1000 – 3.905 µg/ml to determine the non-toxic concentrations using SRB and MTT assays. Results showed more than 80% of viability and overall cell functionality in concentrations 1000 – 3.905 µg/ml. Therefore, the scratch assay was performed using 500, 250, and 125 µg/ml concentrations to examine the wound healing ability of the AE. The cells treated with 125 µg/ml of AE for 48 hours displayed the highest percentage of wound closure (70%) compared to the untreated cells ($P \leq 0.001$). Further, the Griess assay was performed to investigate the effect of AE of *M. cordata* on nitrite levels produced by the scratched cells and results demonstrated that the cells exposed to 125 µg/ml of AE of *M. cordata* for 48 hours displayed 41% reduction compared to positive control ($P \leq 0.001$). Hence, the result of this study suggests that the AE of *Mikania cordata* may have wound-healing properties, indicating the need of further studies to prove its observed activity.

Abstract No: OP18

Cancer stem cell targeted *in vitro* anti-cancer activity and acute *in vivo* toxicity studies of a diterpene isolated from *Caesalpinia pulcherrima*

Wijerathne PKSK, Saliu TP, Rathnayake RK, Rajagopalan U, Senathilake NHKS, Samarakoon SR

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

Cancer stem cell (CSC) subpopulation present in different cancer types plays a key role in recurrence, metastasis, and chemo-resistance. Current anti-cancer drugs do not effectively eliminate CSCs. As an effort to identify CSC targeted drug leads, a diterpene compound of traditionally used anticancer medicinal plant *Caesalpinia pulcherrima* has been computationally predicted as an anti-CSC drug lead that can down regulate Wnt/β catenin pathway that is essential for the maintenance of CSCs. In the current study, the abundance of the said diterpene was investigated in different parts of *C. pulcherrima* using thin layer chromatography (TLC) and the diterpene was isolated from the root bark of the plant. The structure of the compound was confirmed by analyzing proton and carbon nuclear magnetic resonance (NMR) spectra. Wnt/β catenin pathway modulatory activity and anti-proliferative activity of pure diterpene were separately investigated by exposing the diterpene to *in vitro* cultured breast CSCs at different concentrations followed by RT qPCR gene expression assays and Sulforhodamine B (SRB) assay respectively. SRB assay was then conducted for 19 other cancer cell lines after exposing each cell line to pure diterpene. Acute *in vivo* toxicity of the pure diterpene was investigated in Wistar rats according to the Organization for Economic Cooperation and Development (OECD) guideline 420. Strong anti-proliferative activity of the diterpene was observed for breast CSCs (IC_{50} = 49.18 μM), triple negative breast cancer (IC_{50} = 4.905 μM), gastric adenocarcinoma (IC_{50} = 0.99 μM), hepatocellular carcinoma (IC_{50} = 3.101 μM) and ovarian adenocarcinoma (IC_{50} = 3.987 μM). Diterpene modulated Wnt/β catenin pathway target gene expression indicating its ability to effectively down regulate the Wnt/β catenin pathway. *In vivo* toxicity signs were not observed up to the highest dose tested (300 mg/kg) and the diterpene was stable under tested conditions indicating the potential of the diterpene as a good drug lead.

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Abstract No: OP19

Isolation of a potential anti-cancer compound from *Mangifera zeylanica* leaves and investigation of its effects

Perera AADN[1], Samarakoon SR[1], Ediriweera MK[2], Tennekoon KH[1]

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

[2] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo

Lung cancer has been estimated to cause the highest number of cancer deaths in 2023. Of two subtypes of lung cancer, Non-Small Cell Lung Cancer (NSCLC) is the most frequently reported. Currently used treatment options for NSCLC are chemotherapy, radiotherapy and surgery. However, chemo- and radio- therapies result in severe side effects. Therefore, invention of new treatment strategies for NSCLC is timely needed. Investigations on plant derived compounds is becoming a promising approach for discovering potential anti-cancer drug leads. *Mangifera zeylanica* (family: Anacardiaceae) is a plant endemic to Sri Lanka and used for cancer treatment in traditional medicine. We have previously reported that the chloroform extract of *M. zeylanica* leaves is cytotoxic to non-small cell lung cancer cells (NCI-H292) with less cytotoxicity to normal lung fibroblasts. In the present study we isolated an active compound (B-II-c-a) from the chloroform extract using silica-gel column chromatography, size exclusion chromatography and reversed phase preparative high performance liquid chromatography. Cytotoxicity of B-II-c-a on NCI-H292 cells was evaluated using Sulforhodamin B (SRB) assay. The effects of B-II-c-a on cell migration and colony formation was investigated using wound healing assay and colony formation assay respectively. Assessment of potential apoptotic effects of B-II-c-a was carried out using Ethidium Bromide/Acridine Orange (AO/EB) staining. The compound B-II-c-a showed cytotoxic effects on NCI-H292 NSCLC cells and MRC-5 normal lung fibroblast cells following 24h exposure (IC_{50} of 3.22 µg/mL and 7.83 µg/mL respectively). Moreover B-II-c-a resulted in inhibition of colony formation and cell migration. AO/EB staining revealed that B-II-c-a induces apoptosis in NCI-2H92 cells. Structure determination of B-II-c-a is currently in progress.

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Abstract No: OP20

Lactone ring enhances anti-breast cancer activity of three structurally related compounds isolated from *Gardenia crameri*

Wickramaratne NS[1], Thusyanthan J[1], Adhikari A[3], Rajagopalan U[1], Tennekoon KH[1], Karunaratne DN[2], Samarakoon SR[1]

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

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

[3]Central department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Gardenia crameri is an endemic plant used in medical applications to treat various diseases in Sri Lankan traditional medicine. The *Gardenia* genus has also been reported to have anti-cancer properties. Therefore, this study aims to identify compounds from *G. crameri* with anti-breast cancer properties. The isolation was performed on exudate from the buds using chromatographic techniques, mainly normal and reverse-phase wet loading gravity silica columns and preparative thin-layer chromatography according to the polarity of the compounds and the solvent system used for the isolation. Three isolated compounds were identified by using the nuclear magnetic resonance (NMR) spectral data, 1H NMR, 13C NMR and 1H-1H COSY, HSQC, HMBC, NOESY spectral analysis. Anti-cancer activity was tested using the sulforhodamine B assay against highest abundant breast cancer subtypes Luminal A (MCF-7) and triple-negative breast cancer (MDA-MB-231) and normal mammary epithelial cells MCF-10A. The apoptotic effects were tested using fluorescence microscopy and the caspase Glo 3/7 assay. The effects on colony formation and migration ability of cancer cells were tested using colony formation and wound healing assay. Compound 1 showed highly potent cytotoxic activity (<10 µg/ mL) on both MDA-MB-231 and MCF-7 cell lines resulting due to lactone ring. Compounds 2 and 3 with similar structures that do not have a lactone ring exerted less but selective activity (< 70 µg/ mL) on MDA-MB-231. Compound 1 exerted high toxicity whereas compound 2 and 3 have exerted low toxicity on MCF-10A. The all three compounds led to apoptosis of cancer cells. Furthermore, they reduced the colony formation and migration ability of the cancer cells, which would help to prevent the cancer from growing into an invasive stage. The isolated compounds have the potential to be used as tailor-made drugs by modifying their structure to treat different molecular sub types of breast cancer.

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Abstract No: OP21

Investigation of a Termite Nest-Derived Fungus for the Presence of Biologically Active Secondary Metabolites

Rexon SS[1], Punyasiri P.A N[1], Lewke Bandara N[2], Rajagopalan U[1], Senanayake P[3] and de Silva ED[1]

[1] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo
[2] Division of Agriculture and Environmental Science, School of Biosciences, University of Nottingham [3] Entomology and Nematology Division, Tea Research Institute of Sri Lanka

Termites consume decaying plant material. Termites of the family *Termitidae* do not produce wood–digesting enzymes and depend on symbiotic fungi for such enzymes. This study was designed to investigate the capacity of termite-nest-derived fungi to produce biologically active metabolites under laboratory culture conditions. A fungal species isolated from fungal nodules from a termite mound in Ingiriya, Sri Lanka was cultured on potato dextrose agar for 14 days at room temperature. Fungal identification was carried out morphologically and by Sanger sequencing. The cultured fungus was extracted with ethyl acetate and the extract was concentrated under reduced pressure to yield 270 mg of extract which was extensively analysed by normal-, reversed-phase- (RP), and high-performance liquid chromatography. Additionally, it was analysed by ^{13}C- and ^{1}H-NMR spectroscopy and was assayed against the human breast cancer cell line (MCF-7). The crude extract was subjected to solvent/solvent partitioning and the resulting chloroform fraction was separated by RP – column chromatography to yield a pure compound. Morphological and molecular analysis established the fungus as a *Pestalotiopsis* or *Pseudopestalotiopsis* species while the termite was identified as *Hypotermes obscuriceps* belonging to *Termitidae*. The crude extract exerted considerable cytotoxic effects on MCF – 7 cells (IC_{50} = 181 µg/mL) at 24 hours post-treatment. Chromatographic analyses showed the presence of several prominent ultraviolet (UV) active spots indicating that the fungus is producing many UV-active metabolites. Several signals in the low-field regions of the ^{1}H and ^{13}C-NMR spectra and the bioassay results indicated that the fungal metabolites are likely to be secondary metabolites with interesting structural features. The NMR spectra of the pure compound isolated showed that it is a single compound. Further studies to elucidate the molecular structures and bioactivities of the fungal metabolites are in progress.

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

Abstract No: OP22

Prevalence of Iron Deficiency Anaemia and its associated risk factors Among Type 2 Diabetic Patients Attending the Diabetic Centre, Teaching Hospital Jaffna

Thivya K[1], Risla MRF[1], Samiya HM[1], Arasaratnam V[2], Thayananthan[2]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Jaffna, Sri Lanka, [2]Department of Biochemistry, Faculty of Medicine, University of Jaffna, Sri Lanka.

Both iron deficiency and iron deficiency anaemia (IDA) can impair glucose homeostasis by affecting the glycaemic control in diabetic patients. Objective of this study was to evaluate the prevalence of iron deficiency anaemia and its associated risk factors among type 2 diabetic patients, attending the Diabetic Centre, Teaching Hospital Jaffna. This is a descriptive cross-sectional study conducted by systematic random sampling of 300 type 2 Diabetic patients with interviewer-administered questionnaire. Serum albumin, Haemoglobin, Serum ferritin & Total Iron Binding Capacity levels were measured, and peripheral blood smear was prepared. IDA was defined as Hb and serum ferritin levels <130g/dl and < 20ng/ml respectively for males while for females were <120g/dl and < 10ng/ml respectively. Statistical analysis was carried out by multivariable logistic regression analysis. Prevalence of IDA was 10.7% and, 31.3% were males and 68.8% were females. Mean Hb and serum albumin levels of IDA patients were 9.74 (±1.54) and 3.67 (±0.64) g/dl respectively. Medians of serum ferritin and TIBC of IDA patients were 7.35 (4.65-8.30) ng/ml and 564.59 (459.33-746.41) µg/dl respectively. All the patients with IDA exhibited microcytic hypochromic blood pictures. Those from rural areas (AOR= 5.020, 95% CI: 1.449– 6.23), consumed leafy vegetables ≤ 2 times a week (AOR= 12.052, 95% CI: 2.93 – 9.67), have DM for > 10 years (AOR= 4.032, 95% CI: 1.983 – 5.842) and with past family history of IDA (AOR= 7.32, 95% CI: 1.98– 7.45) were significantly associated with the development of IDA. The findings suggested that a high incidence of IDA is likely to occur in patients from rural areas, consumed leafy vegetables ≤ 2 times a week, with DM for > 10 years and with the family history of IDA. Thus, it is essential to evaluate Hb and serum ferritin levels in diabetic patients for a better quality of life.

This work was supported by the Department of Biochemistry, Faculty of Medicine, University of Jaffna.

Abstract No: OP23

Association of Socio-Demographic and Clinical Factors with the Prevalence of Hypertension in Type 2 Diabetic Patients, attending the Diabetic Centre, Teaching Hospital, Jaffna

Thivya K[1], Risla MRF[1], Samiya HM[1], Arasaratnam V[2], Aranraj T [2]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Jaffna

[2]Department of Biochemistry, Faculty of Medicine, University of Jaffna

Hypertension is one of the major threats to type 2 Diabetes Mellitus (DM) patients, which may contribute to morbidity, mortality and aggregated diabetic complications. The objective of this study was to evaluate the association of socio-demographic and clinical factors with the prevalence of hypertension among type 2 DM patients, attending the Diabetic Centre, Teaching Hospital, Jaffna. It was a descriptive cross-sectional study involving a systematic random sampling method to select 300 type 2 DM patients using an interviewer-administered questionnaire. Statistical analysis was carried out by multivariable logistic regression analysis. Majority (64.3%) of the patients were females. The males [60.17 (± 12.07) years] were older than females [57.69 (± 10.86) years]. The BMI of the Females and males were very closer [males 25.39 (±4.68) & females 25.9 (±4.36) kg/m2]. The prevalence of hypertension among diabetic patients was 57.3%. Among them, 59.2% and 40.8% were females and males respectively. Among those with hypertension, 57.3% were > 60 years, 75.2% had secondary education and 57.3% had the monthly income ≤ LKR24,999. Prevalence of hypertension in males from rural areas (64.1%) was more than in females (53.8%) and the opposite tendency was observed from urban areas. More female patients (45.2%) with DM for > 10 years had hypertension than the males (32.4%) (p=0.063). Among the total hypertensive patients, more females (24.2%) had diabetic complications than males (14.6%) (p=0.076). Further, retinopathy was more prevalent among males (27.8%) than in females (14.7%). According to the multivariable logistic regression analysis, age > 60 years (AOR= 2.211, 95% CI: 1.35– 3.62), having diabetic complications (AOR= 2.917, 95% CI: 1.08 – 7.81) among hypertensive DM patients were the independent predictors. Age > 60 years and having diabetic complications remarkably associated with the development of hypertension. Thus, appropriate intervention should be made to prevent and control hypertension among type 2 DM patients.

This work was supported by the Department of Biochemistry, Faculty of Medicine, University of Jaffna.

Abstract No: OP24

Detection of Anti-SARS-CoV-2 Spike Protein Antibodies in COVID-19 Patients and Naive Recipients of Different COVID-19 Vaccines in Sri Lanka

Pathirana SL[1], Deepachandi B[1], Gunasekara P[1], Premawansa G[2], Namalie D[2], Fernando N[1], Perera IC[3], Nanayakkara S[4], Kumarasinghe D[4], Gangani PD[1], Thambyarajah J[1], Perera T[1], Siriwardana S[3], Manilgama S[2], Sumathipala S[5] Muthugala R[4], Rajapakse S[6], Dassanayake D[7], De Silva R[4], Premawansa S[3], Nitsche A[8], Handunnetti S[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo. [2]Colombo North Teaching Hospital, Ragama, [3]Departments of Zoology and Environment Sciences, University of Colombo [4]Medical Research Institute [5]Teaching Hospital, Anuradhapura, Ministry of Health. [6]Post Graduate Institute of Medicine & Faculty of Medicine, University of Colombo. [7]National Hospital, Kandy, Ministry of Health. [8]Center for Biological Threats and Special Pathogens, Robert Koch Institute, Germany.

Antibodies against SARS-CoV-2 spike protein have shown a strong correlation to virus neutralization. It will be interesting to understand trends of immune status in natural infections and vaccinees. This study evaluated serum anti-spike immunoglobulin G antibodies in natural infections and vaccinees using an in-house ELISA. Six groups of patients and a control group were enrolled. Group one (G1) was fifty non-vaccinated individuals recruited within one month from the onset of symptoms of natural infections. G2 was seventeen follow-up individuals of G1 after 6 months from the onset of symptoms (16/17 had 1-2 doses of Sinopharm, 4/17 had a third dose of Pfizer). G3 was eighteen individuals who received two doses of Sinopharm recruited 3 months after the first vaccination. G4 comprised of twenty individuals who had two doses of Sinopharm (13/20 had Pfizer as the third dose, and 4/20 were diagnosed with natural infection at least two weeks before) and whose sera were obtained six months after first dose. G5 and G6 were AstraZeneca vaccinees (n=30 in each) recruited three and six months respectively after the first dose. G6 have had 2 doses of AstraZeneca. The control group (G7) included fifty pre-pandemic healthy individuals. One-way analysis of variance and post hoc multiple comparisons based on Tamhane's T2 tests were applied. G1-G6 had significantly higher antibody levels compared to G7 ($p<0.001$). There was a significant increase of antibodies in G2 compared to G1 ($p=0.003$). G6 showed a significantly high antibody level compared to G3 ($p<0.001$). No significant difference was observed between G2 and G4 ($p=0.052$) or G2 and G6 ($p=0.989$). However, G4 had a significantly higher antibody level compared to G6 ($p<0.001$). These findings were suggestive that AstraZeneca was more effective than Sinopharm. The potency of hybrid immunity would be important to maximise the benefits of vaccines. Monitoring of neutralizing antibodies will be of advantage when formulating vaccination policies.

This work was supported by the IDEA project, Robert Koch Institute (RKI), Germany.

Abstract No: OP25

Expression, isolation, and purification of dengue NS1 protein from bacterial cells

Perera RD, Wickramasinghe N, Wijesinghe KJ

Department of Chemistry, Faculty of Science, University of Colombo

Dengue is a widely prevalent mosquito-borne viral disease that causes explosive outbreaks in tropical and subtropical regions in the world. Early and accurate detection of the disease is important to prevent fatalities as dengue infection can lead to life-threatening severe conditions. This study aims to investigate the suitability of a bacterial expression system to produce recombinant DENV-2 NS1 protein in *E. coli* BL21(DE3) cells and to isolate and purify the recombinant protein. It can be later used to produce monoclonal anti-NS1 antibodies through mice immunization to develop diagnostic rapid assays to detect dengue infections. First, the DENV-2 NS1 sequence was PCR amplified using custom designed primers from a synthetic NS1 construct, and the amplicon was cloned in a modified pET-21a (+) bacterial expression vector. The recombinant expression plasmid was validated by colony PCR and restriction digestion fragment mapping of the isolated recombinant plasmid. Expression of recombinant DENV-2 NS1 (rNS1) protein was carried out in *E. coli* BL21(DE3) cells by inducing the pET expression system with IPTG. The rNS1 protein appeared in the insoluble fraction as inclusion bodies following cell lysis and solubilization of the inclusion bodies was performed in 8 M urea. Expressed rNS1 carrying a C-terminal 6× Histidine tag was purified further using metal-chelating affinity chromatography (IMAC) under denaturing conditions. Through SDS-PAGE, purity and size of the isolated protein were determined and the size was found to be approximately 46 kDa as expected. Final yield of the purified rNS1 protein was estimated using Bradford protein assay which was found to be 1.32 mg per 1 g of cell pellet. Isolated rNS1 which was in the unfolded state was subjected to refolding by dialysis. Subsequently, rNS1 antigens can be utilized to immunize mice.

Abstract No: OP26

Genetic analysis of *Leptospira* from clinically characterized leptospirosis patients from Western Province, Sri Lanka

de Silva, Y[1], Weerasena OVDSJ[1], Fernando N[1], Sathkumara H[1], Chandrapadma N[1], Rajapakse S[2], Premawansa S[3], Handunnetti S[1]

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

[2]Post Graduate Institute of Medicine & Faculty of Medicine, University of Colombo

[3]Departments of Zoology and Environment Sciences, University of Colombo

Leptospirosis remains the most widespread zoonotic disease in the world. It is caused by pathogenic spirochetes of *Leptospira* spp for which there are numerous animal reservoirs. The *Leptospira* genus constitutes different species, classified as pathogenic, intermediate and saprophytic. According to phenotypic classification, there are about 200 pathogenic serovars divided into 25 serogroups. Clinical presentation in patients show a wide variation from asymptomatic, mild and severe/fatal disease. The objective of this study was to determine the genotypes of *Leptospira* that cause mild and severe infections. DNA was extracted from whole blood samples from 53 (21 mild and 32 severe) patients using CEYGEN BactoSpin D™ genomic DNA kit. Leptospirosis was confirmed by Lipl32 based quantitative PCR (Genesis Real-Time PCR detection kit -Primer Design Ltd). Nested PCR was performed using primers for *fla*B gene. Out of 53 samples, 11 were positive with expected amplicon size of 732 bp. The amplicons were purified and subjected to Sanger dideoxy sequencing (Genelabs Medical Ltd). The first sequence obtained from a mild case (NHSL13ML713) was analyzed using MEGA 11. The partial *fla*B gene sequence showed alignment with 100% percentage identity with *L. interrogans* serovar Bataviae. The *fla*B gene sequence was deposited in GenBank and accession number (OQ553816) was obtained. Phylogenetic analysis of the partial *fla*B gene sequence was carried out with 25 *Leptospira* sequences in GenBank using MEGA 11. Sequences were aligned by ClustalW, and phylogenetic inference was carried out using UPGMA method. The sequence analysis of other amplicons is currently in progress. *L. interrogans* serogroup/serovar Bataviae has been previously recorded from a recent study in one isolate from Sri Lanka. The genotype analysis of *Leptospira* from clinically characterized leptospirosis patients would provide data to determine most prevalent genotypes in patients with different disease severity and also target species/serovars for the development vaccine(s) and diagnostics.

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Abstract No: OP27

Aqueous leaf extract of *Vitex negundo* exerts immunomodulatory effects in an *in vitro* model of hypertension

Nyamweya B[1], Rukshala D[1], Fernando N[1], de Silva R[2], Premawansa S[3], Handunnetti S[1]

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

[2]Department of Immunology, Medical Research Institute

[3]Department of Zoology and Environment Sciences, University of Colombo

Although hypertension is a major risk factor for cardiovascular diseases, its control and treatment continue to be ineffective as many patients respond poorly to conventional treatment with a certain proportion developing resistant hypertension. The central drivers of the immunopathogenesis of hypertension are inflammation and oxidative stress. *Vitex negundo* L. is a medicinal plant used in Ayurveda and traditional medicine. However, investigations on its ethnopharmacological potential to effectively curtail the progression of hypertension is limited. This study was aimed at assessing the immunomodulatory effects of aqueous mature leaf extract (ALE) of *V. negundo* in an *in vitro* model of hypertension developed using PMA-induced, THP-1 derived human macrophages stimulated with angiotensin II (Ang II). Non-toxic concentrations of ALE were selected as 15.6 - 500 µg/ml using Sulforhodamine B and MTT assays. Quantitative nitro blue tetrazolium and Griess assays were done to assess the effect of the ALE on Ang II-induced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) respectively. ALE of *V. negundo* inhibited ROS and RNS production in a dose-dependent manner comparable with Telmisartan which was used as the drug control (r=0.910; $p<0.0001$). The effect of the ALE on the expression of TNF-α, IL-6, IL-1β and NF-κB assessed using RT-qPCR showed that both ALE and Telmisartan attenuated the expression of the pro-inflammatory cytokines in Angiotensin II-stimulated macrophages like cells derived from THP-1 cells ($p<0.001$). Our results demonstrate the potential of ALE of *V. negundo* in exerting immunomodulatory effects in this *in vitro* model of angiotensin II-mediated hypertension mainly by limiting production of pro-oxidants leading to inflammation. As inflammation and oxidative stress are known as central drivers of pathogenesis of hypertension, these findings therefore emphasize further studies on validating the antihypertensive potential of *V. negundo* and to develop more effective antihypertensive therapies.

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PP 01

Comparative study of novel human monkeypox virus isolates of the 2022 outbreak

Batagoda BNNT[1], Senanayake KS[2]

[1] Department of Interdisciplinary Studies, Faculty of Information Technology, University of Moratuwa

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

Human monkeypox is a significant zoonotic disease caused by the monkeypox virus (MPXV), the most virulent Orthopoxvirus endangering human public health since the eradication of the variola virus. The virus is endemic in Africa, where its natural hosts are small mammals. Since May 2022, an increasing number of confirmed cases of human monkeypox have been reported from numerous non-endemic countries, declaring a Public Health Emergency of International Concern (PHEIC) by the WHO. As of 25th April 2023, 87113 confirmed cases have been reported with 130 deaths. The MPXV genome consists of a double-strand DNA molecule of around 200 kilobase pairs (kbp) and contains around 200 protein-coding genes. It has been questioned whether the current acceleration in human infections could be attributed to recent changes in the viral genome. Current study tries to address this question by looking at the novel virus strains sequenced during 2023. Complete MPXV genomes sequenced from 1st January to 12th April 2023 (21 sequences) were retrieved from the National Center for Biotechnology Information (NCBI) virus database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/). Multiple alignment was carried out between the 23 sequences which includes two reference sequences NC_003310 (Zaire: Sankuru subregion) and NC_063383 (Nigeria: Rivers State) using NCBI tools. In the phylogenetic tree, NC_003310 formed an outgroup. The sequences were submitted to Nextclade (https://clades.nextstrain.org/) and NC_003310 was grouped into clade I and the remaining sequences along with NC_063383 were grouped into clade II. Sequences showed 71-83 nucleotide substitutions and 32-40 amino acid substitutions relative to the reference sequence NC_063383 in clade II. The gene annotation data of NC_063383 reference genome which consists of 175 genes, were compared with the annotated gene sets of the 21 selected isolates. This analysis showed that 22 genes had identical substitution mutations in all 21 selected isolates when compared to NC_063383. Additionally, 7 genes had similar mutations in the isolates from Germany. These identified variations in the virus genomes could be a contributing factor for the sudden increase of monkeypox disease. However further proteomic studies will be helpful to confirm the above conclusion.

PP 02

Preliminary study to identify shoot and fruit borer (*Leucinodes orbonalis* Guenee) resistance brinjal varieties in Sri Lanka

Jayasooriya JANC[1], Suvanthini T[2], Gajapathy K[1]

[1]Department of Zoology, Faculty of Science, University of Jaffna

[2]Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna

Brinjal is a commercially important vegetable grown in Sri Lanka which is severely affected by brinjal shoot and fruit borer (*Leucinodes orbonalis*). Utilizing host plant resistance is a promising strategy for reducing this pest infestation. This study aimed to assess the resistance of selected brinjal varieties to *L. orbonalis* based on selected physical and biochemical properties. Under natural, consistent management approaches, seven brinjal varieties were grown, such as Amanda F1, HORDI Lenairi 1, Thinnaweli purple, Madduvil muddiyan, Raal kuli, Plastic cultivar, and Eerku vellai. The plots were established with three replications and 25 plants per replicate were selected for the analysis. The resistant status was classified based on the mean fruit and shoot infestation. Simple linear correlation analysis and one-way ANOVA tests were done to explore the influence of selected physical characters, total phenol, and total sugar contents. Results showed significant differences ($p<0.05$) in fruit and shoot infestation levels among the brinjal varieties. Amanda F1 and Madduvil muddiyan showed minimum shoot infestation of 1.13% and 1.39% and fruit infestation of 12.31% and 13.75%, respectively while HORDI Lenairi 1 recorded the highest shoot and fruit infestation of 6.54% and 29.63%, respectively. Physical properties such as short pedicel and calyx, compactly arranged seeds, thin shoot, and thick pericarp were found to be tolerant to *L. orbonalis* infestation. The moderately tolerant Raal kuli showed the highest amount of total phenol (0.81 mg/g) and susceptible HORDI Lenairi 1 had 0.53 mg/g. The resistant Madduvil muddiyan contained 0.59 mg/g of total phenol and 21.59 mg/g of total sugar. Susceptible Eerku vellai had the highest total sugar (22.77 mg/g). Fruit infestation had a positive (0.3832) correlation coefficient with total phenol and a negative (-0.5394) with total sugar. Amanda F1 and Madduvil muddiyan were found resistant to *L. orbonalis*. Physical properties of them are more responsible for resistance than the analyzed biochemical properties. These varieties can be utilized as potential resistance sources in future breeding programmes and these findings might contribute to develop a key for resistant and susceptible brinjal varieties.

PP 03

Comparison of the presence of FLT3 receptor on peripheral blood mononuclear cells between newly diagnosed non-Hodgkin lymphoma patients and healthy individuals

Nandasena CK[1, 3], Jayathilake PWDCC[5], Dharmarathne G[2], Suresh S[5], De Silva AD[4], Perera IC[1], Kottahachchi DU[3]

[1] Department of Zoology and Environment Sciences, [2] Department of Statistics, Faculty of Science, University of Colombo, [3] Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, [4] Department of Paraclinical Sciences, Faculty of Medicine, General Sir John Kotelawala Defence University, [5]Apeksha Hospital, Maharagama

FLT3 (tyrosine kinase receptor) triggers leucopoiesis, especially in the lymphoid lineage. FLT3 mutations led to developing severity and reduced the survival rate in acute myeloid leukemia. Since this was not well-studied in Non-Hodgkin's Lymphoma (NHL), finding the association between FLT3 levels on peripheral blood mononuclear cells (PBMNCs), and full blood count (FBC) parameters in NHLs, and compared with the healthy population was the objective of this study. Ethical clearance from KDU-ERC and the participant's consent were obtained. Peripheral blood samples were analyzed with flow cytometry to detect FLT3 on PBMNCs from new NHL patients (n=11) before starting chemotherapy. Samples from healthy individuals (n=7) were analyzed to detect the normal receptor level. FBC parameters were compared among three populations as FLT3(+) NHL, FLT3(-) NHL, and healthy individuals. SPSS-26 was used to analyze data and $p<0.05$ was the significant level. All patients were monitored after completing chemotherapy cycles. Five NHL patients showed strong positivity for FLT3 while 06 were negative. All healthy individuals were FLT3 negative. The FLT3(-) NHLs and the healthy group showed a significant difference in absolute lymphocyte count (ALC). Examining the PBMNCs of FLT3(+) patients and the healthy group, showed significant differences in total WBC count, absolute neutrophil/eosinophil/ immature granulocyte count, and ALC whereas, the groups of FLT3(+) and FLT3(-) patients showed significant differences between the same with platelet count. Three patients died from the FLT3-positive group during chemotherapy and most of the FLT3(-) patients showed a successful treatment response. Peripheral white blood cell counts were significantly different and the ALC was significantly lower in FLT3(+) NHLs when compared to the healthy population. Therefore, the immunity of FLT3(+) NHLs may be lower than the other two populations. The study population is currently being expanded to establish the results with higher confidence and to investigate the whole FLT3/FL system.

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PP 04

Bio-efficacy and persistence of inert dust formulations as stored-grain protectants against *Rhyzopertha dominica* (F.)

Ganhewa HT, Perera AGWU

Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura

Inert Dust (ID) insecticides make a significant contribution to control storage insect pests. In the present study, the insecticidal activity, progeny inhibition and persistence of three ID formulations, Diatomaceous earth, zeolite and cloisite 20A (modified montmorillonite clay) was evaluated against *Rhyzopertha dominica* (Lesser grain borer) throughout 90 days of storage period. The toxicity and persistence of binary combinations of IDs at their sub-lethal doses was conducted for a storage period of 60 days. Furthermore, the ultrastructural architecture of the test insect pest species was examined via Field Emission Scanning Electron Microscopy (FE-SEM) and Energy Dispersive X-Ray analysis (EDX) to study the penetration/uptake pattern of ID particles by the cuticular layer of target insect pest. The results revealed that all ID formulations exhibited very efficacious toxic and progeny inhibition activities and higher mortality percentages irrespective of the ID used at the end of initial 30 day-long storage period. Thereafter, the mortality percentages and progeny inhibition gradually declined with the progress of the storage time period. Tested ID formulations successfully suppressed F_1 progeny and the lowest average progeny production was recorded at the end of initial storage period. Cloisite 20A was the most efficacious ID followed by zeolite and diatomaceous earth in order. The binary mixtures of IDs induced strong mortality of *R. dominica* adults than each formulation was used alone. FE-SEM and EDX micrographs clearly indicated the presence and distribution of constituting elements of treated IDs on the cuticular layer of the exposed insect pests with the abrasions and scratches that may have led to dehydration and eventual death of respective insect pest. The findings of the present study indicate that diatomaceous earth, zeolite and cloisite 20A and their binary combinations are highly effective for the control of *R. dominica* and could be implemented in management of this destructive storage pest.

PP 05

Molecular phylogenetic analysis on genera *Thrixspermum* (Orchidaceae)

Wijayabandara AMWA [1], Lewke Bandara N[2], Papini A [3], Atthanagoda AG[1]

[1] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo. [2] Division of Agriculture and Environmental Science, School of Biosciences, University of Nottingham. [3]Department of Biology, University of Florence

Orchidaceae, is the second largest family of flowering plants with 736 genera and 22,500 species widely distributed throughout the world. *Thrixspermum,* commonly known as hair seed, is a genus within Orchidaceae *that* comprises epiphytic plants with flat, bilobed and coriaceous leaves, axillary inflorescence, short column continuing into the foot and the presence of 2-4 unequal pollinia. Currently, the genus encompasses nearly 160 species, distributed from the Himalayas to the Philippines, Northern Australia, and the Pacific islands. The small, ephemeral flowers of *Thrixspermum* and the high homology between the species, make morphology-based identification insufficient, necessitating the use of DNA barcoding. The objective of this study was to utilize ITS markers to identify problematic placements of new and existing species of the genera *Thrixspermum*. A phylogenetic tree was constructed using the Bayesian method by MrBayes with ten samples collected from different geographic locations in Sri Lanka, 12 available database sequences from *Thrixspermum,* and two outgroup species (*Dimorphorchis lowii* and *Dimorphorchis rossii*). The combined morphological and phylogenetic evidence supports the delimitation of three clades with six, one and two samples suspected to represent sub-species of *Thrixspermum pulchellum*, *Tx. puginofolium* and *Tx. Walkeri respectively,* and one sample depicting similarities with *Tx. formosanum*. The splitting into subgroups might reflect an early differentiation of the flower colour and the lip. However, further studies are required for the confirmation of the results with suspected herbarium samples and outgroup species sequences. Despite the limitation of ITS partial sequences for intrageneric level classification of the genera of interest, the study findings affirm the suitability of ITS as a molecular marker for generating *Thrixspermum* barcodes. Complete sequences of ITS would further enhance the barcode database, benefiting future taxonomic and biodiversity studies.

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PP 06

In silico investigation of anticancer properties of *Withania somnifera* on cancer stem cells

Perera KDC[1], Faizan M[2], Senathilake NHKS[2], Samarakoon SR[2]

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

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

Cancer is a complex and multifactorial disease and it is the second leading cause of death worldwide. A subset of drug-resistant cells in the cancer cell mass known as cancer stem cells (CSCs) plays a key role in chemo-and radiotherapy resistance, metastasis and recurrence posing a challenge in the fight against cancer. Therefore, search for compounds that destroy CSCs has become an urgent need. The current study aims to investigate the natural drug-like molecules that can be used as CSC-targeted therapy. The pivotal role of β-catenin and Human smoothened receptor in stem cell maintaining Wnt/ β-catenin and hedgehog signaling pathways respectively, and their oncogenic nature makes them important targets in CSC-targeted anti-cancer drug discovery. *Withania somnifera* (Ashwagandha) is a plant used in traditional medicine to treat cancer. Based on the literature and database search, 80 compounds of *W. somnifera* and standard inhibitors were docked with the target β-catenin and Human Smo receptor using Auto Dock Vina in PyRx. The top compounds and the inhibitor complexes were subjected to molecular dynamics simulation (100 ns) using Schrodinger, LLC's Desmond 2022-1 to understand stability and interactions. The top scoring compounds (based on the docking score of higher than −8.0 kcal/mol for beta-catenin and higher than −11.9 kcal/mol for Smo receptor) were evaluated in comparison to the standard inhibitors. The compounds from *W. somnifera* were evaluated based on interactions at the active sites of target proteins with the inhibitors. Physagulin-d, withanoside IV showed high binding stability with the specific amino acid residues of β-catenin and withanolide J, withanolide M showed high binding stability with the amino acid residues of human smoothened receptor.

PP 07

Germline variants in the exon 3 of the *POLG1* gene: optimization of the polymerase chain reaction (PCR) and preliminary analysis in a few selected Sinhalese individuals and breast cancer patients

Kariyawasam CM, Kotelawala JT, Tennekoon KH, Ranasinghe R
Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Mitochondrial DNA (mtDNA) mutations are shown to be profoundly associated with the development of a variety of cancers. Mutations in mtDNA arise during its replication and repair process, which are solely controlled by the human polymerase gamma enzyme. It is encoded by the nuclear encoded Mitochondrial DNA polymerase gamma *(POLG1)* gene. The *POLG1* gene is a frequent target for gene mutations, therefore, is said to play a role in mtDNA instability, leading to mitochondrial diseases and cancers. *POLG1* mutations have also been commonly reported to promote tumorigenesis in breast cancer, however, there are no data for *POLG1* variants for Sri Lanka. The aim of this study was to identify germline variants in exon 3 of *POLG1* which codes for a section of the exonuclease domain (the *exo* motif II) in patients with sporadic breast cancer and healthy individuals in Sri Lanka. Initially, *POLG1* exon 3 specific primers that were designed with primer BLAST were optimized for PCR amplification. Then the DNA were extracted using the Salting out method from 3 breast cancer patients and 3 healthy women [matched for age, body mass index (BMI) and menopausal status]. *POLG1* exon 3 was sequenced and analyzed with reference *POLG1* sequence with BioEdit software, to detect any variants present. No variants were found between the patients and their matched healthy controls. No polymorphism patterns were also observed within the studied samples. Since the present study was carried out using only a few samples due to the limitation of funds, a larger sample needs to be studied to characterize variants in exon 3 of *POLG1* as well as in the rest of the *POLG1* gene in Sri Lankan breast cancer patients and healthy individuals.

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

PP 08

Analysis of *DUOX2* mutations in a cohort of Sri Lankan patients with permanent congenital hypothyroidism

Thayaparan P[1], Hewage S[1], De Silva S[1], Athapattu N[2]

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

[2]Lady Ridgeway Hospital for Children, Colombo 08

Congenital hypothyroidism (CH) is thyroid hormone deficiency from birth, diagnosed at a rate of 1 in 3000 - 4000 live births. Mutations in Dual oxidase 2 (*DUOX2*), a generator of hydrogen peroxide (H_2O_2) which is required for thyroid hormone synthesis, have been identified as a cause of thyroid dyshormonogenesis which can cause permanent CH. Objective of the study was to analyze the presence of selected mutations in the hotspot exon regions of *DUOX2* gene in a cohort of Sri Lankan patients with confirmed permanent CH. The selected four prevalent single nucleotide variants (SNVs) are rs180671269 (exon14), rs774556391 (exon 20), rs181461079 (exon 20) and rs147945181 (exon 30). To achieve the objective, blood samples were obtained from seven children (N=7) aged between two weeks to sixteen years from the Lady Ridgeway Hospital for Children. DNA was extracted from collected samples and the selected four SNVs were screened using allele specific PCR (ASPCR) technique, followed by results of screening the SNV in exon 30 (rs147945181) in all seven samples were verified by sanger sequencing. Out of the SNVs that were screened, all were heterozygous for c.1588A>T (rs180671269) and c.2635G>A (rs774556391). Out of the seven samples, six were heterozygous, and one was homozygous mutant for c.2654G>T (rs181461079). All samples were homozygous wild for c.4027C>T (rs147945181) and this was confirmed with sequencing.

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

PP 09

Analysis of *NKX2-5* mutations in a cohort of Sri Lankan patients with ectopic thyroid

Fonseka WNT[1], Hewage S[1], De Silva S[1], Athapattu N[2]

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

[2]Lady Ridgeway Hospital for Children, Colombo 08

Ectopic thyroid is a form of thyroid dysgenesis. It refers to any thyroid tissue present in unusual locations other than its normal position anterior to the upper trachea. Ectopic thyroid is a rare malformation of the thyroid gland which occur as a result of improper descent of thyroid along its natural course during embryogenesis, affects 1 in 100,000-300,000 individuals. As per the location, size and histopathology of ectopic thyroid its clinical presentation may vary. However, in most cases thyroid ectopy leads to congenital hypothyroidism, requiring lifelong hormone replacement therapy. Mutations in regulatory genes expressed in the developing thyroid could lead to disease development. One such gene expressed in early stages of embryonic development is the NK2 homeobox 5 (*NKX2-5*), a homeodomain-containing transcription factor protein coding gene, involved in the thyroid migration stage of thyroid gland morphogenesis. This study was designed to identify mutations in the *NKX2-5* gene in a cohort of children with ectopic thyroid in Sri Lanka. Study participants were selected from patients already diagnosed as having ectopic thyroid condition and being followed up by the pediatric endocrinologist at Lady Ridgeway Hospital (LRH) for children. The coding regions of *NKX2-5* gene were screened for any possible variations by direct sequencing. When sequences were analyzed, exon 1 of three samples (N=3) gave satisfactory electropherograms and from which one patient was identified to have a reported SNP, c.63A>G /rs2277923. Exon 2 of all five samples (N=5) gave satisfactory electropherograms and from which three patients were identified with previously reported benign variant (c.*61G>T /rs703752 at exon 2 3'UTR region) and a novel variant (c.*147C>A) was detected in one patient. However, a comprehensive investigation needs to be carried out among a larger cohort in future in order to predict the spectrum of *NKX2-5* mutations among ectopic thyroid patients in Sri Lanka.

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

PP 10

Detection of selected SNPs of HOX transcript antisense RNA (*HOTAIR*) gene in a cohort of patients with breast cancer in Sri Lanka

Shafeeu FA[1], De Silva S[1], Hewage S[1], De Silva K[2]

[1]Instititue of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

[2]National Cancer Institute, Maharagama

Breast cancer is a heterogeneous disease that involves a combination of multiple genetic alterations that may lead to breast cancer. Hence, it is necessary to identify genes related to breast cancer aetiology to predict disease susceptibility in a population. There has been much evidence suggesting that the *HOTAIR* lncRNA can act as an oncogenic driver in the initiation and progression of different types of cancer and studies have shown that genetic variants related to its function have been associated with breast cancer risk in certain populations. The aim of the current study was to investigate and screen for two *HOTAIR* genetic variants (rs1899663 G>T and rs920778 T>C) in a cohort of breast cancer patients in Sri Lanka. Nine breast cancer patients (N=9) were screened for the *HOTAIR* intronic variants. Already extracted genomic DNA from tissue samples was used for the current study. Sequence-specific primers were designed and PCR was carried out. Sanger sequencing was used to obtain DNA sequences of patients at the region of interest. Sequence data was analyzed for targeted variants and other variants, if any, by comparing with the reference sequence using bioinformatics software. Previous studies have revealed that TT homozygous and TC heterozygous of rs920778 T>C can be associated with an increased risk of BC susceptibility. Among 9 patients tested for this variant, 7 patients were reported to be homozygous mutants (TT genotype), while the other 2 had heterozygous variant (TC genotype). Among the cohort, the rs1899663 variant was not found in any of the patients, thus showing that rs1899663 had no association with breast cancer susceptibility in the studied cohort. However, further research is needed to evaluate the results in a larger patient cohort.

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

PP 11

Detection of disease-associated variant (n.662G>T) in Colon Cancer Associated Transcript 2 RNA gene in a cohort of patients with colorectal cancer in Sri Lanka

Kehelgamuwa RP[1], De Silva S[1], Hewage S[1], De Silva K[2]

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

[2]National Cancer Institute, Maharagama

Colorectal cancer (CRC) is the third most prevalent malignancy diagnosed worldwide and in Sri Lanka, it is the third most common cancer type in both sexes with many causalities every year. Long non-coding RNAs (lncRNA) have been proven to play a role in the development of CRC by interacting and regulating target genes. The current study was designed to detect disease-related SNP, n.662G>T (rs6983267) in the *CCAT2* gene, based on the studies done in other populations. Previous extracted genomic DNA (N=13) from excised patients' tissues was used for the study. Specific primers were designed to amplify the region containing the selected variant in the gene. Afterward, PCR was performed using designed primers and followed up by Sanger sequencing. The sequencing data were analysed for the selected variant (n.662G>T) in the *CCAT2* gene using bioinformatics software. Analysis of the sequencing data showed homozygous wildtype condition (GG) in four patients, four patients with homozygous mutant (TT), and five heterozygous (GT) patients. However, the wildtype variant (G) is dominant and has been shown to be associated with the development of different forms of cancer including CRC. 69% of patient samples contained the G allele which is associated with the development of CRC. However, a larger sample size along with gene expression studies of affected genes could be carried out to get a comprehensive analysis.

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

PP 12

Neanderthal inherited COVID-19 genetic variations: Assessing the Polymerase Chain Reaction conditions and database-based allele frequency analysis

Galagamaarachchi SH, Fernando AS, Tennekoon KH, Ranasinghe R

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

The COVID-19 pandemic continues to spread worldwide. It was observed that the risk level and the severity of COVID-19 were different among individuals. Since all patients do not carry the risk factors and respond to the infection in a similar manner, it is thought that there are genetic factors modulating the outcome of the disease. Some genes that are associated with the disease have been reported as Neanderthal inherited. The SNP on chromosome 3, rs35044562 (A>G) was reported to predispose to the severe form of the disease while the OAS haplotype on chromosome 12, the SNP rs1156361 (T>C) was reported to protect against the severe form of the disease. The main objective of this study was to optimize PCR conditions of sequence-specific primers that were designed to amplify the regions, including Neanderthal inherited COVID-19-related SNPs chromosomes 3 and 12 in a Sinhalese individual. Moreover, to analyze allele frequencies reported in the 1000 genome database to obtain a better understanding of the risk and severity of COVID-19 associated genes in Sinhalese ethnicity. DNA was extracted manually following the salting out protocol, followed by a conventional PCR amplification carried out using manually designed primers. The designed conventional primers had to be optimized well as they were newly designed primers. 1000 genome results were used to compare the gene frequency among the South Asian populations which indicates the frequency distribution of SNPs in healthy individuals.

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

PP 13

PCR optimization and amplification of selected exons of mitochondrial transcription factor A (TFAM) in sporadic breast cancer patients

Fasha MA[1], Kotelawela JT[2], Ranasinghe R[2]

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

Breast cancer (hereditary or sporadic) is the second most common cancer among women worldwide. Sporadic breast cancer is developed from acquired gene mutations occurring spontaneously after birth and constitutes most cases. Mitochondrial Transcription Factor A (TFAM) is located in chromosome 10q21.1 is a key regulator of mitochondrial DNA replication and transcription and is essential for the maintenance and organization of the mitochondrial genome. As TFAM plays a critical role in regulating expression of mitochondrially encoded proteins that are involved in respiration (oxidative phosphorylation) and energy metabolism. Therefore, dysfunction of TFAM is associated with breast cancer. TFAM variations have been associated with greater lactate production and greater metastasis and also it makes cancer cells more sensitive to radiation. Therefore, altered TFAM expression may increase cell death and suggests that it could be a potential therapeutic target for new cancer therapy. Mutations in the TFAM gene could yield valuable insights into the role of TFAM in breast cancer development and progression. PCR optimization is crucial for achieving efficient PCR amplification. In the present study, primers were designed and optimized to amplify two exonic regions of the TFAM genes (TFAM_1 and TFAM_3). Each primer was optimized separately for the annealing temperature and magnesium concentrations. The optimized temperature of both TFAM_1 and TFAM_3 was 56 ˚C and 60 ˚C respectively, while optimized magnesium concentration of both TFAM_1 and TFAM_3 was 2.0 mM and 2.5 mM respectively. Further analyses of these products with direct sequence technology will be needed to identify variations in the TFAM gene in sporadic breast cancer patients in Sri Lanka.

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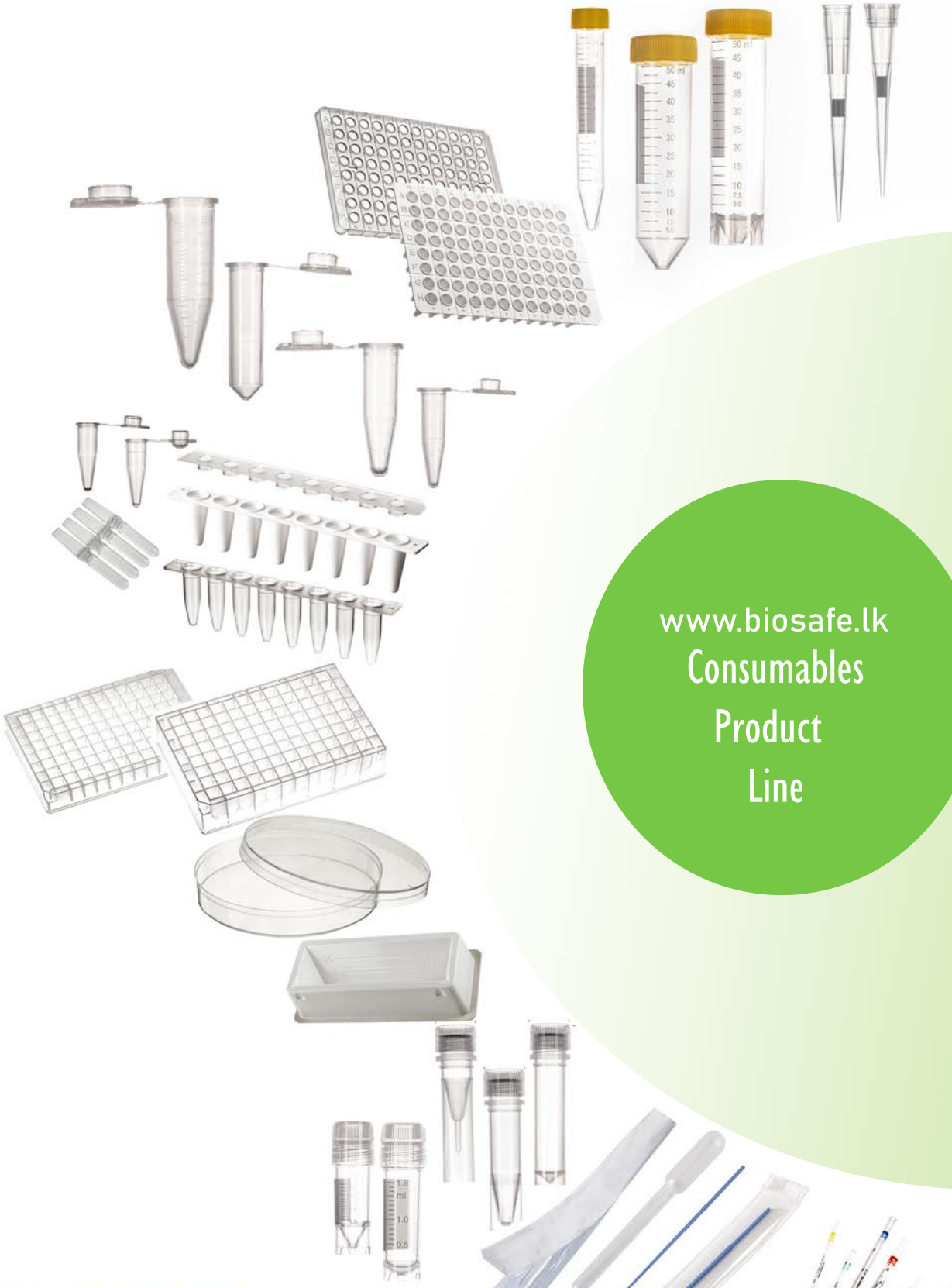

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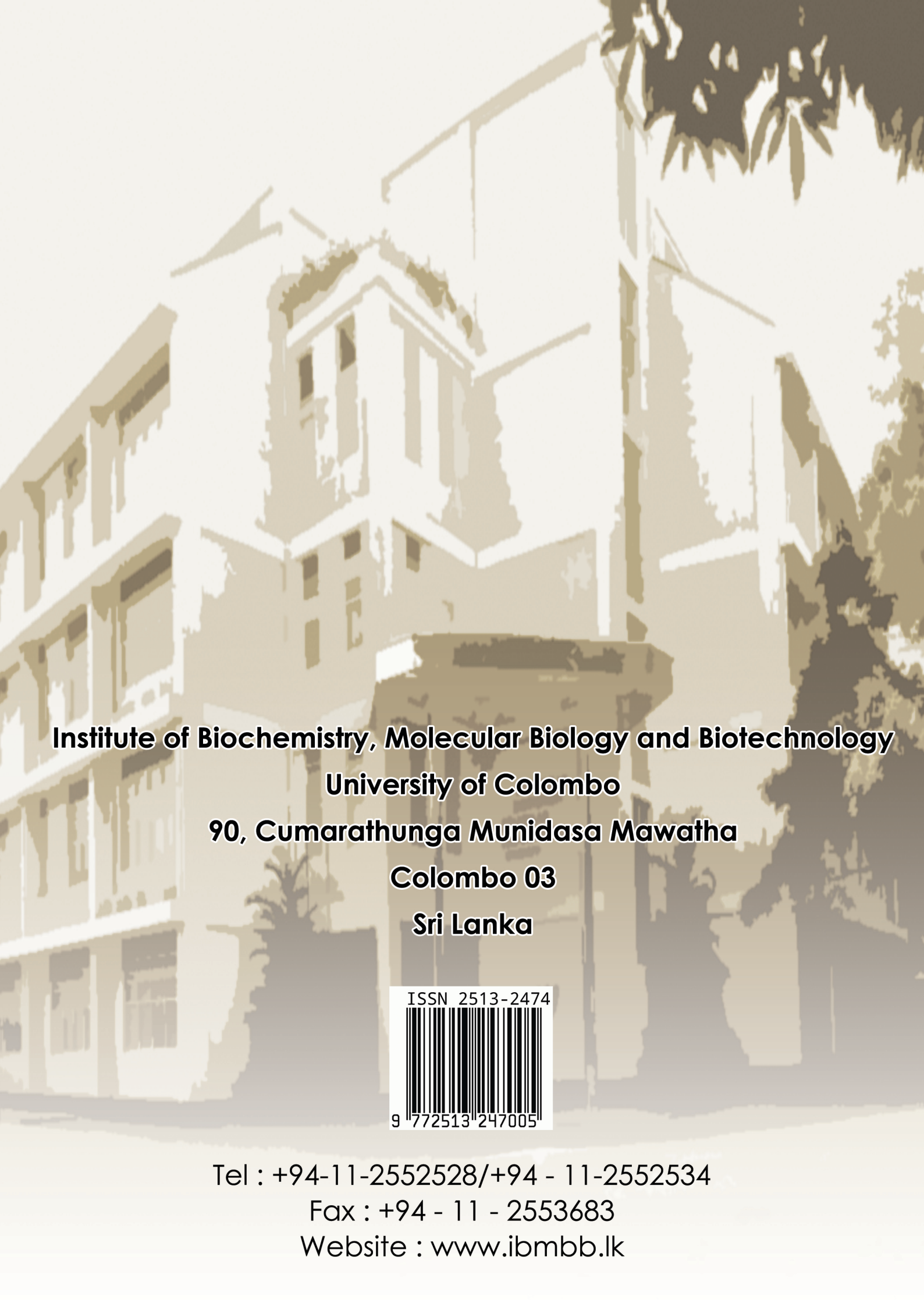

Institute of Biochemistry, Molecular Biology and Biotechnology
University of Colombo
90, Cumarathunga Munidasa Mawatha
Colombo 03
Sri Lanka

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