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E-POSTER PRESENTATIONS

INTRODUCTION: *Eurycoma longifolia* (Tongkat Ali) is a traditional Southeast Asian herb known for its role in enhancing male fertility. Recent studies have demonstrated its potential antiviral properties, with both standardised hot-water extracts and ethanol-isolated quassinoids showing activity against dengue virus. These findings highlight its relevance as a source of natural antiviral agents.

OBJECTIVE(S): This study evaluated the dengue virus serotype 2 (DENV-2) antiviral activity in Huh-7 cells using *E. longifolia* ethanolic extracts.

MATERIALS & METHODS: Extracts were prepared using water, 20%, 50%, 80%, and 100% ethanol. Huh-7 cells were seeded at 1×10^4 cells/well and incubated overnight. Cell viability was measured using the Adenosine Triphosphate assay (ATP assay) following 72-hour exposure to ten two-fold dilutions (starting at 100 µg/mL). For antiviral testing, cells were infected with DENV-2 (MOI = 1) for 1 hour prior to treatment with the same extract dilutions. After 72 hours, viral infectivity was analysed by plaque assay, and infected cell count was evaluated by immunofluorescence assay (IFA) targeting the DENV-2 envelope protein. Data analysis was performed using GraphPad Prism.

RESULTS: Cell viability decreased proportionally with ethanol concentration used in extract preparation. Water extract showed no cytotoxicity at concentrations up to 100 µg/mL, whereas 100% ethanol extract yielded a 50% concentration of cytotoxicity (CC_{50}) of 28.05 µg/mL (ATP assay). Antiviral activity in Huh-7 cells demonstrated a strong correlation with the ethanol concentration used for the extraction. Both IFA and plaque assay results confirmed enhanced viral suppression, with half maximal effective concentration (EC_{50}) values decreasing from 28.2 µg/mL (water extract) to 2.04 µg/mL (100% ethanol extract).

CONCLUSION: Increasing ethanol concentration used in *E. longifolia* extraction resulted in enhanced DENV-2 antiviral activity in Huh-7 cells. This suggests ethanol extraction effectively enriches bioactive compounds—such as quassinoids and alkaloids—that contribute to viral inhibition. These results emphasise the importance of solvent polarity in optimising extract potency for antiviral applications.

KEYWORDS: Dengue Virus, Antiviral, *Eurycoma longifolia* (Tongkat Ali), Ethanol Extraction

EP_056

T-CELL RESPONSE INDUCED BY THE PFIZER-BIONTECH COVID-19 VACCINE AFTER PRIMARY IMMUNIZATION IN MALAYSIA

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INTRODUCTION: The COVID-19 pandemic has accelerated the development and evaluation of next-generation vaccines, including messenger ribonucleic acid (mRNA) platform technologies. Vaccination aims to protect against SARS-CoV-2 infection by eliciting antibodies and T-cell-mediated immunity. While extensive research has predominantly focused on antibody-mediated immunity, a comprehensive understanding of T-cell immunity remains imperative. T-cells play a central role in identifying and eliminating infected cells, establishing durable immune memory and contributing to robust cellular immunity.

OBJECTIVE(S): This study attempted to assess the T-cell response among Pfizer-BioNTech vaccine recipients in Malaysia after completion of the primary dose.

MATERIALS & METHODS: Blood samples were collected before receiving the first dose (T0), 21 days after the first dose (T1) and 14 days after the second dose (completed dose) (T2). We employed the enzyme-linked immunospot (ELISpot) assay to assess the T-cell response in a cohort of 22 healthy subjects. The ELISpot assay enables the detection of individual T cells that secrete interferon gamma (IFN-γ), allowing analysis of immune responses at the single-cell level. Peripheral blood mononuclear cells (PBMCs) were isolated, cryopreserved and subsequently stimulated with SARS-CoV-2 peptides. The secretion of IFN-γ from primed T-cells was quantified as spot-forming units (SFU) per 10^6 PBMCs using the EliSpot plate reader.

RESULTS: Findings showed that T-cell responses were undetectable above the positivity threshold of 33 SFU per 10^6 PBMCs at T0. However, a significant increase ($p < 0.001$) in T-cell reactivity was observed at both T1 and T2, with median (interquartile range, IQR) values of 246 (149) and 550 (386), respectively.

CONCLUSION: These results indicate that the Pfizer-BioNTech vaccine can elicit a robust T-cell response among the recipients. These findings highlight the importance of considering T-cell immunity, in addition to antibody-mediated immunity, when evaluating the efficacy of COVID-19 vaccines.

KEYWORDS: SARS-CoV-2, COVID-19 Vaccination, Cell-Mediated Immunity, ELISpot Assay.

EP_058

TANDUK RUSA WATER EXTRACT - A POTENTIAL NATURAL-BASED BONE DEVELOPMENT STIMULUS

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INTRODUCTION: *Tanduk rusa*, or deer velvet antlers (DVA), has long been utilised in traditional Asian medicine for its various health benefits, including bone health maintenance and regeneration. Nonetheless, the fundamentals of scientific merit is still limited.

OBJECTIVE(S): This study investigates the effects of DVA extract on stem cells derived from human exfoliated deciduous teeth (SHED); capable of differentiating into osteoblasts, which are essential for bone formation and healing. To achieve this, the effect of DVA on the biological activity of SHED and its potential to differentiate into osteoblast-like cells were determined accordingly.

MATERIALS & METHODS: Powdered form of DVA obtained from male Malayan deer (*Cervus timorensis*) was extracted using sterile water, and its impact on SHED was assessed at different concentrations (0 to 200 µg/ml). Cell viability was determined using MTT assay, cell proliferation using AlamarBlue assay while population doubling time (PDT) was determined according to the available protocol, and morphology was observed under the inverted microscope. Osteoblast differentiation was analyzed by measuring alkaline phosphatase (ALP) activity, calcium deposition via Alizarin Red-S staining, and expression of osteoblast markers (*RUNX2, OSX,* and *OCN*) in SHED cultured in osteoblast differentiation medium (ODM) were analysed using RT-qPCR. Statistical analyses were performed using One-way ANOVA and post hoc Tukey's test with $p<0.05$ is considered as significant.

RESULTS: DVA extract was non-toxic to SHED at all tested concentrations, with 3.125 µg/ml identified as the optimal concentration for promoting proliferation and reducing PDT. Morphologically, SHED maintained a fibroblast-like shape at all concentrations analysed. When cultured in ODM, addition of DVA resulted in an increase of ALP activity at Day 21 ($P<0.05$), strong calcium mineralization at Day 14 onwards, and higher expression of osteoblast markers (*RUNX2, OCN*). Changes in morphology into osteoblast-like was observed.

CONCLUSION: DVA stimulates osteoblast differentiation of SHED, particularly during mineralization phases, highlighting its potential as a natural bone development stimulant.

KEYWORDS: Deer Velvet Antler; *Tanduk Rusa*, SHED, Osteoblast Differentiation

EP_059

ANALYSIS OF DENGUE CASES BY SEROTYPE DYNAMICS, CASE DEMOGRAPHY AND CT VALUE TRENDS IN 2024

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INTRODUCTION: Dengue is a mosquito-borne viral infection caused by four distinct serotypes (DENV1–4), primarily transmitted by *Aedes aegypti*. Accurate serotyping is crucial for clinical management, as certain serotypes are associated with more severe disease. Real-time reverse transcription PCR (RT-qPCR) remains the gold standard for early detection and serotyping while cycle threshold (Ct) values provide an indirect estimate of viral load that informs transmission risk. However, local data integrating serotype prevalence with demographics, region, and Ct values remain limited.

OBJECTIVE(S): This study aims to characterize the serotype distribution, patient demographics and Ct values of dengue cases diagnosed in 2024 at the Virology Unit, Institute for Medical Research (IMR).

MATERIALS & METHODS: This was a retrospective, observational study utilizing a dataset of 412 dengue-positive diagnostic samples received for routine RT-qPCR testing at the Virology Unit, IMR from January to December 2024. Data analysis involved descriptive statistics and Chi-Square tests to determine associations between dengue serotypes, patient demographics and Ct values.

RESULTS: DENV2 was the most prevalent serotype in the year 2024 (67.5%), followed by DENV3 (15.0%), DENV4 (9.0%), and DENV1 (8.5%). A significant association was found between serotype and age group ($p=0.005$), with the 26–44 age group having the highest case concentration across all serotypes, indicating age-specific serotype distribution. Serotype distribution varied significantly by locality ($p < 0.001$). DENV2 was the most prevalent serotype in Kedah, Kelantan, Johor, and Selangor. DENV3 was primarily concentrated in Johor, while DENV4 was more common in Selangor and Johor. DENV1 was notably prominent in Kelantan. A significant association was also observed between serotypes and Ct value ranges ($p < 0.001$). DENV4 exhibited a significantly higher Ct range (30.01–40.00), indicating relatively lower viremia, whereas DENV2 showed a lower Ct range (10.01–20.00), suggesting higher viremia.

CONCLUSION: These results show that dengue serotype prevalence varies by age and location, highlighting the importance of sustained epidemiological monitoring to guide public health interventions and track evolving transmission patterns. Incorporating clinical presentations and viremic phase data may yield more clinically relevant insights.

KEYWORDS: Dengue, Serotypes, RT-qPCR

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