ABSTRACTS FOR THE 2ND INTERNATIONAL CONFERENCE ON ORAL MICROBIOLOGY AND ORAL IMMUNOLOGY

NEW FRONTIERS IN MUCOSAL CANCER THERAPY

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MACROPHAGE AND DENDRITIC CELL BIOLOGY: IMPLICATIONS FOR DISEASE

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ABSTRACT

Macrophages, monocytes, and dendritic cells play crucial and distinct roles in tissue homeostasis and immunity, but also contribute to a broad spectrum of pathologies and are thus attractive therapeutic targets. Potential intervention strategies aiming at manipulation of these cells will require in-depth insights of their origins and the mechanisms that govern their homeostasis and activation. Our approach encompasses the integration of high dimensional platforms such as RNAseq, single cell transcriptome analysis using microfluidic RNA sequencing and deep immunophenotypic assessment using state of the art 25 parameters flow cytometry or Cytometry by Time-Of-Flight mass spectrometry (CyTOF). Such high density molecular profiling at the single level and at unprecedented dimensionality and complexity will provide new insights in the biology of DC, monocyte and macrophage cell populations. Defining macrophage and DC populations on the criteria of their origin may aid our understanding of their discrete roles in tissue immunity and homeostasis, as ontogeny of DC and macrophage subsets likely underlie their functional specializations.

PLENARY LECTURES

IMMUNE PRECISION MEDICINE FOR CANCER: A NOVEL INSIGHT BASED ON THE EFFICIENCY OF IMMUNE EFFECTOR CELLS

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ABSTRACT

Cancer cell growth is associated to an immune surveillance failure. The aim to restore an immune control of cancer cells represents an important therapeutic development. Nowadays, immune therapy is improving, due to the recent advances in biological knowledge. One of the most important successes in immune therapy is represented by monoclonal antibodies, particularly the use of rituximab for B-cell lymphoproliferative disorders. More recently, other monoclonal antibodies have been developed, to inhibit immune checkpoints within the tumor microenvironment that limit immune suppression, or to enhance some immune functions with immune adjuvants through different targets such as Toll-receptor agonists. The aim is to inhibit cancer growth proliferation, a situation that could be biologically measured by the diminishing/elimination of cancer residual cells, and clinically by improving the response duration with no or few adverse effects. This effect is supported by enhancing the number, functions, and activity of the immune effector cells (IEC), including the natural killer (NK) lymphocytes, NKT-lymphocytes, γδ T-lymphocytes, cytotoxic T-lymphocytes; directly or indirectly through vaccines particularly with Neoantigens, and by lowering the functions of the immune suppressive cells. Beyond these new therapeutics and their personalized usage, new considerations have to be taken into account, such as epigenetic regulation particularly from microbiota, evaluation of transversal functions, particularly cellular metabolism, and consideration to the clinical consequences at the body level. We plan to discuss these fundamentals, defining simple questions, how, and when to use immune therapeutic tools to support activation, amplification or administration of IEC that control or eradicate tumor cells. In addition, there is a place for a biological dynamic follow-up including the initial inflammatory response, biomarkers for both immune activation and immune exhaustion in order to adapt and to adopt Immune Precision Medicine approach.

ANTITUMOR IMMUNE REGULATION IN COLORECTAL CANCER

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ABSTRACT

Colorectal cancer (CRC) is the second major cause of cancer-related deaths. Notably, immune checkpoint blockade therapy has become a promising treatment for many cancer patients. However, the majority of CRC patients do not respond to this therapy due to poor T-cell infiltration and downregulated immune checkpoint genes. Elucidating tumor-cell intrinsic mechanisms that inhibit antitumor T-cell responses and developing strategies to boost T cell infiltration is critical to improving immune checkpoint blockade therapy in CRC patients. Tumors use a variety of mechanisms to evade, deceive and suppress the host immune system. This presentation will cover, the role of tumor secreted exosomes and the gut microbiome in the regulation of tumor immune response and metabolic interactions. The novel discoveries in this area of investigation will provide insights in stimulating tumor-specific T cell response and form the foundation for a novel anticancer therapeutic strategy.

MICROBIOME AND COLORECTAL CANCER: AN OVERVIEW AND LOCAL PERSPECTIVE

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ABSTRACT

Colorectal cancer (CRC) is an important health problem that is on the rise globally, where it is the fourth most common cause of deaths from cancer. CRC is now the 2nd commonest cancer in men and 3rd commonest in women in Malaysia. Diet, lifestyle, genetics and environmental interaction, together with underlying gut conditions such as inflammatory bowel disease have been reported to contribute to the disease. In addition, the gut microbiome has also been increasingly reported to be associated with CRC development, with dysbiosis of the commensal bacteria observed in CRC patients. Bacterial genera such as Bacteroides, Fusobacterium and Prevotella are more commonly detected in CRC patients compared to healthy individuals. Nevertheless, not much is known about the gut microbiome among Malaysians with different ethnicities. In Malaysia, the Chinese has the highest incidence of CRC, followed by Malays and Indians. The reason behind this difference may be contributed by the differences in the dietary intake that could modulate the gut microbiome and contribute towards the development of CRC. The current knowledge on this field still much depends on reports from individuals of American, European, Chinese, Brazilian and Japanese descendants in origin. The oncogenic potential of bacteria was suggested to include inflammation and the production of mutagenic toxin. A significant increase in certain intestinal microbiota including the genuses Enteroccus and Streptococcus spp. was detected in the advanced stage of colorectal adenoma. However, there are discrepancies in the previous studies, where some bacteria genera might be over-reported or underestimated. It is likely that the gut microbiome differs between populations. There is also no available data on the gut microbiome of the healthy individuals, colorectal adenoma (pre-cancerous) and colorectal cancer patients in the Malaysian population. Recent advancements in next generation sequencing allow faster and more accurate determination of microbial consortium in various niches of the human body and environment. In particular, sequencing of the 16S rRNA gene with specific primers have been reported to allow accurate determination of bacterial orders commonly found in the human gut as well as for those which are not expected in the digestive system. Recent developments in gut microbiome DNA extraction also contributed to the robustness of gut microbiome determination and analysis. All the above will contribute towards an accurate and rapid cataloging process of the Malaysian gut microbiome and also enable comparison between healthy individuals, colorectal adenoma and CRC patients of the Malaysian population.

INVITED SPEAKERS

IMMUNOTHERAPY FOR HEAD AND NECK CANCER

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ABSTRACT

Head and neck cancers have been reported to have high immune infiltration scores, and clinical benefits of the anti-PD1 checkpoint inhibitor have been demonstrated in recurrent and metastatic cancers. Recent genetic signatures of the immune compartment have provided insights to delineate immune-active and -exhausted subtypes, to understand the immune status of OSCC patients that could further drive the development of novel immunotherapies. Vaccination with tumour-associated antigens is an approach to improve tumour recognition which could result in the eradication of cancer cells. Here, I would describe our efforts in developing antigen-specific vaccines for head and neck cancer. Using the B6.Cg-Tg(HLA-A/H2-D)2Enge/J mice bearing established tumours overexpressing the tumour antigens, we demonstrated that the vaccine delayed tumour growth, and in combination with anti-PD1, completely eliminated the tumour. The vaccine increased the expression of PD1 in T cells, and vaccinated animals showed increased antigen-specific responses by the ELISPOT assay. In summary, our data show that antigen-specific vaccine works synergistically with anti-PD1 and could be a promising therapeutic agent for head and neck cancer.

BRIDGING THE REGULATION OF ZINC IN ORAL NUTRITIONAL IMMUNITY

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ABSTRACT

Host induced control of pathogens involves, but not limited to, withholding of essential transition metals as well as releasing the metals at a toxic level. Zinc is one of these transition elements that plays critical role in controlling the pathogens in that manner – a key mediator in nutritional immunity. A number of subcellular and molecular mechanisms such as transport and storage proteins are known to maintain Zn homeostasis and scuffle with the pathogens. Pathogenic bacteria also use a number of mechanisms to combat the scuffle and fight for the right amount of Zn for their survival and growth. From the host perspective, a "delicate" balance of Zn must be maintained for immune surveillance while making the level of Zn either to starve or to intoxicate the pathogens. Metallothionein (MT), a group of low molecular weight proteins, is well known for its Zn transport and storage ability and is expected to play an important role in that nutritional immunity. Zn homeostasis by MT to fight oral pathogens is not unexpected too. Periodontitis and dental caries are two most common oral diseases which are linked to the pathogenic carnival of opportunistic bacteria. Can those culprits be exterminated through nutritional immunity using MT? Or could it be - those human hosts who become the easy prey of those pathogens lack inducible expression of MT in their oral tissues? The synthesis or degradation of MT in response to invading pathogens in oral tissues, the human-MT mediated Zn homeostasis in response to infectious insult in oral tissues are evident. Nonetheless, the cross talk between MT and Zn in oral nutritional immunity is largely unknown.

SELECTIVE CYTOTOXICITY EFFECTS OF CYTOBIOTICS PRODUCED BY LACTIC ACID BACTERIA ISOLATED FROM MALAYSIAN FOODS

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ABSTRACT

Probiotics are live microorganisms and when consumed in adequate amounts will confer health benefit on the host. Probiotic effects of Lactic Acid Bacteria (LAB) have been reported extensively, which rely generally on the viability of LAB cells. However, we have reported extensively the prominent probiotic effects of cell less postbiotics metabolites produced by various strains of *Lactobacillus plantarum* isolated from Malaysian foods on rats, poultry and pigs. *L. plantarum* is a major species of LAB. Despite the emerging evidence of anticancer properties of LAB, very limited information is available on the cytotoxic and antiproliferative activities of cytobiotic metabolites produced by LAB. Recently, we have documented the selective antiproliferative and cytotoxicity of cytobiotic produced by six strains of *L. plantarum* on normal human primary cells, breast, colorectal, cervical, liver and leukemia cancer cell lines via MTT assay, trypan blue exclusion method and BrdU assay. Haemolytic assay was used to determine the toxicity of cytobiotic using human and various animal red blood cells. The cytotoxicity mode was subsequently determined for selected UL4 cytobiotic on MCF-7 cells due to its pronounced cytotoxic effect by fluorescent microscopic observation using AO/PI dye reagents and flow cytometric analyses. The selective cytotoxicity effect on various cancel cells that occurred in a strain-specific and cancer cell type-specific manner whilst sparing the normal cells will be discussed in the presentation. Moreover, the antiproliferative effects and induction of late apoptosis effects against selected malignant cancer cells will be discussed further in the presentation. This report reveals the vast potential of cytobiotics produced by *L. plantarum* strains as functional supplement and as an adjunctive treatment for cancer.

COMPARATIVE REVIEW OF ORAL CANCER AND NASOPHARYNGEAL CANCER: MICROBIOLOGICAL ASPECTS, ETIOLOGY AND GENETIC RISKS

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ABSTRACT

Cancers of the oral cavity are more common worldwide in men than in women, and the same is true for cancer of the nasopharynx region, whereby nasopharyngeal carcinoma (NPC) incidence rate in men is 2.5 times that in women. Different risk factors, including environmental, lifestyle and genetic factors, come into play in terms of contributing towards the development of these cancers. The increased incidence of oral cancers in developed countries in recent years are attributable to rises in the consumption of tobacco and/or alcoholic beverages, in addition to the traditional practice of betel quid chewing in some communities. As for NPC, the risk factors include male sex, overconsumption of preserved salted fish and smoking. In terms of etiology due to microbial agents, the human papillomavirus (HPV) has been linked with oral cancers whereby HPV DNA was found in about 2 out of 3 oropharyngeal cancer cases. In contrast, the Epstein-Barr virus (EBV) has been closely associated with most cases of NPC. Specifically, NPC is categorized by the WHO into two main histological types—keratinizing squamous cell carcinoma (type I) and non-keratinizing squamous cell carcinoma (types II and III), and it is the non-keratinizing type (types II and III) which has very high percentage of EBV DNA. The oncogenicity of these viruses had been studied extensively, and they are now recognized as crucial early triggers of NPC and oral cancers. Genetic factors can also predispose a person to the development of either oral cancer or NPC. Certain HLA class I alleles are associated with increased risks for NPC. Genetic polymorphisms in genes encoding the cytochrome P450 enzymes and glutathione S-transferase had been identified as potential risk factors for NPC. In our studies, we had shown that polymorphism in the XPD gene which encodes a DNA helicase enzyme involved in nucleotide excision repair was linked to risk for NPC in Malaysian population. We also found that the combination of CGC allele from hOGG1, ITGA2 and XPD polymorphisms was significantly associated with increased odds of NPC. In oral cancers, studies by other researchers revealed that gene polymorphisms in HOTAIR gene and the interaction with betel quid chewing are linked to oral cancer risk. Specific COX-2 gene polymorphisms were also found to be associated with increased risk for oral cancer development and progression. Taken together, these studies show a strong correlation between viral etiology combined with the individual's genetic background coupled with certain risky lifestyle behaviours which together contribute towards the development of oral cancer and NPC.

miRNAS AS POTENTIAL BIOMARKERS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA DIAGNOSTICS

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ABSTRACT

MicroRNAs (miRNAs) are small noncoding RNAs that involved in various normal and cancer-related cellular processes. Studies on expression profiling of miRNAs have been performed and the data showed that some miRNAs are up-regulated or down-regulated in cancer. miRNAs play a crucial role in HNSCC development, metastasis, prognosis and survival rate. Several studies have been conducted previously to investigate that use of miRNAs as the biomarkers in disease diagnostic/prognostic and potential therapeutic targets management that may improve the outcomes of HNSCC. Our previous study revealed that upregulation of oncogenic miRNAs including hsa-miR-181a-2*, hsa-miR-29b-1*, hsa-miR-181a, hsa-miR-181b, hsa-miR-744, hsa-miR-1271 and hsa-miR-221* were able to distinguish HNSCC from normal samples. These miRNAs may contribute in a simple profiling strategy to identify individuals at higher risk of developing head and neck cancers, thus helping in the elucidation of the molecular mechanisms involved in head and neck cancer pathogenesis.

ORAL MICROBIOME, NUTRITION AND THEIR ROLE IN ORAL CARCINOGENESIS

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ABSTRACT

A balanced oral microbiome is essential in maintaining a healthy oral cavity. Oral microbiome comprises of various microorganisms that belong to different kingdoms, including bacteria (bacteriome) and fungal (mycobiome). Multiple factors have been shown in oral carcinogenesis including alcohol consumption, tobacco smoking, betel nut chewing and microbial infections. Since the oral cavity comprises of various microbial kingdoms, thus, inter-kingdom interactions are suggested in promoting oral carcinogenesis. Dysbiosis, which is defined as imbalance inter-kingdom microbiome, alone may not cause oral carcinogenesis; thus, it is suggested that nutritional factor may also play a vital role in this disease development. A recent study has shown that sucrose consumption can induce the production of glucosyltransferases (gtfs) by *Streptococcus mutans* which lead to the increasing attachment of *Candida albicans* in polymicrobial biofilms form. The yeast has been reported to be potentially involved in oral carcinogenesis, particularly in the immunocompromised patient. This is due to the inflammation that is caused by candidal infection, which increases pro-inflammatory cytokines such as interleukin-6, interleukin-8 and interleukin-10, that have been linked to oral carcinogenesis. However, further study is needed to conform to the claim. In addition, over-consumption of alcoholic beverages has also been related to carcinogenesis which the ethanol has been reported to be converted into acetaldehyde by *C. albicans* using acetaldehyde dehydrogenases enzymes. In Malaysia, oral cancer has also been related to the consumption of cured and salted fish, which mostly consumed by the Chinese ethnics. However, its relationship to oral microbiome remains unclear. In conclusion, oral microbiome and nutrition may have a role in oral carcinogenesis; however, further study is needed to elucidate the role of both factors in oral cancer development.

POTENTIAL HEALTH DETRIMENT FROM FRUIT-INDUCED IMMUNOMODULATION

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ABSTRACT

The immune system responds to stimulus by activation/increase or inhibition/decrease in activities. These immunomodulatory effects may be triggered by various factors in the environment including cytokines, hormones and growth factors, as well as flavonoids, antioxidants and various antigens in food and the environment. Immunosuppression has a direct effect on the capacity of the immune system to fight against infection and cancer formation. A pro-inflammatory response, however, may induce further progression of tumours that had formed. Inflammation is also associated with many chronic illnesses including pain. The suppressive effects from phytochemicals have been shown in the potential to reduce T-lymphocyte proliferation in vitro and in vivo. Studies have demonstrated inhibition of pro-inflammatory cytokines from flavonoid such as naringenin, green tea polyphenol extract, encapsulated fruit and vegetable juice powder concentrate. *Feijoa sellowiana Berg var. coolidge* fruit juice consumption exerted anti-inflammatory activity on edema-induced mice within first hour of treatment while agipenin, a natural flavonoid reduced neuroinflammation by protection against damage from dendritic cells stimulated T cells in experimental autoimmune encephalomyelitis mouse models. Dietary polyphenols were found to exert a regulatory role on dendritic cell function. Our own study showed immunosuppressive effect from increased T regulatory cells from papaya consumption. Increased regulatory cells are associated with cancer conditions. On the other hand, grape juice consumption mobilized gamma–delta T cells. Ginseng berry extract increased pro-inflammatory molecules in dendritic cells in the spleen while polysaccharide fractions from *Momorica charantia*, an edible medicinal vegetable increased various immune indexes. Fruits may also have endo-immunomodulatory function causing differential effects in male and female. Sex hormones can influence immune changes based on sex as seen in increased NK cells in males and antibodies in females. We observed a population of CD4-CD45RA-CD69+CD25- cells was significantly lower in males. However, none of these studies have been directly conducted on cancers. Investigation into this area may help improve decision making in cancer management.

IMMUNITY AND CELL METABOLISM

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ABSTRACT

Over the past decade, research involving immunometabolism, has been gaining much interest. The immune cell responses of an individual may be influenced by metabolites released by the host or derived from the microbiota. However, the immune response of an individual may vary depending on the health condition of an individual. During infection, the metabolic processes derived from the infectious diseases can effect the function of immune cells and thus determine the response or survival of the host during infection. Immunometabolism also has a role in tumor development although the mechanism of how tumor cells influence immune cell function is not well understood. Among the major meatbolic pathways that have been studied in immune cells include glycolysis, the tricarboxylic acid cycle, the pentose phosphate pathway, fatty acid oxidation, fatty acid synthesis and amino acid metabolism. Understanding the tight connection between metabolomics and immunity in health and disease will be crucial as this could lead to therapeutic interventions or in developing metabolomic biomarkers in immunology.

MESENCHYMAL STEM CELLS: A MODERN TOOL FOR IMMUNOMODULATION

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ABSTRACT

Immunomodulation is essential for controlling the immune system to maintain efficient immune surveillance and inflammation. Both arms of immunomodulation, namely immunostimulation and immunosuppression, are equally crucial in setting the optimal balance of immune response. However, diseases or conditions such as autoimmune diseases, tissue rejection due to transplantation and chronic inflammation require downregulation of overwhelming immune reactions. The conventional immunosuppressive drugs prevent the activation of immune cells, yet create an unsafe condition with toxic adverse effects. In such predicament, mesenchymal stem cells (MSCs) emerged as one of the safe immunosuppressive regiments and widely tested in clinical trials for numerous chronic inflammatory diseases. Mesenchymal stem cells are the origin of the stromal/mesenchymal cells in almost all solid organs, including the pulp of the tooth. In addition to providing structural support to the organ, MSCs participate in the tissue repair and regeneration by ameliorating an overly activated immune response locally and systemically. Regardless of the source, MSCs profoundly suppress the proliferation and effector functions of both innate and adaptive immune cells. The mechanism of inhibition primarily took place in the early phase of cell cycle and mediated via suppression of mainstream signalling pathways that involve cyclins and other cell cycle proteins. The antiproliferative activity of MSCs is not only limited to the healthy immune cells but extends to the various tumour cells of the immune system. Similarly, an array of cell signalling pathways that executed by cell cycle proteins found downregulated in the presence of MSCs. The immunosuppressive activity exerted by MSCs is not specific to particular immune cells where it impairs a group of the common cell signalling pathways or putative cell cycle proteins which are vital elements for the proliferation.

ORAL PRESENTATION

CLASSIFICATION OF CANCER USING DEEP LEARNING TECHNIQUES – A RECENT APPROACH

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ABSTRACT

Introduction: Globally, cancer is the second foremost cause of death, next to heart disease. The name cancer refers to more than a thousand sicknesses illustrated by direct development and uncontrolled replication of multiple cells. In the recent years, the microarray datasets combined with machine learning methods are increasingly used to classify the cancer in clinical conditions. Classification is one of the very broadly-used data mining techniques, to build a model that describes and distinguishes data classes in a manner to be used to predict the class of unseen instances. In machine learning, features are chosen manually for the classifier. Deep learning features extraction and modelling steps that are automatic. **Methods:** Deep learning is one of the most significant forms of machine learning that requires computing systems to iteratively perform calculations to identify patterns by itself. Deep learning uses training data to discover underlying patterns, build models and make predictions based on the best-fit model. Here we review deep learning for classification in bioinformatics; presenting examples of current research. Additionally, we discuss deep learning and convolutional neural network working principles to provide a useful and comprehensive perspective. This paper presents three works DeepGen, SDAE, and Enhance Feature learning in a brief description for each study. **Conclusion:** This review provides a comprehensive outlook and serve as a starting point for a clinical researcher to apply deep learning approaches for classification of gene expression profile in cancer specimens.

MAPPING KRAS GENE IN COLON CANCER PATHWAY USING STRING AND CYTOSCAPE SOFTWARE

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ABSTRACT

Introduction: Colorectal cancer is one of the top three most commonly occurring cancer worldwide with more than 1.8 million cases in 2018. In Malaysia, colorectal cancer is the most common cancer in males and the second most common cancer in females. Albeit being the second most common form of cancer in Malaysia, there is a lack of a formal or structured national colorectal cancer screening programme in Malaysia and it remains a low priority in healthcare planning and expenditure in Malaysia. The risk of developing colon cancer is greatly influenced by factors such as lifestyle habits, genetic inheritance, diet, weight, and exercise. Kras, the most frequently mutated oncogene in cancer, occurs in about 50 percent of colorectal cancers. **Methods:** This study maps the kras gene involved in colon cancer pathway, using bioinformatics applications such as STRING version 11.0 and Cytoscape version 3.7.0 to provide a clear visualisation of all the related and involved proteins and genes that interact with this kras gene in the pathway. **Results:** The 3391 protein interactions were assembled and visualized in y organic form. Six specific non-overlapping clusters of various sizes, which emerged from the huge network of protein-interactors using MCODE version 1.32 clustering algorithm were found. Biological Networks Gene Ontology (BiNGO) was used to determine two ontologies (molecular function and biological process) involved in the protein network. Based on the resulting protein-protein network interaction map, each interaction plays an important role in the cell cycle, metabolic pathways and signal transduction. **Conclusion:** Understanding these interactions provide insight into cellular activities and thus assist in the understanding of the aetiology of disease.

PROGRAMMED CELL DEATH-LIGAND 1, THYMIDYLATE SYNTHASE AND DELETED IN COLORECTAL CARCINOMA BIOMARKERS IN COLORECTAL CARCINOMA

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ABSTRACT

Introduction: It is well known that cancer cells evade the immune system with the help of programmed cell death protein 1 (PD-L1) molecule to remain undetected, causing abnormal proliferation of T-cells. PD-L1 expression on the surface of neoplastic cells inhibits cytotoxic T-cell responses which lead to negative regulation of cytokines and proliferation of T-cells. The deleted in colorectal cancer (DCC) gene belongs to the immunoglobulin superfamily. It is a candidate of the tumour suppressor gene by regulating apoptosis. DCC assessment gives an insight into prognosis in patients with advanced stages of CRC. Thymidylate synthase (TYMS) is a highly conserved enzyme involved in DNA synthesis. TYMS has been an important target for cancer chemotherapy because of its central, rate-limiting role in de novo synthesis of thymidylate. Expression of PD-L1, TYMS and DCC has been demonstrated to confer a prognostic value in CRC but none have been completely validated for patient care. This study aimed to determine the prognostic and predictive potential of PD-L1, TYMS, and DCC biomarkers in CRC. **Methods:** The expression of these biomarkers was evaluated immunohistochemically in 91 formalin-fixed paraffin-embedded (FFPE) archival tumour samples from patients that underwent surgical resection. **Results:** There was high expression of DCC in most cases; 84.6% (77/91). TYMS expression at a high level score was 46.2% (42/91) and at low level was 53.8% (49/91). Majority of cases had low PD-L1 expression in 93.4% (86/91) cases and high expression was detected in 6.6% (6/94) of cases. In addition, there was a significant association between TYMS expression with gender ($P < 0.05$) with distribution of TYMS expression detected at high level was 76.2% in male and 23.8% in female. The Kaplan-Meier survival plot showed mean overall survival in patients with PD-L1 with high expression to be 22 months, which predicts better survival. TYMS low expression showed mean overall survival of 90 which also indicated better survival. DCC high expression showed mean overall survival of 90 which indicated better survival. The correlation between the biomarkers and overall survival were not statistically significant. **Conclusion:** The results from this study suggest that PD-L1, TYMS and DCC expression could be used as biomarkers to predict treatment outcome in CRC. PD-L1 overexpression predicts patients who could benefit from anti-PD-1 and anti-PD-L1 immunotherapy whilst TYMS low expression predicts patients who could benefit from 5-fluorouracil therapy. DCC high expression tumours predicts a better prognosis and overall survival compared to DCC-negative tumours in advanced CRC.

GREEN COFFEE EXTRACT INHIBITS INNATE IMMUNE ACTIVATION VIA TLR TRIGGERING DUE TO NICKEL AND COBALT EXPOSURES

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ABSTRACT

Introduction: Metals are known as contact allergens and are abundantly used in jewellery, orthopaedic, dental construction, cardiac, and other implants. Exposure to alloys in the oral cavity is often associated with both local and systemic adverse reactions. Nickel (Ni) and cobalt (Co) are notorious for inducing immune activation via TLR-4, downstream of the NF-kB pathway. Robusta green coffee (*Coffea canephora*) is expected to inhibit immune activation due to its contents such as flavonoids. It is a promising candidate as an immune blocker and effective antioxidant. The objective of this study was to investigate the potential effect of green coffee against innate immune activation in peripheral blood mononuclear cells (PBMC) exposed to Ni and Co. **Methods:** The metals tested are $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$. The coffee extract is taken from East Java robusta coffee beans, macerated in 97% ethanol and subsequently diluted into three concentrations: 125, 62.5, and 31.25 µg/ml. Innate immune activation was monitored by assessment of release of IL-8 and IL-6 by using ELISA. **Results:** The robusta green coffee at 125 µg/ml was the optimal concentration in reducing immune activation in PBMC cells induced by Ni and Co followed by exposure to 62.5 and 31.25 µg/ml. **Conclusion:** Data obtained in the present study reveals that green coffee has the capacity to inhibit innate immune activation due to Ni and Co exposures, which display potent innate immune capacities. More robust and well-controlled studies are still needed for a thorough understanding of the effect of coffee on other inflammatory markers in humans.

THE EFFECT OF LINUM USITATISSIMUM (FLAX SEED) AND NIGELLA SATIVA OIL ON SELECTED ORAL PATHOGEN (COMPARATIVE STUDY)

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ABSTRACT

Introduction: *Linum usitatissimum* (flax seed) has been cultivated for domestic use since prehistoric times. Its use as a dietary supplement becomes more popular nowadays. *Nigella sativa* seeds and oils have been widely used for centuries in the treatment of various ailments throughout the world. It is an important drug in the Indian traditional system of medicine like Unani and Ayurveda. **Methods:** This is a laboratory experimental in-vitro study using selected oral pathogens (*Streptococcus mutans, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) cultured in nutrient agar. The pathogens were then inoculated in nutrient based broth and incubation for 24hours. *Linum usitatissimum* and *Nigella sativa* extract efficacy was tested by measurement of the zone of inhibition. The result of the extracts antimicrobial activities were compared with positive control (penicillin) and negative control(Dimethyl sulfoxide DMSO). The statistical analysis was done by using SPSS18. **Results:** The antibacterial effect of *Linum usitatissimum* and *Nigella sativa* extract is comparable to the effect of penicillin and this study shows that flax seed extract shows more potent antibacterial effect than *Nigella sativa* on *Streptococcus mutans* and *Pseudomonas aeruginosa* while both extracts didn't show an effect on *Klebsiella pneumoniae*. **Conclusion:** The results of the present study scientifically validate the inhibitory capacity of *Linum usitatissimum* or *Nigella sativa* as antibiotic against selective oral pathogens this will contribute towards the development of new treatment options based on natural base products.

ALTERED MUCOSAL-ASSOCIATED INVARIANT T CELLS PHENOTYPE IN CHILDREN WITH NEWLY DIAGNOSED TYPE 1 DIABETES BUT NOT IN AUTOANTIBODY-POSITIVE AT-RISK CHILDREN

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ABSTRACT

Introduction: Mucosal-associated invariant T (MAIT) cells are unconventional T cells, enriched in the gut. They express an invariant T-cell receptor and recognize riboflavin metabolites from bacteria presented by MHC-Ib-related protein 1 (MR1) molecules. Alterations in gut microbiota have been reported in patients with type 1 diabetes (T1D), even before the onset of the disease. These changes can potentially alter the frequency or phenotype of circulating MAIT cells. **Methods:** We characterized peripheral blood MAIT cells in a cohort of 51 children with newly diagnosed T1D, 27 at-risk children positive for multiple autoantibodies (AAb+) and 113 age-matched healthy children. Using multi-colour flow cytometry, we analysed the frequency, surface phenotype and cytokine production of MAIT cells. In addition, we characterized the frequency and surface phenotype of blood MAIT cells in 26 patients with long-standing T1D and 25 age-matched healthy controls. **Results:** No significant differences in MAIT cell frequency were observed between the study groups. Further phenotyping revealed that the expression of CD8, CD27, CCR5 and β7 integrin on MAIT cells was lower in children with newly diagnosed T1D compared to AAb+ and healthy children. The frequency of MAIT cells producing IFN-γ was also lower in children with newly diagnosed T1D, but the frequencies of IL-17A- and IL-4-secreting MAIT cells were similar in the study groups. Finally, the capacity of MAIT cells to be activated *in vitro* by *E.coli* bacteria through MR1 was comparable between the study groups. However, none of these changes was observed in adult patients with long-standing T1D. In contrast, a decreased frequency of MAIT cells and increased CD25 expression was observed in adult T1D patients with a short duration after diagnosis. **Conclusion:** There are subtle changes in the circulating MAIT compartment in patients with T1D at the onset of the disease as well as after clinical diagnosis, but not in AAb+ at-risk subjects including progression to clinical disease. Consequently, the alterations in blood MAIT cells are likely associated with the clinical manifestation of the disease rather than being features of earlier T1D autoimmunity.

EFFECTS OF DODONAEA VISCOSA LEAF EXTRACTS AGAINST SOME ORAL PATHOGENIC BACTERIA

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ABSTRACT

Introduction: *Dodonaea viscosa* known as Hopwood plant is traditionally used for the treatment of various ailments such as sore throat, wounds, fever, cold, arthritis, sinusitis-flu, boils, dressing for skin diseases, oral thrush, tooth-aches and related problems. *Streptococcus mutans* and *Lactobacillus spp*. are found to be associated in many oral infections. This study was aimed at evaluating the effects of *D. viscosa* leaf-extracts against *Streptococcus mutans* and *Lactobacillus spp*. **Methods:** The crude extract of the plant leaves was prepared using aqueous and methanol fractions by percolation method. Both extracts were screened qualitatively for phytochemicals in which methanolic extract was found to possess high contents of bioactive compounds compared to aqueous extract. The extracts were then tested against two standard strains of oral pathogenic bacteria which are *Streptococcus mutans* and *Lactobacillus spp*. at different concentrations using disc-diffusion method with streptomycin being used as positive control. **Results:** Both extracts of *D. viscosa* have shown to possess antibacterial properties against the test organisms by inhibiting their growth *in-vitro*. The most susceptible organism was *S. mutans* (14mm at 400mg/mL) then followed by *Lactobacillus spp*. (11mm at 400mg/mL), with methanolic extract being more potent compared to aqueous extract. **Conclusion:** Therefore, results of the study suggest that leaf-extract of *D. viscosa* may have the potential to be used alternatively to control *S. mutans* and *Lactobacillus spp*. which are responsible for oral infections and this, may be attributed to the presence of phytochemical components of the leaves.

IMMUNOMODULATORY EFFECT OF PAPAYA, MATA KUCING, DANG SHEN AND PU-ERH TEA ON CYTOKINES PROFILE

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ABSTRACT

Introduction: Immune response against viral infections and tumors not only requires the recruitment of immune cells but also cytokines. Cytokine dysregulation is associated with inflammatory diseases such as cancer, autoimmune diseases, infections and allergy. Intake of fruit and vegetables are known not only to reduce inflammation but may also provide protection against various diseases. **Methods:** Effects of selected fruits and herbs on cytokines profile of IL-8, IL-1β, IL-6, IL-10, TNF and IL-12p70 were examined using the CBA flow cytometric assay. Peripheral blood mononuclear cells (PBMC) obtained from blood samples of twelve healthy subjects aged 20 to 30 years [males = 6 and females = 6] were treated with papaya, mata kucing, dang shen and pu-erh tea, respectively, for 6 and 48 hours at various concentrations. *In vivo* effects was further tested on healthy volunteers [males = 2, females = 4] by 2-days consumption of papaya following 2-days washout period without papaya. The diet of volunteers was controlled with fixed meals. **Results:** *In vitro* results after 6 hours of culture showed that papaya-treated PBMC significantly increased IL-8, IL-1β and IL-6 but reduced IL-10. Mata kucing-treated PBMC significantly increased IL-8 but reduced IL-6 while pu-erh tea significantly reduced IL-8, IL-1β, IL-6 and TNF. Cytokine analysis for dang shen-treated PBMC was only conducted at 48 hours. After 48 hours, papaya extract significantly reduced IL-8, IL-6 (8000 µg/ml), IL-10 and TNF. Significant increase of IL-6 was observed at 4000 and 16000 µg/ml. Mata kucing extract significantly increased IL-1β, IL-6 but reduced TNF. Significant increase of TNF was observed at 16000 µg/ml. Dang shen and pu-erh tea reduced IL-8, IL-1β, IL-6, IL-10 and TNF. However, *in vivo* papaya consumption did not show any significant changes and levels were low. **Conclusion:** This study showed fruits such as papaya and mata kucing had both stimulatory and inhibitory effect on various pro-inflammatory cytokines while effect of herbs such as dang shen and pu-erh tea were inhibitory. Immunomodulatory studies of natural food such as fruits and herbs may provide better understanding and subsequently improve management of inflammatory diseases.

MICROENCAPSULATED FRANKINCENSE ESSENTIAL OIL: A POTENTIAL REMEDY AND PREVENTION OF MOUTH DISEASES

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ABSTRACT

The global burden of disease studies estimated that oral diseases affected half of the world's population (3.58 billion people) with dental caries (tooth decay) in permanent teeth being the most prevalent condition assessed. On the other hand, the increasing resistance of dental caries towards the available antimicrobials and extensive use of the controversial synthetic chemicals to overcome these problems have attracted the scientific community's attention to the search for new cost-effective remedies of natural products. Frankincense or Boswellia species are highly important aromatic plants belonging to the Burseraceae family. The present study will focus on an in-vitro anti-inflammation and anti-bacterial activity of Boswellia carterii (BC) Essential oil (EO) encapsulated into the Gum Arabic (GA) polymer. Thus, certain mouth pathogenic bacteria, which are the main contributors to dental caries and gingivitis, namely (Streptococcus mutans and Lactobacillus species), and their in-vitro responses to the defined micro-particles, will pave the way to introduce a new potential remedy to the forth mentioned problems.

POSTER PRESENTATION

PROTEIN AND GENE EXPRESSION OF LEUKEMIA STEM CELLS MARKERS IN ACUTE MYELOID LEUKEMIA

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ABSTRACT

Introduction: Acute myeloid leukaemia (AML) is a clonal haematological neoplasm characterised by proliferation of immature myeloid cells in the bone marrow resulting in impairment normal cell development in bone marrow. This leads to anaemia, thrombocytopenia and neutropenia. AML primarily affects older adults, with a median age at diagnosis of 69 years but is also seen in all other age groups. AML is recognized as a kind of cancer with marked heterogeneity in both biology of the cells and reactions to treatment. Treatment with intensive chemotherapy regimens of adult AML patients who are ≤ 60 years old results in hematologic remission in about 35% of patients, but at least 30% of these patients will experience a relapse. Mechanism leading to early relapse is still unclear. Leukaemia stem cell (LSC) is shown to correlate with poor prognosis. Biomarkers such as aldehyde dehydrogenase (ALDH) and CD34+CD38- have been identified as potential LSC biomarkers in previous studies. The objective of this study is to examine the expression of such markers for LSC and determine the association. **Methods:** Peripheral blood or bone marrow samples from untreated, newly diagnosed acute myeloid leukemias of all age, gender and race were collected from Hospital Melaka and Kelang. Diagnosis of AML is based on WHO classification which include morphology, cytochemistry, immunophenotyping and cytogenetics. Mononuclear cells were isolated from bone marrow aspirate samples by gradient density centrifugation on Ficoll-Hypaque. Immunophenotyping using CD13, CD14, CD33, CD34, CD38 and ALDH were carried out to identify the presence and proportion of the various populations of interest. **Results:** There was a strong, positive correlation between ALDH and CD34+CD38- cell population, which was statistically significant ($r_s = 0.5989$, $p < 0.05$). **Conclusion:** The strong correlation of ALDH activity and CD34+CD38- expression supported the potential of these biomarkers to identify LSCs cell in AML patients. However, due to the heterogeneity of AML, further studies using more markers and larger sample size are needed to determine the validity and to correlate with disease-free survival rate of AML patients.

TARGETING BLADDER CANCER STEM CELLS USING NEWCASTLE DISEASE VIRUS

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ABSTRACT

Introduction: Bladder cancer is associated with high risk of tumour recurrence and therapeutic resistance. Cancer stem cells (CSC) within a particular tumour are postulated to drive tumorigenesis and influence tumour behaviour. Recent studies have shown that Newcastle disease virus (NDV) is able to selectively kill and exert a strong oncolytic effect against various cancer types. However little is known about the oncolytic effect of NDV against CSC. In this study, the oncolytic effect of NDV against putative bladder CSC was examined. **Methods:** Putative bladder CSC was selectively grown in the form of 3D-spheroids from six different bladder cancer cell lines. The spheroid cells were characterised for their stemness properties to ensure that these cells truly represent CSC. This was conducted via the analysis of CSC associated genes and cell surface markers expression. Subsequently, the oncolytic effect of the wild-type NDV-AF2240 strain against the bladder cancer spheroids was investigated. **Results:** All the spheroids expressed significantly high levels of CSC-associated genes. Flow-cytometry analysis revealed that the expression pattern of the CSC-associated surface markers was different in the spheroid cells; suggesting heterogeneity in the expression signatures of these cells. The infection of spheroids with NDV showed that the NDV was able to target bladder cancer spheroids but there was a spectrum of response across the different spheroids. Intriguingly, NDV was able to persistently infect bladder cancer spheroids that were not sensitive towards NDV infection as the presence of NDV viral genes were detected in the spheroid cells. The NDV persistently infected bladder cancer spheroids were resistant to superinfection and developed an antiviral state by expressing low levels of interferon-beta (IFN-b). NDV persistency of infection affects the process of epithelial to mesenchymal transition (EMT) of cancer cells as the spheroid forming ability of an established NDV persistently infected bladder cancer cell line, EJ28-PI was shown to be impaired. The EJ28-PI cells expressed significantly high levels of the *EN2* gene. Knockdown of the *EN2* expression reduced the viability of EJ28-PI cells; suggesting a role for *EN2* in mediating NDV persistency of infection in cancer cells. **Conclusion:** Bladder CSC gene expression signatures influence the efficacy of NDV-mediated oncolysis. Our current work is focused on identifying genes and signalling pathways that influence NDV-mediated oncolysis using whole-transcriptomic sequencing. The findings of this study can potentially be used to enhance the efficacy of NDV-mediated oncolysis and accelerate the translation of NDV as an oncotherapeutic agent in the clinic.

THE EFFECTIVENESS OF DRY AND WET BRUSHING TEETH TECHNIQUE BY 1.5% ENZYME PASTE AS A PREVENTION OF PLAQUE

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ABSTRACT

Introduction: The purpose of this study was to determine the effectiveness of brushing teeth with the dry compared to wet technique, using a toothpaste containing 1.5% vegetable enzymes. **Methods:** Pre-post test studies was performed with the randomly-selected sampling technique. Samples were divided into two groups, namely, Group 1: Teeth brushing with dry technique, Group 2: Teeth brushing with wet technique. Participants in both groups were instructed to brush with the roll method and use toothpaste containing 1.5% enzymes, and the plaque was then examined by the Patient Hygiene Performance (PHP) Index to score the plaque especially in the aproximal region. The scoring of the plaque was 0 = No plaque; and 1 = plaque (debris). Samples from the dry and wet technique brushing were examined for PHP index after 4 hours. Data was analyzed by parametric statistical and T-test. **Results:** There is a significant difference between dry and wet brushing technique as determined by paired t-test ($p = 0.001$). The dry teeth brushing scores recorded a PHP Index lower than the wet teeth brushing technique. **Conclusion:** Dry teeth brushing technique using a toothpaste that contains 1.5% enzymes is effective for prevention of plaque teeth.

CHARACTERIZATION OF SYNTHETIC GENE ENCODING SECRETORY LEUCOCYTE PROTEASE INHIBITOR CLONED IN ESCHERICHIA COLI HOST CELL TO IMPROVE INHIBITION ABILITY AS A CANDIDATE OF MATERIAL WOUND HEALING

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ABSTRACT

Introduction: Secretory Leukocyte Protease Inhibitor (SLPI) is a protein involved in tissue repair and oral wound healing processes. The gene encoding SLPI was included from amniotic membrane. SLPI is a potent anti-protease, anti-inflammatory, bactericidal, antifungal, and tissue repair agent. It is a non-glycosylated protein of 11.7 kDa and stable in acidic conditions. It has an isoelectric point of more than 9.5. The purpose of this study was to construct a SLPI synthetic gene, and to clone it in *Escherichia coli* for its expression in order to get the optimal expression of recombinant protein of SLPI. **Methods:** The synthetic gene encoding SLPI was designed and commercially synthesized by Genscript in pUC57. This gene was amplified using PCR, then ligated into pET-30a plasmid after digestion with KpnI and XhoI. Ligated mixtures were transformed into *E. coli* TOP10 and the plasmid-containing cells were selected using LB/kanamycine plates. The recombinant plasmid (pET/SLPIopt) was verified using restriction analysis and nucleotide sequence analysis. pET/SLPIopt was then subcloned in *E. coli* BL21(DE3) for its expression. The SLPI protein was expressed by using IPTG induction. **Results:** The synthetic gene encoding SLPI has been successfully constructed in the size of 412 bp and inserted into pET 30a in *E. coli* TOP10. The expression plasmid (pET/SLPIopt) has been successfully sub-cloned into *E.coli* BL21(DE3). The expression result showed that SLPI could be produced by using 25, 50, 100, 500, μM IPTG and mostly accumulated in cell pellet. **Conclusion:** The SLPI synthetic gene cloned in Escherichia coli can express the optimal recombinant protein of SLPI.

DECREASED NUMBER OF MACROPHAGES IN PERIODONTAL WISTAR (RATTUS NORVEGICUS) RATS TREATED WITH GRAPTOPHYLLUM PICTUM (L.) GRIFF. EXTRACT GEL

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ABSTRACT

Introduction: Aggressive periodontitis is a specific type of periodontal disease that is characterized by rapid attachment loss and bone destruction resulting in tooth loss. *Graptophyllum pictum (L.) Griff* is a vine that is widely used as a herbal medicine in Indonesia. *Graptophyllum pictum (L.) Griff* contains flavonoids, steroids, tannins, coumarins, saponins, anthtraquinones, phenols, and sugar. It is known that flavonoids contains quercetin which is known to have anti-inflammatory activity. The aim of this research was to investigate the effect of *Graptophyllum Pictum (L.) Griff.* extract gel on the number of macrophages as an inflammatory indicator on periodontal tissue of Wistar (*Rattus norvegicus*) rats with periodontitis. **Methods:** Twenty-four male Wistar rats were randomly divided into 5 groups: negative control group, positive control group and treatment group treated with 7.5%, 15%, and 30% *Graptophyllum Pictum (L.) Griff.* extract gel for 3 days. The periodontitis was induced by 0.2 ml Aggregatibacter actinomycetemcomitans (Aa) 109 CFU on the right lower molar gingival sulcus, three times a week. The number of macrophages from gingival tissue was analyzed by hematoxylin-eosin staining for histopathologic examination. **Results:** The number of macrophages (mean + SD) were 1.50 + 0.577 in negative control group; 10.25 + 2.062 in positive control group; 5.00 + 2.530, 3.76 + 1.211, 2.50 + 1.975 in treatment group with 7.5%, 15%, and 30% respectively. The significant difference in the average number of macrophages between the negative control group compared with the positive control indicates that the number of macrophages in the inflammatory process was increased to overcome Aa bacteria. In the treatment group, the number of macrophages was lowest at 30%, followed by 15%, and 7.5% of *Graptophyllum pictum (L.) Griff* extract gel. **Conclusions:** *Graptophyllum Pictum (L.) Griff.* extract gel can decrease the number of macrophages in Wistar rats with periodontitis. The concentration of 30% *Graptophyllum Pictum (L.) Griff.* extract gel was the most effective concentration in decreasing the number of macrophages.

DETECTION OF ANTI-CELL MEMBRANE DNA ANTIBODIES BY INDIRECT IMMUNOFLOURESENCENCE TECHNIQUE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) has a broad spectrum of clinical presentations. The diagnosis of SLE remains a challenge and largely depends on the presence of several serum autoantibodies including anti-nuclear antibody (ANA), anti-double-stranded DNA antibody (anti-dsDNA) and anti-Smith antibody (anti-Sm). ANA, a highly sensitive but not specific marker is used for SLE screening Anti-dsDNA and anti-Sm are SLE-specific biomarkers but has lower sensitivity of 80% and 30% for SLE, respectively. However, it is noted that there are SLE patients who are persistently negative for SLE-specific autoantibodies. Anti-dsDNA and anti-Sm were reported to be negative in up to 51.2% and 62.4% of SLE, respectively. This limitation can lead to misdiagnosis and halter proper treatment to SLE patients. Previous studies have suggested that cell membrane DNA (cmDNA) can act as a specific target for the autoantibodies in SLE patients. Autoantibodies towards cmDNA (anti-cmDNA) were reported to have promising value as a reliable biomarker for SLE. In this study, we would like to determine the usefulness of anti-cmDNA in diagnosing SLE as compared to the standard SLE-specific autoantibodies. **Methods:** Serum samples from 83 SLE patients, 86 other connective tissue diseases and 61 healthy subjects were included in this study. The other connective tissue diseases include samples from 10 Sjogren's syndrome, 56 rheumatoid arthritis, 12 scleroderma and eight mixed connected tissues disease (MCTD) patients. All samples were analysed by indirect immunofluorescence (IIF) technique using Raji cells as substrate to detect the presence of anti-cmDNA. Anti-cmDNA was reported as positive if there was presence of a fluorescent ring, either continuous or punctate. Sera from SLE patients were also tested for anti-dsDNA and anti-Sm antibodies by using enzyme-immunoassays. **Results:** Anti-cmDNA positivity was highest in SLE (55.4%) than in other connective tissue diseases (9.3%) and healthy subjects (0%). Anti-cmDNA was 100% specific at differentiating SLE from healthy subjects and 90.7% specific at differentiating SLE from other connective tissue diseases. There was no difference in the sensitivity (55.4%) of anti-cmDNA at differentiating SLE from both groups. Anti-cmDNA were present in 46 SLE samples negative for standard SLE-specific autoantibodies. It was detected in 11 (42.3%) of anti-dsDNA, 23 (63.9%) of anti-Sm and 8 (12.9%) of both anti-Sm and anti-dsDNA negative samples. **Conclusion:** The high specificity of anti-cmDNA detection using IIF method makes it an excellent diagnostic tool for SLE. Anti-cmDNA is potentially a very useful biomarker for SLE with negative anti-dsDNA or/and anti-Sm antibodies.

ADMINISTRATION OF GRAPTOPHYLLUM PICTUM LEAVES EXTRACT IN WOUND HEALING PROCESS ON COLLAGEN DENSITY

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ABSTRACT

Introduction: An incision in the oral cavity is made for gingivectomy. The benefit of using *Graptophyllum pictum* leaves extract as an alternative herbal medicine is that it has fewer side effects. *Graptophyllum pictum* extract contains active substances such as flavonoids, saponins, and tannins. These compounds have anti-inflammatory and antioxidant activities and play a role in wound healing processes. An important marker of wound healing is collagen density. Increased collagen synthesis during the proliferative phase of cells accelerates the wound healing process. This study observed the collagen density in wound healing process of Wistar rats' wound after administration of *Graptophyllum pictum* leaves extract. **Methods:** The study was conducted by making incision wounds in 24 Wistar rats which were divided into control groups and treatment groups. The control groups were left untreated and the treatment groups were given *Graptophyllum pictum* extract on the incision wound once every day for three, seven and fourteen days, and then the animals were sacrificed. Wound tissue was removed and fixed in 10% formalin solution, embedded in paraffin, and stained with Masson's Trichome (MT) to observe the density of collagen microscopically. Statistical analyses of collagen density were performed using Kruskall Wallis and Mann Whitney tests. **Results:** There were significant differences among the groups ($p<0.005$) on the collagen density after treatment for three, seven and fourteen days. **Conclusion:** Administration of Graptophyllum pictum leaves extract on Wistar rats' wound could increase the collagen density in the wound healing process.

LACTOFERRIN POTENTIAL TO INCREASE FIBROBLAST CELL NUMBER AND COLLAGEN IN THE WISTAR RAT WOUND HEALING PROCESS

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ABSTRACT

Introduction: Wounds can be defined as damage or separation of the skin, mucous membrane or tissue caused by the influence of physical, mechanical and biological injury. The healing process is divided into 3 stages, namely: inflammation, proliferation, and remodelling. Lactoferrin is a glycoprotein with iron-binding properties. Lactoferrin is an 80-kDa iron-binding glycoprotein within the transferrin family. Lactoferrin has various biological functions, including roles in iron metabolism, cell proliferation and differentiation, antibacterial, antiviral and antiparasitic activity. Lactoferrin will stimulate the work of macrophages which will produce various pro-inflammatory cytokines and anti-inflammatory cytokines, as well as various growth factors. Lactoferrin can trigger a decrease in pro-inflammatory cytokine processes but also activate anti-inflammatory cytokines, namely Transforming Growth Factor Beta (TGF-β), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF). fibroblast and collagen coir density. The aim of this study is to prove that lactoferrin can increase the number of fibroblast cells and collagen husks in the wounds of Wistar rats. **Methods:** This research is a laboratory experimental type with a post-test only control group research design. The sample consisted of 24 Wistar rats divided into four groups, namely the control group (K), and the treatment group treated with lactoferrin 50% (A1) for 3 days, the treatment group treated with lactoferrin 70% (A2) for 3 days and the group the treatment was treated with lactoferrin 90% (A3) for 3 days. The results of the study were obtained from histopathological examination, namely HE staining to observe the number of fibroblast cells and MT staining to observe the amount of collagen coir density. **Results:** There is an optimal increase in the number of fibroblasts and coir density of collagen by administration of Lactoferrin in the group of 70% (A2). **Conclusion:** Giving lactoferrin in a concentration of 70% can increase the number of fibroblast cells and collagen density in the wound healing process in Wistar rats.

PROSTAGLANDIN-ENDOPEROXIDE SYNTHASE (PTGS2) AND DEFENSIN BETA 1 (DEFB1) GENE POLYMORPHISM IN MALAYSIAN MALAY CHRONIC PERIODONTITIS SUBJECTS

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ABSTRACT

Introduction: *PTGS2* and *DEFB1* single nucleotide polymorphisms (SNP) have been validated to be associated with chronic periodontitis (CP) in European, Japanese and Chinese populations. Polymorphisms of these genes play a role in the pathogenesis of CP. Thus far, no study has been done on the Malay ethnic group. Hence, this study assessed the allele and genotype frequencies of *PTGS2* and *DEFB1* variants in subjects with chronic periodontitis and healthy individuals in Malaysian Malays. **Methods:** Malay CP subjects and periodontally-healthy controls were obtained from Malaysian Periodontal Database and Biobanking system (MPDBS) for this case-control study. Diagnosis for cases was based on case definition by Eke et al (2012). DNA samples were genotyped for 4 candidate SNPs, rs689466, rs5275, rs20417 (*PTGS2*) and rs1047031 (*DEFB1*). Genotyping was carried out using Taqman genotyping method. The association between SNPs and study groups were assessed using logistic regression analysis. **Results:** DNA samples from 140 individuals, 76 CP cases and 64 healthy controls were genotyped. Logistic regression results demonstrated that rs689466 for PTGS2 gene was associated with CP susceptibility in the Malay study group (p=0.03; OR: 1.80; 95% CI=1.05-3.07). The dominant and additive model test showed significant association with rs689466 (C/T) ($p_{dominant\text{-}adjusted}$=0.02; OR: 2.22; 95% CI=1.11-4.43;$p_{additive\text{-}adjusted}$=0.03; OR:1.85; 95% CI=1.07-3.19) after controlling for age and smoking. However, no significant association with CP was observed with other SNPs. **Conclusion:** The results suggest that rs689466 of PTGS2 gene may contribute to CP susceptibility in Malaysian Malay population in our preliminary study.

SALIVARY AND SERUM LEVELS OF CA AND ZN IN PERIODONTITIS WITH OR WITHOUT RHEUMATOID ARTHRITIS

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ABSTRACT

Introduction: While sharing a common causal link, both rheumatoid arthritis (RA) and periodontitis (PD) manifest similar inflammatory responses. With the progression of severity, both diseases result in bone loss. Hence, Ca and Zn, as structural components of the bones, are expected to be altered in saliva and serum in PD and RA respectively. Zinc and calcium concentrations have been studied previously in patients with PD or RA, with PD patients exhibiting increased salivary Ca and decreased Zn concentrations in serum, while RA patients have been reported to express low plasma concentrations of both Zn and Ca. The aim of this study is to evaluate the saliva and serum levels of Ca and Zn in PD patients with or without RA. **Methods:** Serum and saliva samples were collected from 82 patients from the Faculty of Dentistry, University of Malaya and the University Malaya Medical Centre rheumatoid clinic. Patients were grouped according to their periodontal health and RA status (healthy n=21; PD n=21; RA n=21; RAPD n=19). **Results:** Zinc concentration in serum was significantly higher ($p<0.05$) in the PD group (14.54±4.64 μM) compared to the RA group (11.71±2.04 μM), while in saliva samples the zinc concentration in the healthy group (2.07±1.45 μM) was significantly higher ($p<0.01$) than both the PD (1.59±3.44 μM) and RAPD (0.71±0.54 μM) groups. Calcium concentrations in serum were significantly higher ($p<0.01$) in the RA group (19.3±4.28 mg/dL) compared to the control (13.38±1.95 mg/dL) and PD groups (12.36±2.9 mg/dL), while calcium concentrations in saliva were significantly higher ($p<0.05$) in the RA group (5.41±2.25 mg/dL) compared to the other groups (control = 3.18±1.13 mg/dL; PD = 3.41±0.75 mg/dL; RAPD = 3.69±1.25 mg/dL). **Conclusion:** These results suggest that PD and RA affect serum and salivary Zn and Ca concentrations in the course of PD and RA pathogenesis. Further research in this area could uncover more links between PD and RA that as of yet have not been explored.

THE USE OF COLLAGEN BIOMATERIAL IN ORAL CANCER: A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: Oral cancer is the sixth most common malignancy in the world. It is a major concern in Southeast Asia primarily due to betel quid chewing, smoking, and alcohol consumption. In Malaysia, oral cancer related cases accounts for 1.55% of the cause of deaths. Despite recent advances in cancer diagnoses and therapies, the survival rate of oral cancer patients only reached 50% in the last few decades. Tissue engineering (TE) principles may provide new technology platforms to study mechanisms of angiogenesis and tumour cell growth as well as potentially tumour cell spreading in cancer research. The use of biomaterial, appropriate cell source and proper signalling molecules are vital components of TE. Collagen biomaterial are widely used scaffold or membrane in oral application. Nevertheless, no review has been performed on the its usage for the study of oral cancer. This study aimed to systematically review the use of collagen scaffold in oral cancer application. **Methods:** Research articles were searched using Scopus, Pubmed and Web of Science (WOS) databases. The keywords were limited to "collagen membrane OR collagen scaffold" AND "oral cancer". **Results:** Initial search yielded 61 papers (Scopus:37, Pubmed: 12, WOS: 12). Further scrutinization of the papers based on the inclusion criteria resulted total of 3 papers. Two of the papers used collagen membrane for regeneration of oral mucosal defect and increment of alveolar ridge height post-surgery. The remaining paper utilize collagen biomaterial as scaffold for the culture of adenoid cystic carcinoma (ACC) cells. All papers reported significant role of collagen biomaterial in terms of tissue formation, healing scaffold and cellular proliferation. **Conclusion:** Collagen utilization as biomaterial offers potential use for regeneration of oral related structures as well providing useful model for therapeutics anti-cancer research.

THE ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF AQUEOUS OIL PALM (ELAEIS GUINEENSIS) LEAF EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

Introduction: *Staphylococcus aureus* is a Gram-positive staphylococci that form biofilms. Bacteria that dwell in biofilms tend to be highly resistant towards the action of antibiotics. *S. aureus* is a main cause of infections in the oral cavity such as angular cheilitis, endodontic infections, osteomyelitis of the jaw, parotitis and oral mucositis. Previous studies reported that *S. aureus* also spread to the other parts of the body through the circulatory system, which may lead to chronic infections. Hence the search for new antibacterial agents remains high and needs urgent attention to treat this problem. Plants offer a rich source of antimicrobial agents and bioactive compounds. In this study, aqueous oil palm leaf extracts (OPLE) has been used as an alternative antibacterial agent against oral infections mainly caused by *Staphylococcus aureus*. Many studies report the potential use of oil palm leaf extracts in treating bacterial infections such as *Escherichia coli, Salmonella sp., Staphylococcus aureus* (isolated from other part of the body), *Pseudomonas aeruginosa* and *Bacillus sp*. Although previous studies have documented the antimicrobial properties of oil palm leaf extracts, to date no study has been reported on the effect of oil palm leaf extract on oral microbes. **Methods:** The agar diffusion method, minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assay were conducted in order to observe the antibacterial activity of aqueous oil palm leaf extract. The crystal violet assay was used to determine the anti-biofilm activity of the extracts. Chlorhexidine and deionised distilled water were used as the positive and negative control respectively. For agar diffusion method, the diameter of inhibition zone was measured. **Results:** The inhibition zone of the tested bacteria was observed between 0-20mm. The MIC and MBC assay were used to know the lowest concentrations of the extract that inhibit the growth and killed the tested bacteria respectively. The MIC and MBC values for the tested bacteria were observed between 0-7.813mg/mL. While for anti-biofilm assays, OPLE aqueous extract acts as a potent anti-biofilm agent with dual actions, preventing and eradicating the biofilm of the tested bacteria. **Conclusion:** In conclusion, the tested plant extracts could serve as alternative natural antibacterial and anti-biofilm agent against oral infections.

MITRAGYNA SPECIOSA METHANOL EXTRACT (MSME) EXHIBITS POTENTIAL ANTI-OXIDATIVE PROPERTY AND INHIBITORY ACTIVITY ON TUMOUR GROWTH STIMULATING CYTOKINES IN SW480 COLORECTAL CANCER CELL LINES

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ABSTRACT

Introduction: Alternative treatment for cancer from herbal medicine has gained interest due to its benefits on immune modulation, improving the survival and quality of life. *Mitragyna speciosa* (*M. speciosa*) or Kratom is an indigenous plant that can be found in Thailand and northern part of Peninsular Malaysia has becomes popular in recent years due to its ability to exhibit the opioid-like effects of analgesia. Mitragynine is the main alkaloid in M. speciosa which is found to reduce gastrointestinal motility and has been used by local communities as traditional treatment for diarrhoea and many other diseases. However, there is lack of scientific evidence to show that *M. speciosa* has anti-oxidative and anti-cancer properties especially in colorectal cancer. Therefore, our study aims to evaluate the anti-oxidative properties of *M. Speciosa* methanolic extract (MSME) and its effects on colorectal cancer cell line, SW480. **Methods:** The anti-oxidant content and scavenging activity of MSME were determined by total phenolic content (TPC) assay and total flavonoid content (TFC) assay as well as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay respectively. Cytotoxicity and cytokine inhibitory effects of MSME on SW480 cells were determined by (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) and cytokine beads array (CBA), respectively. **Results:** The TPC of MSME (0.1mg/ml = 85.85 ± 8.25 mg GAE/g extract; 1mg/ml = 167.43 ± 13.50 mg GAE/g extract; 10mg/ml = 408.94 ±7.17 mg GAE/g extract) was lower than pterostilbene, the positive control drug (76.37 ± 2.75; 230.52 ± 10.92; 835.44 ± 6.84 mg GAE/g extract). Conversely, the TFC of MSME (0.1mg/ml = 32.17 ± 27.92 mg QE/g extract; 1mg/ml = 347.72 ± 15.97 mg QE /g extract; 10mg/ml = 739.81 ± 5.56 mg QE /g extract) was slightly higher than pterostilbene (ND; 212.73 ± 17.92; 700.50 ± 3.47 mg QE/g extract). In DPPH assay, MSME showed comparatively similar antioxidant scavenging activity (IC50=4.34µg/ml) with pterostilbene (IC50=4.393µg/ml). However, MSME showed lower anti-oxidant scavenging activity (IC50=4.26µg/ml) than pterostilbene (IC50=1.556µg/ml) as measured by ABTS assay. In cytotoxicity assay, IC50 of MSME on SW480 cells was determined to be at 1.486 mg/ml. Overexpression of cytokines such as IL-6, IL-8 (CXCR8) and IL-10 could potentially promote tumour cell proliferation, growth and metastasis. Increased production of these cytokines through LPS stimulation in SW480 was slightly reduced by treatment with MSME. **Conclusion:** MSME could have a potential bioactive compound that possesses anti-oxidative and anti-cancer properties that would be beneficial as an alternative treatment of colorectal cancer.

BODY MASS INDEX (BMI) AND THE SECRETION OF SALIVARY HUMAN β DEFENSINS 1 (HBD-1): A STUDY IN CHILDREN WITH DENTAL CARIES

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ABSTRACT

Introduction: Human beta defensins 1 (hBD-1) is a peptide with antimicrobial activity. Sensitive to both gram negative and gram positive bacteria, hBD-1 is produced by epithelial tissues and produced continuously in many organs, including in the oral cavity. Recently, hBD-1 has been detected in saliva and is linked to oral disease. Alongside other human beta defensins, the role of hBD-1 is important in innate immune responses. Physical condition also plays an important role in innate immune responses, including body mass index (BMI). The aim of this study was to find the relationship between BMI and the secretion of salivary hBD-1 in children with dental caries. **Methods:** This study obtained ethical approval from the local ethics committee. Children aged 9-10 years (n=40) with dental caries were included in this study. The weight (in kgs) and height (in cms) were measured and the BMI was calculated using BMI formula measurement for children. The children were divided into two groups (BMI>17 and 17<BMI). The oral examination was done using DMF-T and def-t index. Unstimulated saliva samples were collected and the secretion of hBD-1 were determined by ELISA (in µg/ml). **Results:** Statistical analysis showed that there was no correlation between BMI and the secretion of salivary hBD-1 ($p>0.05$; $p=0.385$). Further statistical analysis between low BMI (BMI<17) and the secretion of salivary hBD-1 also showed no correlation ($p>0.05$; $p=0.788$). No correlation was shown between high BMI (BMI>17) and the secretion of salivary hBD-1 ($p>0.05$; $p=0.865$. **Conclusion:** From this study, it can be concluded that the secretion of salivary hBD-1 in children with dental caries varies among individuals.

THE EFFECT OF SYNBIOTIC STREPTOCOCCUS SALIVARIUS K12 AND MUSA ACUMINATA ON CANDIDA ALBICANS BIOFILM FORMATION

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ABSTRACT

Introduction: *Candida albicans* is an opportunistic fungus that is associated with oral carcinogenesis. In addition, biofilm formation has been one of the important virulence factors of the yeast. *Streptococcus salivarius* K12 is an oral probiotic while Musa acuminata is a well-known prebiotic. The objective of this study is to investigate the effect of *S. salivarius* K12 and *M. acuminata* skin aqueous extract (synbiotic) on *C. albicans* with the hypothesis that *S. salivarius* K12 and *M. acuminata* inhibit *C. albicans* biofilm formation. **Methods:** To develop mono-species biofilm, *C. albicans* (ATCC MYA-4901 and cancer isolates, ALC2 and ALC3 strains) and *S. salivarius* K12 were standardised to 105 cells and 106 cells, respectively and grown in 96-well plate in nutrient broth (NB) or RPMI at 37 °C for 72 h. Polymicrobial biofilms were developed by inoculating both microorganisms in the same well with similar cell number as in mono-species. To determine the effect of synbiotic, similar protocol was repeated by mixing with 800 mg mL-1 of M. acuminata skin extract and incubated at 37 °C for 72 h. The medium was replenished at every 24 h, aseptically. Finally, the biofilms were assessed using crystal violet assay and the optical density was measured at OD620nm. **Results:** *C. albicans* strain MYA-4901 and ALC3, when grown in polymicrobial with *S. salivarius* K12 in NB that is predominated by yeast-form *C. albicans*, exhibited decreased biofilms by 71.40±11.7% and 49.40±3.9%, respectively when compared to the expected biofilms. Meanwhile in RPMI, which *C. albicans* strain ATCC MYA-4901, ALC2 and ALC3 were predominated by hyphal-form showed decreased biofilms by 72.0±26.7%, 53.4±14.4% and 65.7±6.7%, respectively when compared to the expected biofilms. **Conclusion:** *S. salivarius* K12 and M. acuminata skin extract synbiotic inhibit biofilm formation of *C. albicans* yeast and hyphal forms thus supported the hypothesis of the present study.

LEVEL OF INTERLEUKIN-1β AND INTERLEUKIN-6 IN PREGNANT WOMEN WITH DENTAL CARIES

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ABSTRACT

Introduction: Pregnancy is a physiological process that causes changes in the body, one of which is hormonal changes that cause microbiomes in the oral cavity of the mother to experience dysbiosis. This makes pregnant women more susceptible to dental caries affect IL-1β and the IL-6 response serves as a marker of inflammation. IL-1β and IL-6 will increase if there are active caries, and periapical lesions. Pregnant women will transfer microbiomes to the baby during vaginal delivery or cesarean section, therefore the mother's microbiome during pregnancy will be the same as the baby. The purpose of this study was to analyze the levels of IL-1β, IL-6 and oral microbiome in pregnant women with or without dental caries. **Methods:** A cross-sectional study of 20 pregnant women at Bhayangkara Hospital Surabaya by screening for dental caries and a questionnaire to support the data, after which saliva samples were collected. Saliva was then tested by ELISA for IL-1β and IL-6 levels and 16sRNA PCR. **Results:** The results of the study with the Kruskal-Wallis test obtained $p = 1.00$ ($p > 0.05$) for the IL-1β correlation between pregnant women with or without dental caries. As for the IL-6 correlation between pregnant women with or without dental caries, $p = 0.998$ ($p > 0.05$). PCR 16 sRNA results showed differences in oral microbiome variations in groups of pregnant women with or without dental caries (correlation of microbiome of maternal and infant oral cavities was not included). **Conclusion:** From the results of this study there was no increase in IL-1β and IL-6 in pregnant women with or without dental caries.

STUDYING THE ABILITY OF IMMUNOGLOBULIN Y ANTI-PORPHYROMONAS GINGIVALIS AGAINST PERIODONTOPATHOGEN BACTERIA ADHERENCE

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ABSTRACT

Introduction: Periodontitis is mainly caused by gram-negative bacteria. *Porphyromonas gingivalis* (*P.gingivalis*) is the main bacterium that causes chronic periodontitis and *Aggregatibacter actinomycetemcomitans* (*A.actinomycetemcomitans*) causes aggressive periodontitis. Egg yolk antibodies are an innovative technology that involve the formation of non-invasive polyclonal antibodies from egg yolk. IgY in the form of polyclonal antibodies is used in the form of passive immunization and these antibodies come from egg yolks, colostrum or concentrated cow's milk. IgY antibodies have the same biological role as IgG antibodies in mammals, namely as the main immunoglobulin that provides defense from infectious agents. The aims of this research to prove the ability of egg yolk IgY in inhibiting the occurrence of periodontopathogen bacteria. **Methods:** *P.gingivalis* ATCC 33277 was cultured in MHA and incubated at 37°C for 48 hours using an anaerobic jar. The bacterial culture was centrifuged at 6000 rpm, at 4°C for 15 minutes then suspended in PBS containing 1% BSA. The bacterial content was made to 108/ml, IgY anti-*P.gingivalis* was made to 7 concentrations, with each containing 50 µl IgY solution. Fifty µl enterocyte suspension was added to each concentration and shaken slowly in a shaking water bath at 37°C for 30 minutes. The bacterial suspension (108/ml) was added as much as 50 µl and incubated in a 'shaking incubator' for 30 minutes at 37°C. The samples were subsequently centrifuged at 1500 rpm, at 4°C for 3 minutes, smeared on a glass slide and stained with Gram staining. The preparations were observed under a 1000x magnification microscope, and the number of bacteria attached to the enterocytes were counted for each observation of 100 enterocytes. **Results:** This study shows that IgY anti-*P. gingivalis* can significantly reduce the adherence index value of *P.gingivalis*, *A.actinomycetemcomitans*, and can reduce the adherence index value of *F. nucleatum* but not significantly. **Conclusion:** IgY anti *P. gingivalis* can inhibit *P. gingivalis* and *A. actinomycetemcomitans* adherence, but cannot inhibit *F. nucleatum* adherence.

ANTIBACTERIAL ACTIVITIES OF GREEN SILVER NANOPARTICLES-STROBILANTHES CRISPUS (AgNP-SC) AGAINST CLINICALLY IMPORTANT BACTERIA

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ABSTRACT

Introduction: Silver nanoparticles has been proven to be an effective agent for antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms. Green synthesis is one of the methods that has been developed to synthesize silver nanoparticles in environmentally-friendly conditions. It uses plant extracts as reducing and capping agents. Besides act as reducing and capping agents, bioactives such as phenolic compounds may bind to silver nanoparticles and enhance its medicinal properties. Strobilanthes crispus is a Malaysian native plant. Previous studies had shown that *S. crispus* contains polyphenols, catechins, alkaloids, caffeine, tannins and vitamins. Therefore, the aim of this study is to determine antibacterial activities of silver nanoparticles-*Strobilanthes crispus* (AgNP-SC) against clinically important pathogens such as *Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus mutans*. **Methods:** The disc diffusion assay (DDA) was performed to investigate the inhibition zone of AgNps-Sc towards *E. coli, P. aeruginosa* and *S. mutans*. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) was used to determine bactericidal/bacteriostatic profile of AgNP- SC against *E. coli, P. aeruginosa* and *S. mutans*. **Results:** AgNP-SC (40mg/mL) shows the greatest inhibition properties (12.67±0.6mm) against *S. mutans* when compared to *Strobilanthes crispus* leaves extract (6.0±0.001mm) and blank silver nanoparticles (6.0±0.001mm). MIC values for AgNP-SC against *S. mutans* and *E. coli* were at 0.625 mg/mL and 1.25 mg/mL, respectively. Whereas the MIC value of AgNP- SC against *P. aeruginosa* was at 2.5 mg/mL. MBC values of AgNP-SC against *E. coli, P. aeruginosa* and *S. mutans* were at 1.25, 2.5 mg/mL respectively. Results are concentration-dependent, with higher concentration demonstrating better inhibition property. **Conclusion:** It can be concluded that AgNP-SC possesses bactericidal properties against *S. mutans*, *E. coli* and *P. aeruginosa*.

SUPPRESSION OF NON-ALBICANS CANDIDA SPECIES (NAC) BIOFILM FORMATION BY PROBIOTIC STREPTOCOCCUS SALIVARIUS

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ABSTRACT

Introduction: *Candida spp*. are most common opportunistic pathogenic yeast that inhabit human oral cavity, epidermis, gastrointestinal tract, and vagina leading to candidiasis. The transition of this yeast from commensal to potent pathogen is facilitated by numbers of virulence factors including biofilm formation. While most reports on candidiasis are associated with formation *Candida albicans* biofilms, however, non-*albicans Candida* species prevalence is of growing concern. Recently, the use of probiotics as antifungal and antibiofilm has gained an increasing attention. As such, we aim to evaluate the inhibitory effect of monomicrobial and polymicrobial of *Streptococcus salivarius* on six strains of NAC namely *Candida dubliniensis, Candida glabrata, Candida krusei, Candida lusitanaei, Candida parapsilosis* and *Candida tropicalis*. **Methods:** Antifungal activity of *S. salivarius* on NAC species was performed using well diffusion method on Mueller Hinton Agar (MHA) and the diameter of inhibition zone were assessed. For formation of monomicrobial biofilm, standardized cell suspensions of NAC species (1 x 10^5 cells/ml) and probiotic Streptococcus salivarius (1 x 10^6 cells/ml) were grown in RPMI or nutrient broth media at 37°C for 72 h. Meanwhile to study polymicrobial biofilm of both NAC and *S. salivarius*, similar protocol was employed by inoculating both microorganisms with a similar cell density as in monomicrobial. Finally, biofilm formation was assessed through quantification of total biomass by crystal violet (CV) assay and the absorbance of adherent biofilm was measured in triplicate at 620nm. **Results:** Antifungal susceptibility testing of S. salivarius on all six NAC species discerned no zone of inhibition. Furthermore, our results showed variability of monomicrobial and polymicrobial biofilm biomass between NAC species and growth medium. All six polymicrobial NB-grown and RPMI-grown exhibited decreased of the biofilm formation. *C. parapsilosis* co-cultured with *S. salivarius* in NB medium had shown lowest biofilm biomass by 75.51+_1.34% while in RPMI medium, *C. lusitanaei* demonstrated with most reduced biofilm biomass by 67.03+_5.19. **Conclusion:** Our study elucidated the antagonistic relationship between *Streptococcus salivarius* and non-*albicans Candida* by supressing the growth of polymicrobial biofilm and pseudohyphae/hyphae of NAC species.

ANTIBACTERIAL ACTIVITY OF SPILANTHES ACMELLA FLOWER EXTRACTS (SAFE) AGAINST STREPTOCOCCUS MUTANS

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ABSTRACT

Introduction: *Spilanthes acmella*, also known as "subang nenek', has been used traditionally in Malaysia to treat toothache. A previous study has shown Spilanthes acmella leaves extracts (SALE) inhibit *Streptococcus mutans* growth. *Streptococcus mutans* is commonly found in the human oral cavity and is the main contributor to tooth decay. There is no study on the antibacterial effects of *Spilanthes acmella* flower extracts (SAFE) against *Streptococcus mutans* reported to date. Therefore, the objective of this study is to investigate antibacterial properties of SAFE against *S. mutans*. **Methods:** *S. mutans* was subcultured in Muller Hinton (MH) broth and agar. Sequential extractions of *S. acmella* flowers were conducted using four different solvents with increasing polarity, [n- hexane, dichloromethane (DCM), acetone, methanol (MeoH)] and tested with different concentrations against *S. mutans* via the disc diffusion assay, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Sodium fluoride (NaF) was used as a positive control while DMSO was used as a negative control. **Results:** The disc diffusion assay shows SAFE inhibited Streptococcus mutans growth. SAFE-DCM shows the greatest inhibition properties (12.33±2.30 mm) followed by SAFE-n-hexane (11.33±0.57 mm). Meanwhile, SAFE-Meoh and SAFE-acetone show no inhibition zone (6.00±0.001 mm). MIC value for SAFE-DCM and SAFE-n-hexane is 12.5 mg/mL respectively. Whereas, MBC value SAFE-DCM and SAFE-n-hexane is 50.0 mg/mL respectively. **Conclusion:** It can be concluded SAFE-DCM and SAFE-n-hexane possesses bactericidal properties against *Streptococcus mutans*.

CORRELATION BETWEEN CORRESPONDING PROTEIN AND MRNA IN ACUTE MYELOID LEUKAEMIA (AML)

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ABSTRACT

Introduction: Protein and gene expressions are intensively profiled for potential biomarkers in diagnosis or prognosis of diseases. The correlation between corresponding protein and mRNA of a gene is important to establish whether transcript levels of a given gene can be used as proxies for the corresponding protein levels. mRNA profiling is more commonly utilised as this method is cheaper and the technology more advanced. Acute myeloid leukaemia (AML) is a heterogeneous group of malignant precursors of the myeloid lineage that leads to death if not treated. Cytokines and death receptors are commonly evaluated in this disease in search of potential biomarkers; however, the mRNA/protein correlations of these biomarkers are still unclear. **Methods:** Semi-quantitative expression of mRNA expression and protein levels of IL-1β, IL-18Rα, IL-6, TNF-α and DR5 were measured by conventional polymerase reaction (PCR) and flow cytometry in 11 cases of AML at diagnosis. Correlation in the intensity of the PCR amplicon and corresponding mean fluorescence intensity of protein was determined by Spearman's rank correlation test. **Results:** None of the cytokines/death receptor was significantly correlated except IL-6 (Rs= -0.6287, p=0.038). Unexpectedly, this was also a significant negative correlation. **Conclusion:** For the majority of selected biomarkers in AML, whether secreted or surface-expressed, mRNA and protein expressions were not significantly correlated. The strong negative correlation for IL-6 is worth further investigation.

MICROBIOME OF TRACHEOESOPHAGEAL PROSTHESIS: WHO ARE THEY?

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ABSTRACT

Introduction: Laryngectomy patients undergo voice rehabilitation that requires implantation of trachea-oesophagal speech valves (TESV). Usually, laryngeal cancer patients require insertion of these devices post-operatively to improve their quality of life. Implantation of TESV dates back to 1979 by pioneering work of Blom and Singer. There are cases of aspiration of TESV wearer reported, and obstruction of the TESV causes leakage through the valve and is suggested as a main reason for replacement of the device. The dysfunctional failure may be caused by microbial colonization on the valve or physical malfunction and requires immediate replacement is desirable. The aim of this study is to identify the microbial community members of selected TESVs using both culture-independent techniques (Next-generation sequencing) to analyse the microbiota, including unculturable species, and routine microbiology techniques (culture-dependent method) and to obtain representative isolates that can form the basis for experiments to enable increased understanding of the community. **Methods:** Biofilms were harvested from 16 explanted speech valves from patients visiting the ENT clinic in Freeman Hospital, Newcastle, UK. Routine microbiology techniques (culture-dependent method) including ChromeID® plates and Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) Mass Spectrometry were used for identification of TESV microbiome. Sequencing of the samples was performed at MR DNA (www.mrdnalab.com, USA) on a MiSeq following the manufacturer's guidelines in order to determine the bacteria and candida composition in the biofilm community. **Results:** The most frequently isolated fungal species was *C. albicans*, which was cultured from 11 out of 16 TESVs (79%), followed by five TESVs with *C. tropicalis* (36%), three TESVs had *C. glabrata* (21%) and only one TESV contained *S. cerevisiae* (7%). Interestingly no biofilm communities contained more than two fungal species and 2 TESVs (12%) possessed only bacterial species. There were only 16 species of bacteria cultured and identified by MALDI-TOF MS. This was far lower than the 91 species that were detected by NGS. Species from the genus *Lactobacillus* were found in 10 of 16 TESVs (63%), the highest frequency of any bacterial genus isolated from TESVs followed by *S. aureus* found in eight TESVs of 16. S. epidermidis was identified in two TESVs (13%), *Streptococcus spp., K. oxytoca* and *O. anthropi* were both identified in five different TESVs, while the gut bacterium *E. faecium* was found in four TESVs. Only one TESV contained *E. coli*. **Conclusion:** TESV biofilm composition was dominated by *Candida* spp. and occasionally contained other types of eukaryote such as *Saccharomycetes*. It was not uncommon for more than one *Candida* species to be present. The biofilms also harboured a mixture of bacteria, with lactic acid producers (*Lactobacillus* sp. and *Streptococcus* sp.) normally accompanying *Candida* sp. in the biofilm.

THE EFFECT OF BROTOWALI (TINOSPORA CRISPA) EXTRACT ON NUMBER OF MACROPHAGE CELL OF WISTAR RATS (RATTUS NORVEGICUS) PERIODONTITIS

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ABSTRACT

Introduction: Periodontitis is an inflammatory disease of the periodontal tissues commonly caused by the bacteria *Actinobacillus actinomycetemcomitans*. In the healing process of periodontitis, macrophages have an important role in the inflammatory phase. Brotowali (*Tinospora crispa*) contains flavonoids that can accelerate the healing of periodontitis. **Methods:** Brotowali extract was taken from brotowali dried stems. The dried specimens were processed into powder and macerated with 80% ethanol. The extraction was prepared in three concentrations, namely, 25, 50 and 100%. Wistar rats were divided into 5 groups, the negative control group: healthy Wistar rats; the positive control group: Wistar rats with periodontitis; treatment group 1 = Wistar rats with periodontitis given brotowali extract concentration of 25% (dose 0.1 ml); treatment group 2 = Wistar rats with periodontitis given brotowali extract concentration of 50% (dose 0.1 ml); Group 3 = Wistar rats with periodontitis given brotowali extract concentration of 100% (dose 0.1 ml). Histological slides were prepared to calculate the number of macrophages. **Results:** One-Way ANOVA test showed significant differences in each group. Test Tukey HSD Post Hoc Test showed no significant difference between group 1 and group 2. Conclusion: Brotowali extract may affect the number of macrophage cells of Wistar rats induced by bacteria *Actinobacillus actinomycetemcomitans*.

BUCCAL SWAB AS A SUITABLE SOURCE OF GENOMIC DNA FOR SCREENING OF SELECTED HUMAN LEUKOCYTE ANTIGEN (HLA) ALLELES

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ABSTRACT

Introduction: Human leukocyte antigens (HLA) are a group of unique transmembrane glycoproteins that are expressed on the surface of virtually all types of cells within the human body. These molecules are encoded by a set of highly polymorphic gene sequences known also as the major histocompatibility complex (MHC) and play an essential role in the presentation of antigenic peptides to immune cells for recognition and response. In recent years, various HLA alleles have been found to be associated with different autoimmune and inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and allergic rhinitis. Identification of these alleles via HLA typing is necessary for initial screening and diagnosis purposes. Besides that, HLA typing is also used to determine compatibility matching between a donor and a recipient for tissue/organ transplantations in order to prevent graft rejection. Therefore, good quality and quantity of genomic DNA is required. In most scenarios, peripheral blood is chosen as the most reliable source of DNA for analysis, however this approach is seen as invasive and may cause pain and anxiety among the patients, particularly young children and weak subjects. Hence, derivation of genomic DNA from buccal cells as an alternative source material is becoming increasingly popular, especially in PCR-based genetic assays. Some of the most commonly described methods to collect buccal cells include using oral swabs, cytological brushes, mouthwashes and treated cards. Each technique yields varying quantities of DNA with diverse purity levels. In this study, we aim to evaluate the amount and purity of genomic DNA extracted from buccal swabs and brushes as well as blood for screening of selected HLA class II alleles. **Methods:** Cheek cell samples were collected using sterile foam tipped buccal swabs (Whatman) and buccal collection brushes (Gentra Puregene) whereas peripheral blood samples were withdrawn following routine venipuncture techniques. All samples were subjected to DNA extraction according to modified commercial kit protocols. Screening of selected *HLA-DRB1* alleles was conducted via PCR with sequence-specific primers as established by Bunce et al. 1995. **Results:** There was no significant difference ($p > 0.05$) in the total DNA yield obtained from blood and buccal swab samples, which were 17.57µg (± 8.66) and 13.28µg (± 4.81), respectively. All samples exhibited similar 260/280 ratios of about ~1.80 ($p > 0.05$). However, buccal brush samples contributed the least amount of DNA (0.29µg, ± 0.12) compared to other sources ($p < 0.05$). The pure genomic DNA isolated from both blood and buccal swab samples were successfully typed for low resolution *HLA-DRB1* alleles. **Conclusion:** Buccal swabs provide good quantity and quality of DNA for screening of HLA alleles with high accuracy and thus can be utilized as a non-invasive substitute for venipuncture.

COMBINATION EXPRESSION OF Gα12 AND MAGED4B ASSOCIATED WITH POOR PROGNOSIS IN OSCC

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is a major health problem worldwide. The overall survival rate remains at 50% despite numerous studies and various treatment modalities in OSCC. The presence of lymph node metastasis in OSCC is well established as an independent prognostic factor. This present study aims to investigate the association of four tumour antigens; FJX-1, GNα12, IFITM3 and MAGED4B with the sociodemographic and clinicopathological parameters of OSCC. The potential use of these markers as a prognostic indicator of patient survival and lymph node metastasis in OSCC was explored. **Methods:** 35 cases of OSCC with available formalin-fixed paraffin-embedded (FFPE) specimens involving the tongue, buccal mucosa, gingiva, alveolus and floor of mouth were evaluated by immunohistochemistry for FJX-1, GNA12, IFITM3 and MAGED4B expression. Assessment of the expression of these tumour antigens was based on the cellular sub-site, intensity and percentage of staining in the OSCC samples. **Results:** The expression of all four tumour markers were expressed in all samples (n=35) but none statistically associated with any clinicopathological or socio-demographic parameters. Survival analysis using Kaplan-Meier test showed high expression of GNA12, IFITM3 and MAGED4B individually with poor prognosis in OSCC patients. A combination of markers, GNA12 and MAGED4B demonstrated a significant association with patient survival in OSCC (p=0.014). Multivariate analysis after adjustment for selected socio-demographic factors (age, gender, risk habits and sub-sites of the oral cavity) revealed that high expression of both MAGED4B and GNA12 remained as an independent prognostic factor for poor prognosis in OSCC (HRR =5.231, 95% CI 1.601,17.084; p=0.006). **Conclusion:** We concluded that high combined expression of both marker (Gα12 and mAGED4B) might be used as an independent prognostic indicator in OSCC.

CIRCULATING INTERLEUKINS IN LEPTOSPIROSIS WITH PULMONARY INVOLVEMENT PATIENTS

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ABSTRACT

Introduction: Leptospirosis is a re-emerging zoonotic disease caused by Leptospira bacteria. The clinical manifestations of leptospirosis include mild-fever to a severe or even fatal. Increased levels of inflammatory cytokines produced in response to the Leptospira infection by the host immune system were hypothesized as among the causes of severity in leptospirosis. Besides the classical presentation with the triad of febrile, jaundice, and renal failure, patients with leptospirosis also can pose with predominant sign and symptoms of pulmonary involvement. This study aimed to compare the levels of TNF-α, IL-1b, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-18, and IL-22 In the plasma of samples of leptospirosis patients with and without pneumonia. **Methods:** Circulating cytokine levels in plasma were measured in seventeen patients hospitalized and diagnosed with leptospirosis in Malaysia (January 2016 – December 2017) and nineteen healthy individuals as controls. Patients were categorized into leptospirosis without pneumonia (n=12) and with pneumonia (n=5). Cytokine was measured using SimplePlexTM assays (San Jose, CA, USA). Measurement was performed in triplicate and statistical analysis was conducted using Graphpad® Prism v6 (San Diego, CA, USA). **Results:** Elevation of plasma TNF-α, IL-6, IL-8, IL-10, IL-18, and IL-22 levels were observed among leptospirosis patients with pneumonia compared to without, although no statistical differences were observed between these two groups. **Conclusion:** There are no significant differences observed between the levels of plasma TNF-α, IL-6, IL-8, IL-10, IL-18, and IL-22 in patients with pneumonia compared to without.

EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR 4 (FGFR-4) IN NASOPHARYNGEAL CARCINOMA (NPC) CELL LINES AND ITS POTENTIAL AS THERAPEUTIC TARGET

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ABSTRACT

Introduction: Nasopharyngeal carcinoma (NPC) is a prevalent cancer among human population in Southern China, Hong Kong and Southeast Asia. In Malaysia, NPC is the fourth most common cancer in both sexes, predominantly in the Chinese. Epstein-Barr virus (EBV) infection is known to be highly associated with NPC. Fibroblast growth factor receptor-4 (FGFR4) is part of the family of tyrosine kinase receptors that regulate cell survival, differentiation and proliferation. The binding of FGFR4 ligands such as fibroblasts growth factors (FGFs) has been shown to activate various oncogenic signalling pathway including MAPK, Ras and PI3K-Akt pathways. In the past, FGFR4 has been shown to promote tumorigenesis and tumour progression in various cancers such as liver, colon, breast and pancreatic and gastric cancers. However, its role in NPC establishment and pathogenesis is under-explored. This study aimed to evaluate the FGFR4 expression in NPC using various cell lines and its potential as a therapeutic target for NPC treatment by gene silencing. **Methods:** The basal FGFR4 level of NPC (EBV-positive: C666-1 and EBV-negative: HONE1 and HK1) and nasopharyngeal epithelial (NPE) normal (NP69 and NP460) cell lines was determined by western blot analysis and RT-qPCR. FGFR4 level at different time points (0, 24, 48, and 72 hours) in HONE1 and C666-1 cell lines were determined by western blot analysis. Luminescence-based assay was performed to determine the cell proliferation of NPC cells in correlation with the FGFR4 expression. NPC cells were then treated with the optimised FGFR4 siRNA or FGFR inhibitor, BLU-9931 and the silencing/ inhibition of FGFR4 expression was confirmed by western blot analysis. The effect of FGFR4 inhibition on the cell proliferation and aggressiveness of NPC cells was then investigated through wound healing assay and invasion marker analysis. **Results:** Out of the five tested cell lines, HONE1 and C666-1 highly expressed FGFR4, NP69 showed very low expression while HK1 and NP460 did not express FGFR4. In the time-point study, the FGFR4 level of HONE1 and C666-1 peaked at 24-48 hours which is the exponential phase of cells. Following that, the FGFR4 level decreased corresponding to the decreased cell growth rate due to the nutrient deprivation. siRNA experiments showed that 6.25nM of four siRNAs (5, 6, 9 and 10) could effectively target and silence the FGFR4 expression of HONE1, but not in C666-1 even up to 250nM was tested. When BLU-9931 was used, only modest inhibition was observed in both cells at 3uM. Compared to the untreated control, FGFR4-inhibited HONE1 exhibited decreased cell proliferation rate. Cell migration and invasion capabilities of HONE1 were also significantly reduced following the FGFR4 silencing, suggesting the potential of utilising FGFR4 as the therapeutic target. **Conclusion:** FGFR4 is highly expressed in C666-1 (EBV-positive) and HONE1 (initially EBV-positive, but lost EBV genome in subsequent in vitro passage) NPC cells, but not in EBV-negative HK1 NPC cell and normal NPE cells. FGFR4 gene silencing effectively inhibited the cell proliferation, migration and invasive potentials of NPC cell line. These findings highlight the therapeutic value of targeting FGFR4 for NPC treatment. Further investigations are warranted to reveal the molecular mechanism and the possible role of EBV in regulating FGFR4 pathway.

ANTI-PROLIFERATION EFFECTS OF BENZIMIDAZOLE ANALOGUES ON GASTRIC CANCER: STRUCTURE ANALYSIS RELATIONSHIP

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ABSTRACT

Introduction: Benzimidazole analogues are bicyclic compounds that had been synthesized comprising the fusion of benzene and imidazole. It gains interest in research as it poses numerous therapeutic potential such as anti-ulcer, anti-malarial, anti-helminthic, anti-fungal, anti-inflammatory, and anti-cancer. Hence, this work aims to screen novel benzimidazole analogues using MTT assay for potential anti-proliferation activities on gastric cancer, which is the second cause of cancer-related death. **Methods:** MTT assay was conducted following standard protocol on HGT-1 gastric cancer cells. Cells were seeded and allowed to attach overnight before being introduced with various concentration of benzimidazole analogues up to 72 hours and the optical density of the MTT was recorded using 560 nm wavelength. Two-Way ANOVA was used to analyse all data, followed by post-hoc Tukey test and the structure analysis relationship was analysed using MTT result. **Results:** From five analogues, only compound 4 showed anti-proliferation activity with IC50 8.212 ± 0.813 μM at 72 hours. Compound 4 had hydroxyl group at ortho- and para- position and remarkably, compound 2 which contained the hydroxyl group at ortho- and meta- position together with compound 5 which contained the combination of meta- and para- induced proliferation on gastric cancer. **Conclusion:** Different position of hydroxyl group on the benzene ring gives different activities on gastric cancer and from the experiment, only compound 4 had the anti-proliferative activity.

HALAL & ORGANIC MOUTH-WASH: FRANKINCENSE ESSENTIAL OIL & HYDROSOL BOSWELLIA MOUTH-WASH ESSENTIAL OIL (BOMEO)

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ABSTRACT

Introduction: This is a proto-type product which is based on Frankincense essential oil and hydrosol. **Methods:** Three oleo gum resin species, namely; *Boswellia carterii* (BC), *Boswellia frereana* (BF), and *Commiphora myrrha* (CM) of Burceraceae family were extracted for their essential oil by hydro-distillation. They were screened for their potential of anti-cariogenic activity by in-vitro experimental study of two main bacterial species (*Streptococcus mutans* and *Lactobacillus spp*), which are considered the main cause of dental and mouth diseases. **Results:** Methanol and acetone extracts of the three plants inhibited the growth of the bacteria. However, BF-methanol extract shows the greatest inhibition followed by BC and CM respectively. Hence, the obtained result encourages proceeding further thorough investigation to benefit the positive outcomes of these plant extracts in terms of introducing new potential antimicrobial formulations, such as mouth wash which can be used for mouth cleansing and protection from the diseases such as mouth ulcers, gingivitis, sinusitis, glandular fever and brucellosis as well as dental caries. This result can be converted to Boswellia Mouthwash Essential Oil (BosMEO) and Boswellia Mouthwash Hydrosol (BosMoHy) based products. This new plant extract product can be exploited for further research for its potential used as moth infection natural treatments such as mouth ulcers, gingivitis, sinusitis, glandular fever, brucellosis as well as respiratory problem. It is free of synthetic chemicals, organic, natural, plant based, and halal with no major health side effects. **Conclusion:** Plant-based product which is free from synthetic chemicals and with minimal side effects will satisfy its quality efficiency.