



IBMBB



Institute of Biochemistry, Molecular
Biology and Biotechnology
University of Colombo

**Proceedings of the 13th
Annual Scientific Sessions of the IBMBB**

24th October 2025

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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Programme

Time	Event
8.30 h - 9.00 h	Registration
9.00 h - 9.05 h	Inauguration
9.05 h - 9.10 h	Lighting of the Traditional Oil Lamp
9.10 h - 9.20 h	Welcome Address by Director - IBMBB
9.20 h - 9.30 h	Address by Vice Chancellor - University of Colombo
9.30 h - 9.40 h	Best Teacher Awards 2023/2024 & IBMBB Research Excellence Awards 2023/2024
9.40 h - 10.30 h	Professor Stanley Wijesundera Memorial Lecture: “Brains, Budgets, and Beds: Aligning Health HR, Finance, and Education for a Healthier Sri Lanka” Professor Dileep De Silva
10.30 h - 10.40 h	Address by a Member of Professor Wijesundera family
10.40 h - 10.45 h	Vote of Thanks
10.45 h - 11.30 h	Tea
11.30 h - 13.15 h	Oral Presentation; Session 1 (Medicinal Plants)
13.15 h - 14.00 h	Break
14.00 h - 15.15 h	Oral Presentation; Session 2 (Health Sciences)
14.15 h - 16.15 h	Poster Presentation
15.15 h - 16.15 h	Oral Presentation; Session 3 (Biotechnology)
16.30 h - 16.45 h	Tea
16.45 h - 17.00 h	Award Ceremony & Closing Remarks

Message from the Director – IBMBB

Professor Prasanna Galhena

Professor in Biochemistry and Clinical Chemistry

Director

IBMBB, University of Colombo



On behalf of 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 13th Annual Scientific Session of the IBMBB, University of Colombo.

This is one of the key events at the IBMBB, that facilitates an interactive dialog among all the stakeholders in the field of Life Sciences. Annual Scientific Session 2025 primarily focuses on advancement in health sciences, Biotechnology applications and developments in Natural products, giving opportunities for the student community to interact with expertise in the field.

Professor Stanley Wijesundera memorial lecture 2025, delivered by Prof. Dileep De Silva, Professor of Community Dentistry, Faculty of Dental Sciences, University of Peradeniya will grace the session.

I would like to express my sincere gratitude to the distinguished invited speakers for their presence and contribution to this year session. 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 appreciation to the team at IBMBB who worked tirelessly in bringing you a productive session despite all the challenges encountered.

Message from the Chairperson

Professor Nimal Punyasiri

Professor and Chair of Biochemistry

IBMBB

University of Colombo



The Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo, held its 13th Annual Scientific Sessions on 24th October 2025. The Chief Guest at the event was Professor Indika Mahesh Karunatillake, Vice Chancellor, University of Colombo. The sessions were graced by distinguished invitees, including Deans and Directors of the University of Colombo, the Registrar, Bursar, and many other esteemed guests.

The Annual Scientific Sessions provide an important platform for disseminating the research findings of IBMBB students as well as scientists from other higher educational institutions. It is a matter of pride that these sessions have been conducted continuously since 2006, except in the years when the IBMBB hosted its International Conferences in 2009, 2014, and 2024.

A key highlight of the Annual Research Sessions is the Professor Stanley Wijesundera Memorial Lecture. This year, the lecture was delivered by Professor Dileep De Silva, Chair Professor and Board-Certified Specialist in Community Dentistry, Faculty of Dental Sciences, University of Peradeniya, and an eminent authority in Health Finance and Health Management.

The 2025 Annual Scientific Sessions featured a total of 25 presentations, comprising 16 oral presentations and 9 poster presentations.

I take this opportunity to extend my sincere thanks to Professor Indika Mahesh Karunatillake, Vice Chancellor of the University of Colombo, for gracing the occasion as Chief Guest, and to Professor Dileep De Silva for delivering the memorial lecture. I also wish to express my heartfelt gratitude to all those who contributed to the successful organization of this event.

Organizing Committee

Main Committee Appointments

Position	Name
Chair, Symposium Committee	Prof. Nimal Punyasiri
Secretary	Dr. Visula Abeysuriya
Joint-Secretary	Dr. N. H. K. S. Senathilake
Co- Editor	Dr. Narmada Fernando
Co-Editor	Dr. R. A. C. R. Ranasinghe
Coordinator (Sponsorship)	Prof. S. S. R. Samarakoon
Coordinator (Scientific Presentation Logistics)	Prof. W. S. De Silva
Coordinator (Abstract Submission to ARS of UoC)	Dr. A. S. Hewage
Coordinator (Media/IT/Publicity)	Dr. K. S. Senanayake

Sub-Committees

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Ms. R. K. H. A. Jayaweera
Ms. S. M. Withana Arachchi

Editorial Committee

Ms. Anjana Welikala
Ms. Kavindi Sandamali Sayakkara
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Ms. A. T. N. Somaratne
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Food & Beverages Committee

Ms. A. A. Jayasoma
Mr. Y. B. M. N. Y. Bandara

IT & Media Committee

Mr. G. G. S. Niranjana
Mr. Dimantha Ranaweera

Invitations Committee

Ms. W. L. Damayanthi
Ms. H. W. Eranga Umayangi

Oral Presentations

Session 1 - Medicinal Plant

- | | | |
|-----|---|--|
| OP1 | Anti-glycemic activity of ethanolic extract and essential oils obtained by solvent-free microwave extraction and hydro-distillation of rhizomes and leaves of <i>Alpinia calcarata</i> Roscoe | <u>Aazif NMT</u> , Samarasekera JKRR, Liyanaarachchi GD, Fernando N |
| OP2 | Synergistic anticancer potential of <i>Andrographis paniculata</i> and <i>Datura metel</i> combined with Doxorubicin in inhibiting breast cancer cells | <u>Kanagasundaram A</u> , Rajagopalan U, Samarakoon SR |
| OP3 | Anti-cancer potential of a supercritical CO ₂ extract of Vernolac nutraceutical, a viable polyherbal formulation | <u>Rathnayake PC</u> , Samarakoon SR, Rajagopalan U, Senathilake KS |
| OP4 | DC3B, a diterpene obtained from <i>Caesalpinia pulcherrima</i> modulates stemness in NTERA-2 cancer stem-like cells | <u>Senavirathna DMDC</u> , Senathilake KS, Rajagopalan U, Samarakoon SR |
| OP5 | Inhibition of the production of reactive oxygen and reactive nitrogen species in RAW 264.7 cells by aqueous extract of <i>Artocarpus heterophyllus</i> | <u>Perera WGMCH</u> , Rukshala BAD, Vijithsingh NN, Handunnetti SM |
| OP6 | The in vitro effect of <i>Andrographis paniculata</i> on the viability and morphological changes induced by Vascular Endothelial Growth Factor on endothelial cells | <u>Rodrigo JK</u> , Jagoda D, Vijithsingh NN, Wanigarathna US, Hadunnetti SM, Fernando N |
| OP7 | <i>In vivo</i> pharmacokinetic and tissue distribution studies of a natural anticancer diterpene isolated from <i>Caesalpinia pulcherrima</i> | <u>Gunawardane SDV</u> , Oshadi EA, Wijerathne PKS, Punyasiri PAN, Senathilake KS, Rajagopalan U, Samarakoon SR |
| OP8 | QSAR-driven discovery of novel anticancer derivatives from a natural halogenated scaffold | <u>Rathnayaka RMRK</u> , Samarakoon SR, Tennekoon KH, Gunasekara DS |

Session 2 - Health Sciences

- | | | |
|------|--|--|
| OP9 | Diagnostic value of BAT-25 and BAT-26 qPCR markers for microsatellite instability in colon cancer | <u>Yadeeshani HDL</u> ,
Abey Suriya V, Rajagopalan U, Abey Suriya V, Akram FN |
| OP10 | A preliminary study targeting exon 12 MSH2 mutations in FFPE rectal tumors: feasibility and challenges | <u>Nufla MAF</u> , Yadeeshani HDL, Abey Suriya V, De Silva S, Abey Suriya |
| OP11 | Identification of genetic variants of <i>PHEX</i> gene in a cohort of children with hereditary hypophosphatemic rickets in Sri Lanka | <u>Milhan MM</u> , Hewage AS, De Silva S, Atapattu N |
| OP12 | Detection of serum levels of human telomerase reverse transcriptase (hTERT) mRNA in non-alcoholic steatohepatitis (NASH) related hepatocellular carcinoma (HCC) in Sri Lanka | <u>Umar UL</u> , Hewage AS, De Silva S, Samarasinghe S, Tennekoon KH, Siriwardana RC, Niriella MA |

Session 3 - Biotechnology

- | | | |
|------|--|--|
| OP13 | Extraction, characterization and optimization of marine algal-derived hydrogels for three-dimensional biomaterials | <u>Darshanamala TMD,</u>
Rajagopalan U, Samarakoon SR |
| OP14 | Isolation of nitrogen fixing and phosphate solubilizing bacteria from diverse soil ecosystems | <u>Prabaharan M,</u> Gunaratna LNR, Weerasena OVDSJ, Premanandarajah P |
| OP15 | Comparison of protein profiles of medically important vipers in Sri Lanka | <u>Perera RMTN,</u> De Kostha YBNS, Handunnetti SM, Gnanathasan CA, Weeratunga PN, Fernando N |
| OP16 | Comparative analysis of flavonoid composition and expression of flavonoid biosynthesis enzymes in <i>Clitoria ternatea</i> varieties | <u>Shanmugaprabha S,</u>
Punyasiri PAN, Weerasena OVDSJ, Gunarathne LNR |
| OP17 | Development of a colorimetric RT-LAMP assay with novel primers for detection of Dengue virus in <i>Aedes aegypti</i> mosquitoes | <u>Janaki MDS,</u> Sanchez-Vargas I, Ratnayake OC, Elmegerhi S, Weerasena OVDSJ, Aryaprema VS, Fernando N, Handunnetti SM, Pathirana PPSL, Tissera HA, Rovnak J, Perera R |

Poster Presentations

- | | | |
|-----|--|--|
| PP1 | Assessing awareness and knowledge of endometriosis and effects of endometriosis on daily functioning and emotional well-being among 18-30 aged women in Colombo District Sri Lanka: A cross-sectional survey | <u>Gamage JV</u> , Dilrukshi SHB, Jayawardena JMTN |
| PP2 | Preliminary genetic analysis of selected PYGL variants in two children with glycogen storage disease Type VI in Sri Lanka | <u>Nadaraja S</u> , De Silva S, Hewage S, Punyasiri N, Jasinge E, Warawita D |
| PP3 | Isolation and molecular identification of lipase producing bacteria in natural oil substrates | <u>Wickramarathna AMP</u> , Weerasena OVDSJ, Gunaratna LNR |
| PP4 | Green synthesis of silver nanoparticles incorporating <i>Bougainvillea glabra</i> leaves: Evaluation of multifunctional properties for industrial use | <u>Naleem H</u> , Kandiah M |
| PP5 | Anti-inflammatory, antibacterial activity and chemical composition of essential oil extracted from <i>Citrus aurantium</i> L leaves using solvent-free microwave extraction technique | <u>Jagoda D</u> , Liyanaarachchi GD, Fernando N, Handunnetti SM, Weeratunge HD, Divisekera W, Samarasekera JKRR |
| PP6 | Comparison of diagnosis of Leptospirosis by microscopic agglutination test and polymerase chain reaction | <u>Gangani PD</u> , de Silva Y, Fernando N, Rajapakse S, Premawansa S, Handunnetti SM |
| PP7 | Therapeutic potential of a nutraceutical formula (Vernolac) in cancer therapy through integrated network pharmacology and computational simulations approach | <u>Gunawardane SDV</u> , Milhan MM, Rathnayaka PC, Gurusinghe PS, Gunaratne KS, Kanagasundaram A, Darshanamala TMD, Senevirathne DC, Senathilake KS, Rajagopalan U, Samarakoon SR |
| PP8 | Synergistic effects of triple combination therapy with thymoquinone, <i>Datura metel</i> and <i>Andrographis paniculata</i> extracts on the proliferation of human lung cancer cells | <u>Milhan MM</u> , Rajagopalan U |

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 Professor Dileep De Silva, Chair Professor and Board-Certified Specialist in Community Dentistry, Faculty of Dental Sciences, University of Peradeniya.

Professor Stanley Wijesundera Memorial Lecture

Professor Dileep De Silva

Chair Professor and Board-Certified Specialist in Community Dentistry

Faculty of Dental Sciences

University of Peradeniya



“Brains, Budgets and Beds: Aligning Health HR, Finance and Education for a Healthier Sri Lanka”

This memorial lecture, delivered in honour of the late Professor Stanley Wijesundera-the inaugural Vice Chancellor of the University of Colombo and a distinguished academic and administrator-critically examines the structural and financial dimensions of Sri Lanka’s allopathic health system, with particular emphasis on human resource development. The lecture presents an analytical overview of both the public and private healthcare sectors, delineating their respective contributions to service delivery and health outcomes. Special focus is placed on the public health sector, which remains central to ensuring equitable and universal access to healthcare services.

A detailed evaluation of national health expenditure is undertaken, including an assessment of health spending as a proportion of Gross Domestic Product (GDP). The principal sources of health sector financing-namely government budgetary allocations, out-of-pocket household expenditures, and external donor contributions-are identified and examined. The distribution of funds between recurrent and capital expenditure is analysed, with reference to its implications for service efficiency, system sustainability, and infrastructure development.

The lecture further addresses the training and deployment of the health workforce, with primary focus on the medical, nursing, and allied health professions. It assesses current capacities in undergraduate and postgraduate education, curricular trends, and the institutional frameworks governing health professional training. Issues related to employability, workforce retention, and international migration are also explored within the context of prevailing fiscal and infrastructural constraints.

In commemorating Professor Wijesundara’s enduring legacy in higher education and public service, this lecture aims to foster a critical discourse on the trajectory of Sri Lanka’s health system. It seeks to identify strategic priorities for reform, with a view to enhancing the efficiency, equity, and quality of healthcare delivery in the country.

Anti-glycemic activity of ethanolic extract and essential oils obtained by solvent-free microwave extraction and hydro-distillation of rhizomes and leaves of *Alpinia calcarata* Roscoe

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Alpinia calcarata Roscoe is a rhizomatous herb used in Ayurveda and traditional Sri Lankan indigenous medicine for treatment of metabolic disorders, including diabetes. Although its anti-diabetic properties have been preliminarily investigated, comprehensive studies evaluating the glycemic regulatory properties of both rhizomes and leaves have not been reported. This study evaluated the anti-glycemic activity of rhizomes and leaves of *A. calcarata* ethanolic extracts (EE) and essential oils (EOs) obtained by hydro-distillation (HD), and Solvent-Free Microwave Extraction (SFME). HD was performed with a Clevenger apparatus, while SFME used a NEOS GR microwave extractor to obtain EOs. EEs of rhizomes and leaves were obtained following standard protocols. Anti-glycemic activity of EOs and EEs were assessed through α -glucosidase and α -amylase inhibition, and an advanced glycation end-product (AGE) formation assay with acarbose as the positive control. The leaf EE exhibited the highest α -glucosidase inhibitory activity ($IC_{50} = 7.05 \pm 0.37 \mu\text{g/mL}$), followed by the rhizome EE ($IC_{50} = 23.79 \pm 0.56 \mu\text{g/mL}$) and both were more active than the standard drug acarbose ($IC_{50} = 430.55 \pm 0.14 \mu\text{g/mL}$). α -amylase inhibitory activity of the rhizome EE was notably higher ($IC_{50} = 5.70 \pm 0.09 \mu\text{g/mL}$) compared to acarbose ($IC_{50} = 76.28 \pm 0.25 \mu\text{g/mL}$). In contrast, the EOs extracted by HD and SFME showed weak inhibition in both enzyme assays, with inhibition values below 10% at 500 $\mu\text{g/mL}$. However, in the anti-AGE formation assay, EOs from leaf HD (13.08 mg RE/g) and leaf SFME (10.17 mg RE/g) showed higher inhibition compared to leaf EE (4.5 mg RE/g) and rhizome EE (3.42 mg RE/g). The EEs of *A. calcarata* rhizomes and leaves are rich in bioactive compounds and indicate strong anti-hyperglycemic effects. Additionally, the moderate anti-AGE activity observed in the EOs may further enhance therapeutic potential of this plant. Overall, these results highlight *A. calcarata* as a promising candidate for studies to identify phytochemicals, isolation and characterization of bioactive constituents and *in vivo* studies for development of natural anti-diabetic formulations.

Keywords: *Alpinia calcarata*, Antiglycemic, Inhibition, Essential oils, Ethanolic extracts

Acknowledgements: Financial assistance by Treasury grant Nos TG 21/203 and TG 23/229.

Synergistic anticancer potential of *Andrographis paniculata* and *Datura metel* combined with Doxorubicin in inhibiting breast cancer cells

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Sri Lanka

Breast cancer remains one of the prevalent malignancies affecting women worldwide. This study investigates the synergistic anticancer potential of triple-combinatorial phytotherapeutic agents, *Andrographis paniculata* and *Datura metel*, along with the chemotherapeutic drug doxorubicin (DOX), on estrogen-receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast carcinoma cells and normal breast epithelial cells (MCF-10A). The objective was to identify a synergistic drug–natural product combination that enhances cytotoxic efficacy while minimizing off-target toxicity. Sequential ultrasound-assisted extractions were performed using hexane, ethyl acetate, methanol, and aqueous-ethanol. SRB-based cytotoxic screening revealed that ethyl acetate extract of *A. paniculata* (AP) and methanolic extract of *D. metel* (DM) were most potent, demonstrating IC₅₀ values at 168.2 µg/mL (MCF-7) and 90.26 µg/mL (MDA-MB-231) for *A. paniculata*, and 0.23 µg/mL (MCF-7) and 21.59 µg/mL (MDA-MB-231) for *D. metel* after 72h, with relatively low cytotoxicity toward MCF-10A. Bioactivity-guided fractionation of *A. paniculata* ethyl acetate extract was performed using normal-phase column chromatography with a 10% gradient solvent system consisting of hexane and ethyl acetate. Methanolic extract of *D. metel* was subjected to Kupchan's solvent-solvent partitioning to isolate active constituents. Fraction C8 of *A. paniculata* (AP-C8) exerted denotable cytotoxicity with IC₅₀ values at 32.99 ± 15.27 µg/mL on MCF-7 and 4.674 ± 0.73 µg/mL on MDA-MB-231 at 48h post-treatment. The chloroform fraction of *D. metel* (DM-F2) demonstrated the highest cytotoxicity with IC₅₀ at 16.04 ± 0.33 µg/mL and 2.535 ± 0.09 µg/mL on MCF-7 and MDA-MB-231, respectively. Triple-combination treatments (DOX + AP-C8 + DM-F2) were assessed in various fixed dose ratios using the SRB assay. CompuSyn software analysis confirmed that the highest synergistic interactions were observed at a ratio of 1:64:256 on MCF-7 (combination index [CI]=0.71846) and at 1:1.25:10 on MDA-MB-231 (CI=0.77291). Both combinations demonstrated higher dose reduction index (DRI) values in a favorable range, especially for DOX, indicating potential for therapeutic dose minimization. Mainly, the combinations exhibited minimal cytotoxic effects in MCF-10A cells, supporting their selective anticancer potential in the selected combination ratios.

Keywords: Synergism, Breast cancer, *Andrographis paniculata*, *Datura metel*, and Doxorubicin

Abstract No: OP3

Anti-cancer potential of a supercritical CO₂ extract of Vernolac nutraceutical, a viable polyherbal formulation

Rathnayake PC, Samarakoon SR, Rajagopalan U, Senathilake KS

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Sri Lanka

Therapeutic resistance and adverse side effects limit the efficacy of conventional cancer treatments, highlighting the need for safer, more potent alternatives. Natural bioactive compounds have gained attention due to their synergistic mechanisms, low toxicity, and reduced potential for resistance. However, conventional extraction methods often fail to extract the spectrum of phytochemicals, which can lead to a loss of bioactive compounds. Supercritical CO₂ extraction provides a safer and more efficient alternative, yielding higher concentrations of active compounds. This study evaluated the anti-proliferative potential of an optimized supercritical CO₂ extract of 'Vernolac', a commercially available polyherbal nutraceutical containing aerial parts of *Vernonia zeylanica* and *Leucas zeylanica*, seeds of *Nigella sativa*, roots of *Hemidesmus indica*, and rhizomes of *Smilax glabra*. Cytotoxicity was assessed by using Sulforhodamine B (SRB) assay on Caco-2, MCF-7, NTERA-2 cl.D1, and normal MCF-10A cells. Induction of apoptosis was evaluated by acridine orange/ethidium bromide staining using fluorescence microscopy and caspase-3/7 activity assays. The scratch assay measured effects on cell migration, expression of apoptosis-related genes (*TP53*, *Survivin*), and autophagy-related gene *mTOR* was quantified and analysed using reverse transcriptase-quantitative PCR. Antioxidant capacity was measured using the DPPH assay, and intracellular ROS levels were determined using the nitroblue tetrazolium assay. The supercritical CO₂ extract showed potent cytotoxicity against NTERA-2 cl.D1 cells (IC₅₀ at 41.12 µg/mL, 48 h) with negligible toxicity toward MCF-10A cells (IC₅₀ > 1000 µg/mL). Based on these results, NTERA-2 cl.D1 cells were selected for further assays. The extract induced early apoptosis, as evidenced by fluorescence microscopy and elevated levels of caspase-3/7 activities. In Vernolac extract-treated cells, up-regulation of *TP53* expression and downregulation of *survivin* and *mTOR* expressions were evidenced, which confirms apoptosis regulation after Vernolac exposure. Additionally, the extract significantly inhibited cell migration and increased intracellular ROS levels. These findings demonstrate that the Vernolac extract possesses strong anticancer activity against cancer stem-like NTERA-2 cl.D1 cells, which supports its potential as a therapeutic candidate for targeting cancer stem cells.

Keywords: *Vernolac, Supercritical CO₂ extraction, Anti-proliferative, Nutraceutical, Cancer stem-like cell*

DC3B, a diterpene obtained from *Caesalpinia pulcherrima* modulates stemness in NTERA-2 cancer stem-like cells

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Cancer is a multi-stage, heterogeneous disease characterized by the uncontrolled proliferation of cells, leading to tissue invasion and metastasis. A growing body of evidence attributes the malignant traits of cancer, including therapy resistance and recurrence, to a subpopulation of tumor cells known as cancer stem cells (CSCs). CSCs possess stem-like properties such as self-renewal, differentiation, sphere formation, and high tumorigenic potential, making them critical contributors to metastasis and treatment failure. The effectiveness of modern cancer therapy is often limited by high toxicity and failure to eliminate CSCs. Natural products have long served as a valuable source of anticancer compounds due to their ability to modulate multiple signaling pathways with relatively lower toxicity. *Caesalpinia pulcherrima*, a plant widely used in traditional medicine, contains several bioactive compounds, among which 6 β -cinnamoyl-7 α -hydroxyvouacapen-5 α -ol (DC3B) has demonstrated significant antiproliferative activity across various cancer cell lines. Previous studies carried out at the Institute of Biochemistry, Molecular Biology, and Biotechnology (IBMBB) revealed that DC3B inhibits the Wnt/ β -catenin pathway, a critical regulator of CSC maintenance, by disrupting β -catenin/TCF interactions. The present study aimed to investigate the stemness-modulating potential of DC3B using NTERA-2 cancer stem-like cells as an in vitro CSC model. Treatment with DC3B significantly reduced colony formation ($p < 0.0001$) indicating suppressed self-renewal. Gene expression analysis further revealed that DC3B markedly downregulated the expression of key stemness-associated genes, *OCT4*, *SOX2*, and *NANOG*. A substantial reduction in AKT2 expression was also observed, suggesting an additional mechanism by which DC3B may impair CSC characteristics through modulation of the PI3K/AKT pathway. Collectively, these findings highlight the potential of DC3B as a CSC-targeting therapeutic agent.

Keywords: *CSCs, Natural products, Wnt/ β -catenin, PI3K/AKT*

**Inhibition of the production of reactive oxygen and reactive nitrogen species in RAW 264.7 cells
by aqueous extract of *Artocarpus heterophyllus***

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Sri Lanka

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) are highly reactive molecules that are secreted by immune cells, during inflammatory responses. ROS and RNS are required for host defense and intracellular signaling. However, overproduction of them can cause damage to macromolecules such as lipids, proteins and DNA leading to cellular damage and dysfunction as well as leading to oxidative and nitrosative stress potentially resulting chronic inflammatory diseases. Medicinal plant-based natural products are rich in flavonoid and phenolic compounds and have gained attention for their potential to modulate ROS and RNS production and to inhibit inflammation. Aqueous preparations of different parts of *Artocarpus heterophyllus* Lam. (Moraceae) are traditionally used in Sri Lankan medicinal practices for treatment of various diseases including immune related diseases such as skin diseases and asthma. In this study, aqueous root extract (ARE) of *A. heterophyllus* was used to determine its effect on ROS and RNS production in stimulated RAW 264.7 murine macrophages. Using MTT assay, ARE concentrations of 62.5-2000 µg/mL were determined as non-toxic to RAW cells and suitable to perform the inhibition assays. Inhibitory effects of ARE on ROS and RNS production in RAW cells were assessed by quantitative nitro blue tetrazolium and Griess assays respectively. ARE treatment significantly inhibited phorbol 12-myristate 13-acetate-stimulated ROS production and lipopolysaccharide-stimulated NO production in dose-dependent manner ($r=0.724$; $p<0.001$ and $r=0.686$; $p<0.001$ respectively). The highest inhibition of ROS production was 71.2 ± 0.65 ($p<0.001$) at 2000 µg/mL of ARE with $IC_{50}=169.04$ µg/mL whereas highest inhibition of RNS production was 38.3 ± 4.9 ($p<0.001$) at 2000 µg/mL of ARE. Further, a strong positive correlation was observed between ROS and RNS inhibition by ARE ($r=0.972$; $p=0.001$). These results demonstrate that ARE can effectively suppress macrophage-derived ROS and RNS validating its anti-inflammatory potential and the use of *A. heterophyllus* in indigenous medicinal practices for immune related diseases.

Keywords: *Artocarpus heterophyllus*, Anti-inflammatory activity, Reactive nitrogen species and Nitric oxide, RAW macrophage

Acknowledgements: This work was supported by the MSc program in Cellular and Molecular Immunology, IBMBB, University of Colombo and constitutes a part of MSc studies of WGMCHP.

Abstract No: OP6

The *in vitro* effect of *Andrographis paniculata* on the viability and morphological changes induced by Vascular Endothelial Growth Factor on endothelial cells

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Vascular leakage, a hallmark of dengue hemorrhagic fever, is attributed to complex interactions between dengue viral (DENV) factors and the host immune response. Significantly elevated levels of Vascular Endothelial Growth Factor (VEGF) have been continuously reported in dengue hemorrhagic fever (DHF) patients, rendering it a potential biomarker of severity. Further, VEGF is known to drive the endothelial hyperpermeability, resulting in vascular leakage. *Andrographis paniculata* is a widely used medicinal plant in Sri Lankan traditional medicine, demonstrating promising antiviral activities against dengue. In this study, we examined its ability to modify the viability and morphological changes of the VEGF-induced EA.hy926 cells (EC). The effects of selected VEGF concentrations (50-2000 pg/mL) on EC were assessed using the Sulforhodamine B (SRB) assay, which were then compared with the results obtained after *A. paniculata* post-treatment. EC viability was >80% across all VEGF concentrations tested, indicating that VEGF was not toxic across all concentrations. Morphological observations revealed a prominent gap formation in the EC monolayer at high VEGF concentrations (>500 pg/mL), marking a 30-60% cell reduction in 50% of the observable view under the inverted light microscope at 20X magnification. The *A. paniculata* treatment of the VEGF-induced EC monolayer yielded over 80% EC viability at all VEGF concentrations (similar to that of VEGF-only). Further, the morphological observations revealed that VEGF-induced EC treated with *A. paniculata* maintained a tight monolayer with a 5-20% increase in the number of cells observed under the microscope than at high VEGF. These results show that *A. paniculata* post-treatment had effectively reversed the VEGF-induced morphological changes of the EC monolayer, highlighting its potential therapeutic use in DHF.

Keywords: *Dengue, VEGF, Andrographis paniculata, Endothelial hyperpermeability, Vascular leakage*

Acknowledgements: *This work was supported jointly by the MSc program in Cellular and Molecular Immunology, IBMBB, University of Colombo and authors JKR.*

Abstract No: OP7

In vivo* pharmacokinetic and tissue distribution studies of a natural anticancer diterpene isolated from *Caesalpinia pulcherrima

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A cassane-type diterpene, 6 β -Cinnamoyl-7 α -hydroxyvouacapen-5 α -ol (DC3B), is isolated from *Caesalpinia pulcherrima* and has demonstrated promising *in vitro* anticancer potential. This study aimed to evaluate the pharmacokinetics and tissue distribution of DC3B in male Wistar rats. A reverse-phase high-performance liquid chromatography method (RP-HPLC/UV) was developed and validated according to the United States Food and Drug Administration (US FDA) guidelines for bioanalytical method validation, using a C18 column with a methanol–water gradient and UV detection at 275 nm. DC3B and the internal standard (propyl paraben) eluted at 8 and 4 minutes, respectively. The RP-HPLC method was fully validated for specificity, selectivity, linearity ($R^2 > 0.999$), sensitivity, accuracy, precision, extraction recovery, matrix effect, carryover, and dilution integrity across all matrices. The validated HPLC method was applied to quantify DC3B in rat plasma and tissues. DC3B was administered orally at a single dose of 200 mg/kg using corn oil as the vehicle. Plasma and tissue samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, and 24 h post-dose. The compound was absorbed with a T_{max} of 4 h, a C_{max} of 1314.12 ± 31.41 ng/mL, and a plasma elimination half-life ($t_{1/2}$) of 1.37 h in plasma. DC3B was distributed to major organs, with peak concentrations (C_{max}) observed in the liver (1752.41 ng/mL), stomach (27549.02 ng/mL), small intestines (23377.15 ng/mL), lungs (1139.84 ng/mL), kidneys (702.47 ng/mL), heart (1807.90 ng/mL), and spleen (704.56 ng/mL). Notably, DC3B penetrated sanctuary sites such as the brain and testes, with C_{max} of 408.94 and 2881.99 ng/mL, respectively. Although DC3B exhibited delayed absorption, it demonstrated substantial and widespread tissue distribution. Its accumulation in the above organs highlights its capacity to reach protected or metastatic niches, reinforcing its potential as a cancer stem cell-targeting agent. These findings support oral administration as a viable route and underscore the need for formulation and dosing strategy refinements to improve systemic exposure and therapeutic outcomes.

Keywords: *Cassane diterpene, Caesalpinia pulcherrima, pharmacokinetics, tissue distribution, RP-HPLC/UV*

QSAR-Driven Discovery of Novel Anticancer Derivatives from a Natural Halogenated Scaffold

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Natural halogenated compounds have emerged as promising scaffolds for anticancer drug discovery. A previously reported halogenated compound (CMF) from *Mangifera zeylanica*, an endemic plant of Sri Lanka, demonstrated selective cytotoxic activity against triple-negative breast cancer cells (MDA-MB-231; IC₅₀ < 50 µg/mL), with lesser cytotoxicity towards normal breast epithelial cells (MCF-10A; IC₅₀ = > 100 µg/mL). However, its moderate potency limited its therapeutic potential. Therefore, the present study aimed to improve the anticancer efficacy of CMF. A quantitative structure–activity relationship (QSAR) model was developed to predict the activity profiles of structurally modified predicted derivatives of CMF. Computational screening revealed several candidate molecules with higher predicted activity compared to the parent compound. Guided by these results, selected derivatives were synthesized using standard synthetic protocols and structurally characterized. In vitro cytotoxicity assays demonstrated that these novel derivatives exhibited significantly enhanced activity against MDA-MB-231 cells while maintaining favorable selectivity over MCF-10A cells. These findings validate the predictive power of the QSAR model and highlight the value of integrating computational design with synthetic chemistry to optimize natural product–derived anticancer leads. This study establishes three among ten synthesized halogenated derivatives (Compound K1, K2, and K3) of CMF, as potential candidates for further development against TNBC, a highly aggressive breast cancer subtype lacking effective targeted therapies.

Keywords: *Mangifera zeylanica*, triple-negative breast cancer, QSAR, natural product derivatives, drug design

Diagnostic value of BAT-25 and BAT-26 qPCR markers for microsatellite instability in colon cancer

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Colon cancer is a leading cause of cancer-related morbidity and mortality worldwide. Microsatellite instability (MSI), reflecting deficient DNA mismatch repair, is present in a subset of colon cancers and may serve as a useful diagnostic marker. This study aimed to detect MSI in colon cancer patients by analyzing BAT-25 and BAT-26 microsatellite markers using quantitative PCR (qPCR) in a Sri Lankan cohort. In a cross-sectional design, DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissues of ten patients who were confirmed as colon cancer patients by histopathological investigations. Five patients whose biopsy samples were confirmed negative for colon cancers, colon or rectal polyps were selected as controls. Comorbidities which effect on the MSI status wasn't reported in the cohort. qPCR was performed to determine cycle threshold (Ct) values for BAT-25 and BAT-26. Group comparisons used t-tests, Mann-Whitney U, and ANOVA as appropriate. Logistic regression assessed the diagnostic potential of Ct values. ROC analysis determined the discriminatory performance of BAT-25 and BAT-26. Both BAT-25 and BAT-26 showed significantly different Ct values between patients and controls ($p < 0.05$). Lower (more negative) BAT-25 and BAT-26 values were associated with earlier T, N, and M stages. Logistic regression identified BAT-25 as a significant predictor of patient status ($p = 0.041$, OR = 0.82, 95% CI: 0.50–0.94). ROC analysis showed good discriminatory power for both markers, with area under the curve (AUC) values of 0.72 (BAT-25) and 0.80 (BAT-26), and specificity up to 80% in BAT 25 and 100% for BAT-26. qPCR-based analysis of BAT-25 and BAT-26 effectively distinguishes colon cancer patients from controls and correlating with disease stage. These findings support the utility of BAT-25 and BAT-26 as molecular markers for MSI detection in colon cancer, with potential diagnostic applications in clinical practice.

Keywords: *Colon Cancer, Microsatellite instability, BAT-25, BAT-26, qPCR*

Abstract No: OP10

A preliminary study targeting exon 12 *MSH2* mutations in FFPE rectal tumors: feasibility and challenges

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Rectal cancer constitutes a major burden of colorectal malignancies in Sri Lanka. Despite growing disease burden, Sri Lanka lacks molecular data and genomic profiles, particularly on Mismatch repair (MMR) gene mutations. *MSH2* is a frequently mutated MMR gene, associated with microsatellite instability-high (MSI-H) tumors. This pilot study explores the feasibility and limitations of detecting *MSH2* exon 12 mutations using formalin-fixed paraffin-embedded (FFPE) tissue the most accessible archival resource in local clinical settings. Twelve FFPE tumor samples and four control blocks were sectioned and processed using the QIAamp DNA FFPE Tissue Kit. DNA was quantified via NanoDrop spectrophotometry and agarose gel electrophoresis. Primers targeting exon 12 (471 bp) were designed using Primer-BLAST, BioEdit, and IDT OligoAnalyzer. PCR optimization was initially conducted with high-quality blood-derived DNA, then adapted for FFPE DNA using incremental changes in annealing temperature, MgCl₂ concentration (2–3.5 mM), and template pre-treatment (dilution, 95 °C incubation). Amplified products were visualized, gel-purified using low-melting agarose, and sequenced via Sanger method. Blood DNA consistently yielded specific 471 bp products. In contrast, FFPE DNA yielded smeared products or weak bands, often accompanied by primer-dimers. Gel-purified products from FFPE samples were successfully extracted but sequencing chromatograms showed poor peak resolution, baseline noise, and unreadable sequences, confirming insufficient template quality for Sanger sequencing. Amplification and sequencing of *MSH2* exon 12 from FFPE rectal tumor DNA proved technically unviable due to extensive fragmentation and chemical modifications inherent to formalin fixation. Large-amplicon PCR from FFPE was not consistently achievable despite multi-parameter optimization, indicating that prolonged/formalin fixation and block age are dominant constraints on amplifiability in this setting. Future efforts should employ Standardizing fixation, enforcing block-age thresholds shorter amplicon designs, FFPE-specific polymerases, and enzymatic repair steps to enable reliable mutation detection from archival tissue. By systematically confronting the technical barriers of FFPE tissue genomics in a Sri Lankan context, this pioneering study not only highlights the urgent need for protocol innovation but also lays the foundation for accessible, population-scale molecular diagnostics in underrepresented cancer cohorts.

Keywords: Rectal cancer, *MSH2* exon 12, FFPE, DNA fragmentation, optimization

Abstract No: OP11

Identification of genetic variants of *PHEX* gene in a cohort of children with hereditary hypophosphatemic rickets in Sri Lanka

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Hereditary hypophosphatemic rickets (HHR) comprises a group of rare genetic disorders characterized by renal phosphate wasting leading to chronic hypophosphatemic rickets. The most common form, X-linked hypophosphatemic rickets (XLHR), arises from inactivating mutations in the phosphate-regulating endopeptidase homolog X-linked (*PHEX*) gene, which disrupts phosphate homeostasis through dysregulation of the phosphaturic hormone FGF23. Although more than 800 pathogenic *PHEX* variants have been reported globally, the genetic variants in Sri Lankan patients remain unexplored. This study aimed to identify the genetic variants of XLHR in Sri Lankan pediatric patients. Eleven children clinically diagnosed with hypophosphatemic rickets underwent comprehensive evaluation, including biochemical analyses, radiological assessments, and phenotypic characterization. PCR amplification followed by targeted Sanger sequencing was performed on *PHEX* exons 17, 19, and 22, along with flanking intronic regions. Pathogenicity predictions were conducted using MutationTaster and SpliceAI algorithms. The index patient (Male) harboring a novel variant was diagnosed at one year and five months of age and exhibited hypophosphatemia (0.9 mmol/L, approximately 25% below the normal lower limit for age), markedly elevated alkaline phosphatase (682 U/L, nearly twofold above normal range), serum calcium within normal limits (2.31 mmol/L), and elevated parathyroid hormone (8.06 pmol/L). Radiographs revealed classic rickets features. Genetic analysis identified a novel intronic deletion variant, c.1900-31_1900-30del, within the polypyrimidine tract 30 base pairs upstream of exon 19. This two-nucleotide deletion reduces a consecutive thymine stretch from 12 to 10 bases, potentially disrupting splice acceptor site recognition and pre-mRNA splicing. MutationTaster predicted the variant as disease-causing with possible cryptic splice site generation, while SpliceAI assigned low pathogenicity scores, illustrating the complexity of non-coding variant interpretation. This is the first report of an intronic polypyrimidine tract deletion in the *PHEX* gene in Sri Lankan patients. The variant's associated clinical phenotype supports its pathogenicity. Functional validation studies are needed to confirm these findings.

Keywords: *X-linked hypophosphatemic rickets, PHEX gene, polypyrimidine tract, splice site, hypophosphatemia*

Detection of serum levels of human telomerase reverse transcriptase (*hTERT*) mRNA in non-alcoholic steatohepatitis (NASH) related hepatocellular carcinoma (HCC) in Sri Lanka

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NAFLD is a spectrum that can progress from simple liver steatosis to NASH-HCC, which is the most advanced and life-threatening stage, with increasing incidence and prevalence globally. This is attributed to its several predisposing risk factors, including genetic factors. Dysregulation of one of the critical key driver genes called human telomerase reverse transcriptase (*hTERT*) was reported to influence HCCs' pathogenesis. Traditionally, *hTERT* catalyses the insertion of the short DNA sequences (TTAGGG) to newly replicated telomeres ends during normal cell division. However, its overexpression prevents the hepatocytes from undergoing traditional apoptosis, making them immortal. Currently, there is no study on the impact of *hTERT* expression levels specifically from NASH-HCC patients in Sri Lanka. Hence, this study aimed to be the first study to quantify the serum level of *hTERT* mRNA from NASH-HCC patients, hoping to contribute to identifying it as a potential non-invasive diagnostic biomarker for early diagnosis of NASH-HCC. Quantitative PCR (qPCR) was used to quantify the expression of *hTERT* mRNA levels from NASH-HCC and cirrhotic age, BMI and gender - matched controls. The analysis revealed an elevated expression of *hTERT* mRNA in the NASH-HCC cohort, with a mean level of 9.11 ± 8.44 , representing a sevenfold increase compared to the cirrhotic age, BMI, and gender-matched controls, which exhibited a mean expression level of 1.3 ± 1.49 . Furthermore, alanine aminotransferase (ALT) and tumor nodularity correlated with *hTERT* mRNA expression from NASH-HCC patients. We realized higher expression of *hTERT* in NASH-HCC patients than in the cirrhotic matched-control cohort. However, it is recommended to further evaluate the link between *hTERT* mRNA expression levels and some of its predisposing genetic driving risk factors, such as pathogenic *hTERT* promoter's SNPs and related key transcription factors, using a larger sample size of NASH-HCC patients; this may suggest *hTERT* level as a promising NASH-HCC diagnostic biomarkers.

Keywords: HCC, NASH-HCC, *hTERT* mRNA, Diagnosis, Biomarker

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Extraction, characterization and optimization of marine algal-derived hydrogels for three-dimensional biomaterials

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Marine algae are valuable sources of polysaccharides with wide ranging applications in biomaterial development. In the present study, four marine algae species, *Kappaphycus alvarezii*, *Sargassum* spp. (two species), and *Turbinaria conoides* were selected for evaluation as potential biomaterial sources. Identification of the algal species was made using morphological characteristics and molecular barcoding using *ITS* primers. Algal polysaccharides were extracted using the hot alkaline extraction method. Carrageenan was extracted from the red alga *K. alvarezii* with a high yield of 51.78%. Alginate was extracted from the brown algae with a yield weight of 42.65% and 42.59% from the two *Sargassum* spp. and *T. conoides* respectively. The chemical composition of the polysaccharide extracts was confirmed by fourier transform infrared (FTIR) spectroscopy, revealing characteristic sulphate ester peaks in carrageenan and guluronic and mannuronic acids in alginate. Optimization studies were conducted to enhance the properties of hydrogel formation. Parameters relevant to gelation properties were evaluated including solubility, gelation capacity and time, calcium chloride concentration, pH stability, swelling ratio, turbidity and melting and freezing temperatures. Optimum hydrogel formulations were achieved with 1.0 M CaCl₂ in carrageenan 2% (w/v) and with 0.5M CaCl₂ in alginate 1% (w/v). The optimized hydrogel formulation combined high gel strength, low turbidity, stable swelling behaviour, and thermal stability suitable for physiological conditions. The materials produced in this study present a promising, sustainable, and biocompatible platform for future applications in biomedical engineering and advanced three-dimensional cell culture systems.

Keywords: *Algal hydrogels, Carrageenan, Alginate, Three-dimensional cell culture, Biomaterial*

Isolation of nitrogen fixing and phosphate solubilizing bacteria from diverse soil ecosystems

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The plant growth promoting microorganisms stimulate the plant growth by enhancing nutrient uptake, nitrogen fixation and phosphate solubilization. However, the identification and application of indigenous soil bacteria with these potential is in emerging stage. The objective of the study was to isolate and identify high nitrogen fixing and phosphate solubilizing bacteria from different regions in Sri Lanka, to determine their potential in formulation of a biofertilizer. In this study, nitrogen-fixing and phosphate-solubilizing bacteria were isolated from soil samples collected from 11 geologically different locations, including paddy fields, coconut estates and agricultural lands. A total of 32 morphologically different bacteria were isolated after serially diluting soil samples and screening them on selective media (Burk's nitrogen-free medium and NBRIP agar medium) yielding 16 nitrogen-fixing and 18 phosphate-solubilizing isolates, respectively. Among them, two isolates—Isolate I-30 and Isolate I-25 showed the highest activities of nitrogen fixation and phosphate solubilization. Quantitative assays confirmed that Isolate I-30 released 70.03 mg/L of ammonia and solubilized 213.20 mg/L of phosphate, while Isolate I-25 released 54.92 mg/L of ammonia and solubilized 174.50 mg/L of phosphate. Using 16S rRNA gene sequencing, Isolate I-30 was identified as *Bacillus aryabhattai* and Isolate I-25 as *Priestia megaterium*. Both isolates are non-pathogens, and a bacterial antagonism assay indicated their compatibility by showing their non-antagonistic interaction. These results suggest the combination of both the isolates hold promise for biofertilizer formulation, to increase soil fertility and advance sustainable agricultural practices. The formulation is currently in progress.

Keywords: *Plant growth-promoting bacteria, Nitrogen fixation, Phosphate solubilization, Biofertilizer formulation*

Comparison of protein profiles of medically important vipers in Sri Lanka

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Snakebite envenomation is a neglected tropical disease causing over 80,000 deaths globally each year. In Sri Lanka, approximately 80,000 bites and 400 deaths occur annually, mostly affecting rural communities. Russell's viper, Hump-nosed pit viper, and Saw-scaled viper are considered as the medically important vipers in Sri Lanka. Accurate identification of the envenomed species remains challenging due to overlapping clinical symptoms and the lack of species-specific diagnostic tools resulting in challenges in patient management. This study compared the protein profiles of the three viper venoms using SDS-PAGE. Crude venom (25 µg) of each viper was electrophoresed with 15% SDS-PAGE under reducing conditions at 60 V for 5 hours alongside pre-stained molecular weight marker (10-250 kDa). Proteins were visualized using Coomassie Brilliant Blue staining. SDS-PAGE analysis resulted in distinct protein banding patterns in all three venoms, and the detected proteins ranged from 10 to 118 kDa. Several conserved and species-specific venom components were identified in protein comparison. Common bands across all species were detected at 83, 39, 21 and 10–12 kDa positions and those could be possibly implicated as L-amino acid oxidase, snake venom metalloproteinase, snake venom serine protease, and phospholipase A2, respectively, consistent with previous literature. Unique proteins observed in Russell's viper ranged from 118 – 106 kDa and 58-68 kDa, in Hump-nosed pit viper between 47–37 kDa and 33–29 kDa, in Saw-scaled viper between 118 - 110 kDa. These differences highlight venom diversity with implications for venom-specific diagnostics and antivenom development.

Keywords: *Snakebite, Viper venom proteins, Russell's viper, Hump-nosed pit viper, Saw-scaled viper*

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Comparative analysis of flavonoid composition and expression of flavonoid biosynthesis enzymes in *Clitoria ternatea* varieties

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Clitoria ternatea is a medicinal plant valued for its flavonoid profile and pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. Although the blue-flowered variety, characterized by anthocyanins such as delphinidin derivatives, has been studied extensively, biochemical and molecular differences among flower colour variants remain less understood. This study aims to compare flavonoid composition and expression of key biosynthetic enzymes: dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and flavonol synthase (FLS) in blue, purple, and white-flowered varieties. Flavonoids were extracted from flowers and leaves and quantified via HPLC using authenticated standards of myricetin, quercetin, kaempferol, luteolin, apigenin, delphinidin, and cyanidin. Gene-specific primers for DFR, ANS, and FLS were designed, and relative expression was analyzed by RT-qPCR using the Livak method, with the white variety as calibrator due to its minimal pigmentation. Reference gene primers were adapted from a validated study and verified against the *C. ternatea* genome (PRJNA1016062; accession KAK7264054.1). Myricetin and kaempferol were more abundant in leaves, whereas quercetin and luteolin were higher in flowers. Quercetin content was highest in the white variety and lowest in the blue. Apigenin predominated in leaves, delphinidin was exclusive to blue flowers, and cyanidin was absent in all samples. DFR was upregulated in blue (18.176-fold) and purple (4.257-fold) relative to white flowers. ANS was upregulated in blue (2.488-fold) but downregulated in purple (0.595-fold) flowers. FLS remained relatively stable (0.765 and 0.883-fold in blue and purple flower varieties respectively) and slightly downregulated across all variants. Due to limited reagents and time, p-values were not generated. Blue flowers exhibit high ANS and DFR expressions, which promotes the production of anthocyanin, particularly delphinidin, responsible for their blue coloration. Purple flowers exhibit moderate DFR but downregulated ANS, indicating alternative pigment or regulatory pathways despite visible pigmentation. White flowers display higher FLS expression, favouring flavonol synthesis over anthocyanins, consistent with their elevated flavonol content, highlighting gene-specific contributions to flower colour variation.

Keywords: *Clitoria ternatea*, Flavonoids, Dihydroflavonol 4-reductase, Anthocyanidin synthase, Flavonol synthase

Development of a colorimetric RT-LAMP assay with novel primers for detection of Dengue virus in *Aedes aegypti* mosquitoes

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Dengue poses a threat to public health both globally and locally. Rapid detection of the Dengue virus (DENV) in mosquitoes is crucial for prompt and proactive control measures and interventions. Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) provides a rapid, sensitive, and affordable solution for this purpose. RT-LAMP for detection of all four DNV serotypes using 24 primers (6 primers per each serotype) is currently available. The objective of this study was to find out the possibility of developing a new RT-LAMP assay by reducing the number of primers required. A comprehensive bioinformatic analysis was carried out using Geneious software to identify a conserved region in all four serotypes (DENV 1-4). However, consistently conserved regions for all serotypes was not found to develop a simple assay to detect all four serotypes. Therefore, based on previous studies which targeted 3' untranslated region (3'UTR) of DENV 2, two separate sets of primers (5 primers per each set) were designed; Primer set 1 using Eiken's Primer Explorer V4 and primer set 2 using NEB LAMP Primer Design Tool. Both primer sets were assessed using RNA from cultures of all four dengue serotypes and WarmStart Colorimetric LAMP master mix (NEB, USA) including UDG and Syto 9 green, fluorescent dye. Primer set 1 showed positive color changes and peak amplifications specificity only for DENV 2 RNA within 40 minutes. Primer set 2 showed positive reactions with DENV 2 first, followed by DENV 3 and 4, within 40 minutes. Based on the results primer set 2 (5 primers) was selected and as a further development, DNV 1 detection primer set (6 primers) from above currently available LAMP assay was added for the detection of DNV 1. However, when the assay was conducted with extracts (homogenates) from *Aedes aegypti* mosquitoes infected with each of the four serotypes, the assay was positive only with extracts of mosquitoes infected with DENV 1 or 2, indicating that mosquito extracts resulting weaker reactions compared to purified viral RNA, emphasizing the need of further optimization of assay with the novel primers.

Keywords: Dengue, Dengue virus serotypes, RT-LAMP, *Aedes aegypti*, primer optimization

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Assessing awareness and knowledge of endometriosis and effects of endometriosis on daily functioning and emotional well-being among 18-30 aged women in Colombo district Sri Lanka: A cross-sectional survey

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Endometriosis affects women globally, particularly in low-resource countries like Sri Lanka, due to poor awareness and improper reproductive health practices, leading to diagnosis delays and severe cases. The aim of this research is to analyze the awareness level of endometriosis among young women and impact on their quality of life. A total of 200 women aged 18-30 from various demographic backgrounds participated, because educating women at this age promotes early recognition, timely care, and reduces negative impacts on quality of life while preparing them to guide future generations. A short, closed-ended questionnaire based on the Endometriosis Health Profile-30 was given to each participant. The findings revealed a significant gap in awareness and knowledge of endometriosis among young women in the Colombo District. Only 80 individuals reported awareness of the condition and 60% of diagnosed individuals reported initial misdiagnoses or uncertainty about their diagnosis. Statistical analysis using the Chi-square test confirmed a significant association between education level and awareness ($p = 0.005$), indicating that women with postgraduate and undergraduate education are more likely to be aware of endometriosis compared to those with certificate or diploma qualifications. Women who recently used healthcare services were 4 to 12 times more likely to be aware of endometriosis than women who did not use healthcare services recently. A Mann–Whitney U test revealed that individuals diagnosed with endometriosis experienced significantly more limitations in managing daily activities, higher levels of pain affecting daily functioning (median = 2, IQR = 2), and a significant difference in perceived emotional support (median = 1, IQR = 0) compared to undiagnosed individuals' daily activity management (median = 2, IQR = 1) and emotional support (median = 1, IQR = 0). The research findings emphasize the immediate need to acknowledge the reproductive age women population to improve their quality of life.

Keywords: *Awareness Level; Endometriosis; Low-resource countries; Quality of life; Reproductive health*

Preliminary genetic analysis of selected PYGL variants in two children with Glycogen Storage Disease Type VI in Sri Lanka

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Glycogen Storage Disease Type VI (GSD VI), or Hers disease, is a rare autosomal recessive metabolic disorder resulting from pathogenic variants in the *PYGL* gene, which encodes liver glycogen phosphorylase, an essential enzyme in glycogenolysis. Despite its clinical significance, the genetic landscape of GSD VI remains largely unexplored within the Sri Lankan population. The current study aimed to conduct a preliminary molecular investigation by screening three selected *PYGL* variants: c.280C>T, c.434T>G, and c.1620+1G>A in two pediatric patients clinically diagnosed with GSD VI. Genomic DNA was extracted from peripheral blood samples, followed by primer design, polymerase chain reaction (PCR) optimisation, and Sanger sequencing. Both patients presented with classical clinical features, including hepatomegaly and elevated hepatic transaminases; however, none of the targeted *PYGL* variants were identified in either patient. These findings suggest potential genetic heterogeneity and the likelihood of uncharacterised or novel *PYGL* mutations within the Sri Lankan cohort. The current study can be extended to develop a mutation spectrum for GSD VI, thereby improving genetic testing and counselling, which may assist in treatment management for affected individuals in Sri Lanka.

Keywords: *Glycogen Storage Disease (GSD), GSD Type VI, PYGL gene, liver glycogen phosphorylase and hepatomegaly*

Isolation and molecular identification of lipase producing bacteria in natural oil substrates

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Lipases are biocatalysts with deserving ability to catalyze hydrolysis (of triglycerides), esterification (between free fatty acids and alcohols) and transesterification (between esters and alcohols) reactions under mild conditions. The increasing demand for lipases across various industries highlights the need of microbial sources that are capable of making high enzyme yield and activity. The main objective of this study was to isolate and molecular identification of the lipase producing bacteria from natural oil substrates; coconut, peanut, corn and grated coconut meat residue (GCMR). Different bacterial isolates obtained from oil substrates were cultured separately in nutrient agar (NA) medium. Bacterial isolates were then screened for lipase activity using phenol red and tributyrin agar media. The isolate obtained from corn demonstrated highest lipase activity and the activity was further assessed quantitatively (as 153.48 U/mL) by spectrophotometric assay, using p-nitrophenyl palmitate (pNPP) as the substrate. The highest lipase producing bacterium was subjected to molecular identification. The bacterial deoxyribonucleic acid (DNA) was extracted using modified boiled cell method and the 16S rRNA universal barcoding region was amplified by polymerase chain reaction (PCR). The amplicon was sequenced bidirectionally and the sequence was searched over GenBank database using Basic Local Alignment Search Tool (BLAST). The bacterium was identified as *Staphylococcus pasteurii*. The lipase produced by the bacteria was partially purified using ammonium sulfate ((NH₄)₂SO₄) precipitation.

The results demonstrate that the potential of *Staphylococcus pasteurii* as efficient microbial sources of enzyme lipase, highlighting their relevance in future industrial and biotechnological applications.

Keywords: *Lipase, Staphylococcus pasteurii, Molecular identification*

**Green synthesis of silver nanoparticles incorporating *Bougainvillea glabra* leaves:
Evaluation of multifunctional properties for industrial use**

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Recent advancements in nanotechnology have led to groundbreaking discoveries in various scientific fields, emerging as a powerful tool for solving global challenges in industrial waste management. This study explores the eco-friendly green synthesis of silver nanoparticles (AgNPs) and its multifaceted applications. The aim of this research was to synthesize AgNPs using five varieties of *Bougainvillea glabra* (white, red, variegated magenta, pink and orange). Out of which, three varieties (red, variegated magenta and orange) yielded AgNPs while using water extracts (WE) of leaves and silver nitrate at the optimum conditions of 90° C and 60 minutes. Characterisation by UV-Visible spectrophotometry and scanning electron microscopy confirmed AgNPs peak at 400 nm and spherical Orange-AgNPs between 30-50 nm, respectively. AgNPs showed significantly greater total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) than WE. Catalytic and photo-catalytic activities were tested by para-nitrophenol (PNP) degradation, and methylene blue (MB) and methyl orange (MO) degradation under sunlight respectively. All AgNPs (4000 ppm) degraded PNP within 15 minutes, where the highest degradation was observed with red-AgNPs ($k=0.0627$). Whereas, only variegated-magenta-AgNPs (4000 ppm) showed some MO degradation ($k=0.0041$). Cytotoxicity was evaluated using *Artemia salina* and presented 100% viability. The antibacterial activity of AgNPs was assessed using *Escherichia coli* and *Staphylococcus aureus*, demonstrating WE and AgNPs had no significant differences ($p=0.450712$ and 0.629398 respectively). Melamine adulteration (4 ppm) in spiked milk was detected by Orange-AgNP (4000 ppm). The present study revealed that *B. glabra* leaves mediated-AgNPs have diverse functional properties, presenting a promising avenue for future research and potential practical applications that contribute to sustainably solve major world problems such as antibacterial resistance, melamine adulteration and AgNPs toxicity.

Keywords: *Silver nanoparticles, Bougainvillea glabra, Catalysis, Photodegradation, Cytotoxicity*

Anti-inflammatory, antibacterial activity and chemical composition of essential oil extracted from *Citrus aurantium* L leaves using solvent-free microwave extraction technique

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Citrus aurantium is known for its therapeutic properties in managing inflammation associated with disease conditions. Its essential oil (EO) is valued for antiseptic, antimicrobial, astringent properties and is used in aromatherapy, to alleviate anxiety and pain. This study presents the first systematic evaluation of the chemical composition, anti-inflammatory and antibacterial activity of EO extracted from the leaves of *C. aurantium*, using solvent-free microwave extraction (SFME) technique. Fresh leaves (200 g) were subjected to SFME using the NEOS GR microwave extractor to obtain the EO. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the chemical composition. Anti-inflammatory activity was evaluated by the inhibitions of enzymes arachidonate 5-lipoxygenase (A5-LOX), arachidonate 15-lipoxygenase (A15-LOX) activities, and inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages. Antibacterial activity of EO was evaluated by agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhimurium*, and *Candida albicans*. GC-MS analysis identified D-limonene (25.35%) and α -Citral (17.00%) as major compounds in *C. aurantium* EO. Dose-dependent inhibitions of A5-LOX and A15-LOX activities were recorded with IC₅₀ values of 6.97 $\pm$ 0.85 and 411.24 $\pm$ 2.23 μ g/mL respectively ($r=0.995$ & 0.979 ; $p<0.05$) which was significantly higher compared to the reference standard baicalein (IC₅₀: 2.09 $\pm$ 0.19 μ g/mL; $p<0.05$). The *C. aurantium* EO dose-dependently inhibited NO production ($r=0.987$; $p<0.05$), with 74.44 $\pm$ 0.58% inhibition at a non-toxic concentration of 1000 μ g/mL in LPS-stimulated RAW 264.7 cells, with IC₅₀ value of 391.06 $\pm$ 31.43 μ g/mL. The EO exhibited moderate antimicrobial activity with an inhibition zone of 10 mm for both *E. coli*, and *B. subtilis* at 1000 μ g/mL compared to the standard antibiotic, ciprofloxacin (5 mcg/disc). These findings on significant inhibition of A5-LOX, and NO production may support the therapeutic potential of *C. aurantium* leaf EO and emphasize the importance of sustainable and efficient extraction methods for its industrial applications in pharmaceuticals and cosmetics.

Keywords: Solvent-free microwave extraction, Gas Chromatography-Mass Spectrometry, Essential oil, Anti-inflammatory activity

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Comparison of diagnosis of Leptospirosis by microscopic agglutination test and polymerase chain reaction

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Leptospirosis is a zoonotic disease caused by spirochaetes of genus *Leptospira*. Sri Lanka is considered as a hotspot for leptospirosis. Leptospirosis includes wide spectrum of clinical features ranging from mild fever to multi-organ dysfunction which mimics many other infectious diseases. The objective of this study was to detect *Leptospira* DNA using *flaB* nested PCR in a set of patient samples (n=105) which had been previously tested for leptospirosis using Microscopic Agglutination Test (MAT). Patient blood samples collected during May 2012 and October 2014, having days of onset of symptoms <10 and clinically characterised as severe or mild were used. MAT positivity was considered as having a titer of ≥ 400 in acute serum, a four-fold rise in MAT titer between acute and convalescent sera and seroconversion ≥ 100 in paired sera. DNA was extracted from whole blood samples and *flaB* nested PCR was carried out as described previously. Of the 105 samples, 75 (71%) showed MAT positivity and 41 samples (39%) were positive in *flaB* nested PCR. No significant differences were observed in days of onset of symptoms in both MAT positive and negative patients as well as PCR positive and negative patients. Within the MAT positive group, 45% were negative by *flaB* nested PCR. Among the MAT positive patients, the proportion of PCR positive, severe leptospirosis patients (68%) was significantly higher than those who were PCR positive and having mild leptospirosis ($p < 0.005$). Considering MAT as a reference standard, *flaB* nested PCR showed 54.66% sensitivity and 100% specificity. Together, these findings are consistent with previous studies indicating that a combination of positivity through MAT and PCR is more suitable as a diagnostic option for confirming leptospirosis which is defined as a biphasic infection.

Keywords: *Leptospira*, Sri Lanka, Microscopic Agglutination Test, *flaB* nested PCR, days of symptoms

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Therapeutic potential of a nutraceutical formula (Vernolac) in cancer therapy through integrated network pharmacology and computational simulations approach

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Vernolac is a commercially available polyherbal nutraceutical formulation comprised of *Vernonia zeylanica* aerial parts, *Nigella sativa* seeds, *Hemidesmus indicus* roots, *Leucas zeylanica* aerial parts, and *Smilax glabra* rhizome. The present study employed an integrative network pharmacology-based approach with *in vitro* validation to explore the anticancer potential of Vernolac. Phytochemicals present in Vernolac were retrieved from public databases and filtered based on drug-likeness using SwissADME. Protein targets of phytochemicals were predicted using SwissTargetPrediction and intersected with cancer-related targets retrieved from GeneCards to identify common targets. Common targets were used to construct a protein-protein interaction network using the STRING database and analyzed in Cytoscape to identify core targets based on degree. Clustering, topology, hub node analysis, and formula-herb-compound-target-disease network construction were performed on core targets using Cytoscape. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed to identify key pathways regulated by phytochemicals in Vernolac. Molecular docking and dynamics simulations were conducted to evaluate the binding affinities of key ligand-target interactions. The *in vitro* antiproliferative activity of Vernolac was assessed in MCF-7, Caco-2, NTERA-2 cl.D1, and MCF-10A, using the Sulforhodamine B assay. A total of 155 drug-like phytochemicals and 137 core overlapping targets were identified. Key phytochemicals, including vernolactone, thymoquinone, quercetin, nigellidine, and carvacrol, were found to interact with key hub nodes, including AKT1, BCL2, CASP3, CTNNB1, EGFR, JUN, SRC, TNF, and STAT3. Pathway enrichment analyses indicated a significant involvement of targets in multiple cancers, such as prostate, endometrial, bladder, pancreatic cancer, and leukemia. The molecular docking and dynamics simulation studies identified novel target-ligand interactions. *In vitro* experiments demonstrated substantial antiproliferative activity against Caco-2 ($IC_{50} = 173.2 \mu\text{g/mL}$), MCF-7 ($IC_{50} = 124.5 \mu\text{g/mL}$), and NTERA-2 cl.D1 ($IC_{50} = 61.5 \mu\text{g/mL}$), while sparing normal MCF-10A cells ($IC_{50} = 1075 \mu\text{g/mL}$). Vernolac may exert anticancer effects through apoptosis induction, immune modulation, antioxidant, anti-inflammation, antiproliferative, and chemoradiosensitizing mechanisms. This study suggests that Vernolac may exhibit chemoradioprotective potential by alleviating therapy-induced toxicity, supporting its promise as a potential adjunct to conventional cancer treatments.

Keywords: Nutraceuticals, cancer, Network pharmacology, Molecular docking, Molecular dynamics simulation

Synergistic effects of triple combination therapy with thymoquinone, *Datura metel* and *Andrographis paniculata* extracts on the proliferation of human lung cancer cells

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Lung cancer remains one of the leading causes of cancer-related mortality worldwide. Conventional therapeutics often exhibit limitations in providing complete cure for cancer and occurrences of drug resistance is evidenced in solid tumors. Combination therapies that target multiple oncogenic pathways have emerged as a promising strategy to overcome chemoresistance and minimize treatment-associated toxicity. Thymoquinone (TQ), a bioactive compound derived from *Nigella sativa*, has been reported for its potent anti-cancer properties. *Datura metel* (DM) and *Andrographis paniculata* (AP) are medicinal plants, containing several compounds with anti-cancer properties. Therefore, this study investigated the synergistic anti-proliferative effects of TQ in combination with *D. metel* and *A. paniculata* extracts against human lung cancer cells (NCI-H292). Using sequential ultrasound-assisted extraction, aerial parts and leaves were extracted with hexane, dichloromethane, ethyl acetate, and methanol. SRB-based cytotoxic screening revealed that ethyl acetate extract of *A. paniculata* and dichloromethane extract of *D. metel* were most potent, demonstrating IC₅₀ values at 53.46 µg/mL and 63.04 µg/mL at 24 h exposure, respectively. Triple combination treatments in nine different combination ratios (from 1:1:1 to 16:16:1) were assessed using SRB assay. Synergistic interactions were quantified using CompuSyn and SynergyFinder software. The combination 2:2:1 of TQ:DM:AP demonstrated prominent cytotoxicity (IC₅₀ values at 2.411 µg/mL) and strong synergistic effects with combination index values of 0.466 (CI < 1) at fractional affected (Fa) 0.5. This synergistic combination showed remarkable drug reduction index (DRI) values of 2.793, 65.234, and 25.684 for TQ, DM, and AP, respectively, demonstrating substantial dose reduction potential while maintaining cytotoxic efficacy. SynergyFinder analysis further confirms these findings, with all four reference models yielding negative scores with synergism (ZIP: -11.52, Loewe: -1.45, HSA: -0.62, Bliss: -10.96), indicating prominent synergistic interactions. This finding demonstrates the potential of the selected triple combination to be developed as an improved therapeutic agent against lung cancer.

Keywords: *Synergism, Lung cancer, Thymoquinone, Andrographis paniculata and Datura metel*

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