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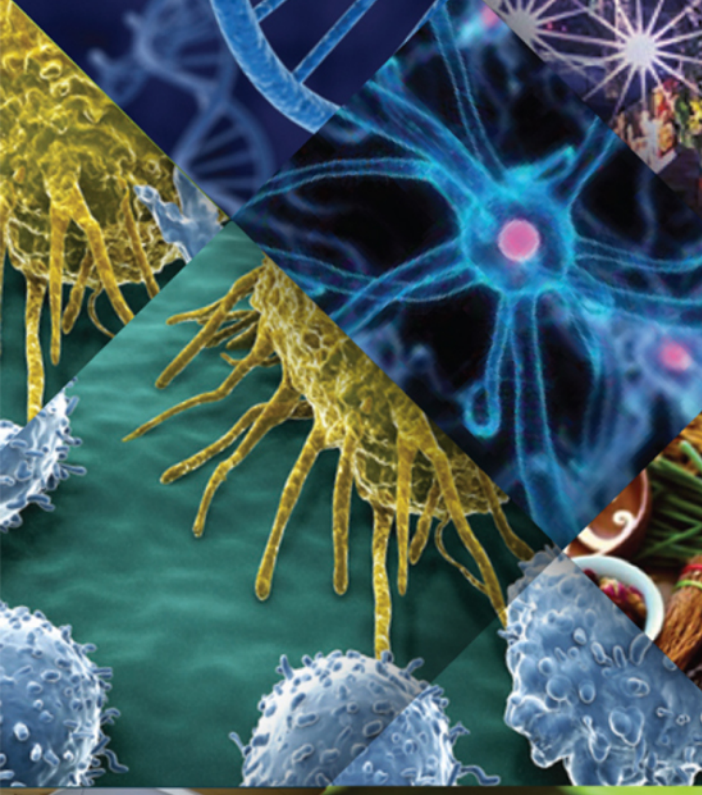

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VENUE: Riverside Majestic Hotel
Kuching, Sarawak, Malaysia

Pre-conference: Abstracts for Oral Presentation

001

Bioassay-guided isolation of a new anti-colorectal carcinoma agent, Rubbing-Mercapto-Nitrile, isolated from *Nicotiana glauca*

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Purpose: Our previous investigations showed that the n-hexane extract of both stem and leaves of Nicotiana glauca exhibited the highest cytotoxicity against the colon (HCT-116) and breast (MCF-7) cancer cell lines in comparison with ethanol, methanol, and water extracts. In the present study, we report for the first time the active anticancer principle that underlies the observed activity.

Methods: A new compound called Rubbing-Mercapto-Nitrile (RMN) was isolated from N. glauca and characterized using anticancer bio-assay guided isolation. the effects of RMN on HCT-116 cells was studied using cell cytotoxicity, inducement of apoptosis, anti-tumorigenicity, and hanging drop assays, and the effects on cell signaling pathways were evaluated using the Cignal Reporter Assay.

Results: RMN was selectively cytotoxic against HCT-116 cells (half maximal inhibitory concentration (IC50 = 0.056 μM) and significantly induced apoptosis in these cells via nuclear condensation, chromatin degradation, and damage to the mitochondrial membrane potential. RMN displayed a dose-dependent inhibitory effect on the HCT-116 tumor spheroids. It also caused down-regula¬tion of the TGF and HIF signaling pathways and up-regulation of the WNT, NOTCH, NF-κB, ERK, P53, and JNK signaling pathways.

Conclusion: This study describes a new anti-colon cancer compound and its mechanisms of action *in vitro*. An *in vivo* study is recommended to obtain a full understanding of RMN as a potential chemotherapeutic agent.

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Keywords: *Nicotiana glauca;* Rubbing-Mercapto-Nitrile (RMN); Colorectal carcinoma; Apoptosis

002

Practices In Manufacturing Premise That Elevate The Risk Of Toxicity To Consumers Of Herbal Based Products

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Purpose: Traditionally human has consumed plants in various ways benefiting from phytochemicals contained in the different parts of the plant namely fruit, seed, leaf, and root. Nowadays, these bioactives have been formally commercialized in food, cosmetic and medicinal products. These products are regulated by laws to ensure the consumer's rights are protected in term of product's quality, safety and efficacy. Responsible manufactures have implemented good manufacturing system namely MESTI, ISO 9001, HACCP, ISO 22000, WHO-GMP and ICH-GMP to establish a systematic reliable controlled production. The presentation shares some practices seen at manufacturing premises which require preventive actions among herbal product manufacturer to improve products safety.

Methods: Walk-thru visits at several manufacturing premises in Peninsular Malaysia that produce herbal based products under the categories of food, beverages and traditional medicine. The visit is part of efforts to assist local industry of small and medium enterprises (SME).

Result(s): The implementation of the good manufacturing system in the premises visited has obviously created controlled production of herbal based products which is beneficial to assure product quality. However, there are some practices seen at the manufacturing premises which may elevate the risk of toxicity.

Conclusion(s): The effectiveness of good manufacturing system to assure secure and safe manufacturing of quality herbal based products is dependent on the scientific knowledge of the production key personnel.

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Acknowledgement: Heartiest appreciation to management of the manufacturing premises for the kind permission and trust of confidentiality.

Keywords: Herbal Product, Manufacturing, Toxicity, Safety, Quality, GMP

003

Cytotoxicity of plant-mediated metallic nanoparticles: A systematic review

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Purpose: This systematic review aims to compare the cytotoxicity of plant-mediated metallic nanoparticles based on its potency, therapeutic index and cancer cell type susceptibility in finding the most promising anti-cancer agents to be developed. This study also correlates nanoparticle size and morphology with the potency of cytotoxicity.

Methods: A literature search was conducted on electronic databases including Science Direct, Elsevier, PubMed, Springer Link and Google Scholar. Keywords such as biosynthesis, plant synthesis, plant-mediated, metallic nanoparticle, cytotoxicity and anticancer were used in the literature search. All types of research which met the inclusion and exclusion criteria were considered regardless of the results being positive, negative or null.

Results: Data from 76 selected articles were extracted and synthesised. Most research showed the cytotoxicity property of plant-mediated metallic nanoparticles and cytotoxicity being time and/or dose-dependent. Silver nanoparticles demonstrated higher cytotoxicity potency as compared to gold nanoparticles from the same plants, irrespective of the cell types used. Size and morphology determine the resultant cytotoxicity potency of the studied nanoparticles. *Butea monosperma, Melia azedarach, Annona squamosa, Couroupita guainensis, Indoneesiella echioides,* and *Gossypium hirsutum* metallic nanoparticles were acceptably safe as anti-cancer agents, having a therapeutic index of 2.0 and above when tested on both cancer cells and normal human cells.

Conclusion: The listed plant-mediated metallic nanoparticles are among the most promising anti-cancer agents to be developed. Results from this study suggest a focus on the listed potential anticancer agents for further investigations hoping to reduce the Global Burden of Diseases (GBD) and the second leading cause of mortality.

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Keywords: anti-cancer, cytotoxicity, nanoparticles, plant, therapeutic index

007

Micropropagation of SuperFruits in Malaysia for Nutritional Security

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Purpose: The term superfruit is defined as fruits that contain high concentrations of nutrients in the superfood category mainly due its rich sources of phytochemicals and antioxidants. In Malaysia, the fig (*Ficus carica*), lemon (*Citrus* × *limon*) and gac (*Momordica cochinchinensis*) are categorised as superfruits that are currently being introduced to the local market. The fig fruit is well known for its high levels of calcium, phenolic antioxidants and dietary fibre with its leaves possessing anti-diabetic properties. The lemon is known for the high amounts of vitamin C and health-promoting phytochemicals such as amines, flavonoids and limonoids whereas the gac fruit has been proven to contain exceptionally high levels of lycopene that are beneficial in reducing cardiovascular diseases and prevention of prostate cancer. The aim of the project is to micropropagate the fig, lemon and gac for commercial purposes.

Methods: Sterilized explants of the fig, lemon and gac were subjected to culture and propagation in media supplemented with plant growth regulators for multiple shoot induction. Shoots obtained were rooted in the rooting media and acclimatized for field adaptation.

Result(s): The current study has successfully established the micropropagation steps for fig. Tissue culture propagated fig plants were found to grow and produce fruits at a faster rate compared to the cuttings grown in the greenhouse.

Conclusion(s): Ongoing work involves improvement and establishment of micropropagation methods for lemon and gac. The *in-vitro* superfruit plantets can also be further investigated in other studies related to the pharmaceutical industry and production of natural products.

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Keywords: fig, lemon, gac

008

Assessing Elateriospermum tapos for efficacy and safety: pharmacological screening and toxicology on 3T3-L1 and developmental toxicity zebrafish (Danio rerio) embryo.

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Purpose: This study focuses on antioxidants activities, and inhibitory effects of *Elateriospermum tapos* on digestion and absorption of key digestive enzymes. Despite numerous biological and pharmacological activity exerted by natural products, some may carry toxicological properties. To achieve safe treatment, in vitro model (3T3-L1) and in vivo model (zebrafish embryo) were monitored.

Methods: To explore the potential effectiveness of E.tapos, cold aqueous (C), hot aqueous (H) and 70% ethanol (E) extracts of both the seed (D) and shell (S) were used in this study. The total phenolic (TPC) and flavonoid content (TFC) and its inhibitory effect on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and beta-carotene bleaching assay were quantified. The extracts were screened for inhibitory effects on pancreatic lipase, alpha-amylase and alpha-glucosidase. Toxicity studies were assessed on 3T3-L1 cells and zebrafish embryo survival, hatching rate, also heart rate.

Result(s): Among all types of extraction, hot aqueous extraction of E.tapos shell (SH) has the highest TPC (176.9±0.6mg) and TFC (218.4±6.4mg) which also significantly ($p<0.05$) inhibited DPPH and beta-carotene. The seed extracts inhibited pancreatic lipase, alpha-amylase and alpha-glucosidase with IC50 48.9mg/ml (DC), 0.03mg/ml (DC) and 0.02mg/ml (DE) respectively. However, pancreatic lipase, alpha-amylase and alpha-glucosidase was more sensitive to shell extracts with IC50 0.03mg/ml (SE), 0.03mg/ml (SH) and 5.6mg/ml (SH) respectively. The SH and SE exhibited the minimum cytotoxicity and embryotoxicity across all extract types. The IC50 result from 3T3-L1 cell cytotoxicity test is highly correlated (r=0.96) to LC50 of zebrafish embryo acute toxicity (FET) test at 72 hours post-exposition, considered non-toxic. In FET, hatching rate and heart rate of the treated embryos were similar to normal control group.

Conclusion(s): The outcomes validates E.tapos as an antioxidant and inhibitor of digestive enzymes for lipid (pancreatic lipase) and carbohydrate (alpha-amylase and alpha-glucosidase) which useful to combat obesity and diabetes. Correlation confirmed E.tapos is non-toxic at optimum dose level, suggesting future potential obesity and diabetes therapeutics.

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Keywords: 3T3-L1, alpha-amylase, alpha-glucosidase, antioxidant, pancreatic lipase, zebrafish embryo acute toxicity test.

011

The Use Of Natural Resources As Traditional And Complementary Medicine (Tcm) Among The Rural Community In Ramsar Site, Sabah, Malaysia

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Purpose**:** Available evidences on the practice of traditional medicine have largely been derived from the Malay, Chinese and Indian communities in Malaysia. Through the narrative review of previous literature, we discovered that limited empirical documentation has been made to study the practices of Traditional and Complementary Medicine (TCM) among the rural communities in Sabah, especially those residing along the Ramsar site. Thus, this article aims to document the natural resources used in TCM by the rural communities in Dagat village in the Lower Kinabatangan-Segama area.

Methods**:** Using qualitative ethnography research, a series of in-depth interviews was carried out with two experienced traditional medicine practitioners (both women) from the Dagat village which is located within the Ramsar site, Lower Kinabatangan-Segama, Sabah. These two women are very experienced in traditional medicine practices and still play the role as 'emergency doctors' for the villagers in Dagat village. After the interviews, the raw data were analysed by using the thematic analysis technique.

Results**:** This research has successfully identified the natural resources used for TCM practices by the local community in the study site. The informants revealed that almost all resources in traditional medicine practices fully depend on forests surrounding in their living area. These plants are pivotal for the informants treating the patients in the village and this acknowledges the natural resources in surrounding area are priceless sources.

Conclusion**:** This study discovered that the local communities within the Ramsar site, particularly in Dagat village, are highly dependent on natural resources to solve their health-related issues. It is also believed that these floras have significant therapeutic values as perceived by the study's informants. It is also our hope that the findings from this study could be used to identify the potential floras that can be used to complement the conventional healthcare industry in Malaysia.

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012

Protective effects of *Elacteriospermum tapos* against obesity in high-fat diet Sprague-Dawley rats.

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Purpose: This study aims to determine the preventive effects of *E. tapos* extract on rats fed with high-fat diet and its potential as an anti-obesity agent through inhibition of lipoprotein lipase and triglyceride absorption.

Methods: Thirty-six male Sprague-Dawley rats were randomly assigned to six groups as follows: a negative control group (C) fed with normal diet, while positive control (HFD), drug control (HFD-ORL), and treatment groups (ET10, ET100 and ET200) were fed with high-fat and cafeteria diet. The HFD-ORL group was given Orlistat of 10mg/kg bodyweight and the treatment groups were supplemented with *E.tapos* extract of three different concentrations (10 mg/kg, 100mg/kg and 200mg/kg bodyweight). Bodyweight and calorie intake were measured weekly. After 4 weeks, the rats were euthanized and the weights of organs, triglycerides level, and plasma lipid profiles were measured.

Results: Group fed with high fat diet had significantly higher bodyweight, calorie intake and weight of adipose tissue compared to C group. The extract significantly lowered the bodyweight and deposition of retroperitoneal adipose tissue in treatment group as compared to HFD group. Triglycerides level of adipose tissue in ET10 and ET100 groups were significantly lower compared to HFD. The extract (ET100 group) also significantly reduced low density lipoprotein as compared to HFD group.

Conclusion: Based on this study, the aqueous extract of *E. tapos* shell has exhibited preventive effects on high-fat and cafeteria diet-induced obesity in rats.

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Acknowledgement: The work was supported by a project grant GP-IPM Universiti Putra Malaysia.

Keyword: Obesity, *Elacteriospermum tapos*, anti-obesity, lipoprotein lipase and triglyceride inhibition

013

Metabolite profiling of mangosteen (*Garcinia mangostana* Linn.) pericarp, aril and seed tissues using GC-MS based metabolomics approach

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Purpose: Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit with unique taste and pleasing aroma, known to possess phenolic compounds with anti-inflammatory and anti-cancer properties. However, there is less emphasis in other mangosteen tissues such as aril and seed. Potential bioactive compounds including primary metabolites from these various tissues are still inadequately documented and previous research were mainly focused on certain secondary metabolites. Hence, a global metabolomics study focusing on primary metabolites has been conducted to obtain an overview of the biochemical profiles of this fruit.

Methods: This study utilized GC-MS analysis to elucidate the metabolite composition in mangosteen pericarp, aril and seed during the final stage of ripening (dark purple stage). Two extraction methods were compared which mainly differ in their methanol/chloroform/water solvent ratios (3:1:1 v/v or 2:1:2 v/v). The results from both analyses were then further analyzed using a correlation analysis.

Result(s): In total, 43 metabolites were found from both extraction methods and different tissues. Various types of metabolites were also identified ranging from sugars (51% of total metabolites), organic acids (14%), sugar acids (12%) as well as others. Correlation analyses further suggested that nine metabolites such as thymol-α-d-glucopyranoside, α-D-galactofuranose, galacturonic acid, butanoic acid, arabinopyranose, myo-inositol, β-hydroxypyruvic acid, D-ribose and D-xylopyranose were well correlated between the different extraction methods while others were either weakly or negatively correlated.

Conclusion(s): This current study highlights the importance of method optimization in a metabolomics study particularly when different tissues of a plant species are of interest.

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Keywords: GC-MS, mangosteen fruit, metabolomics

014

Metabolomics: A New Insight to Unravel the Potential of *Polygonum minus*, a Local Herb

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Purpose: Rapid development in botanical drugs, including traditional herbal medicines, analysis of their bioactive components is becoming more popular. One of the goals of metabolite profiling is to detect all metabolites (defined as the metabolome) in a biological sample with high accuracy in terms of quality and quantity. Thus, metabolomics approaches here have the benefit of building on present knowledge and research.

Methods: A variety of techniques, including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) are applied to carry out metabolite detection to the maximum extent possible.

Result(s): *P. minus* of different lowland and highland origin were grown under a controlled environment with different temperature regimes to study the effects on secondary metabolites. Further studies on antioxidant, total phenolic content, anticholinesterase and antimicrobial activities were also determined.

Conclusion(s): This study focuses on the applications of metabolomics platform in unraveling the local herb potential in pharmaceutical and nutraceutical industry.

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Acknowledgement: The study was funded by UKM RU grants (UKM-GUP-2013-028 and DLP 2013-024). We acknowledged the Chulalongkorn University, Thailand for the Visiting Lecturer Fellowship 2017 awarded to Syarul Nataqain Baharum.

Keywords: Metabolomics, *Polygonum minus*, Local Herb

015

Authentication and Quality Control of *Clinacanthus nutans* (Burm. f.) Lindau (Acanthaceae)

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Purpose: *Clinacanthus nutans* (Burm. f.) Lindau (CN), a native plant from the family of Acanthaceae is endemic in Southeast Asia especially in Thailand and Malaysia. It has been used traditionally for treatment of inflammation, herpes viral infection and cancer. Research to date has shown some anti-inflammatory, anti-oxidative, antibacterial antiviral, anti-proliferative and antitumorigenic effects. Regardless of pharmacological researches, there is a need of ensuring the authentication, consistency and reproducibility of the herb to assure the public safety. Hence, in order to cope with the international trend, this study aimed to develop authentication and quality specification of *Clinacanthus nutans* (Burm. f.) Lindau according to the pharmacopeias standard.

Methods: Ten batches of *Clinacanthus nutans* (Burm. f.) Lindau were collected from the herbal retailers, wholesalers or direct from the herbal plantations around Malaysia and Taiwan. A series of experiments from morphological (macroscopic and microscopic) identifications, chemical analysis (thin layer chromatography, high performance thin lipid chromatography), to safety assessments were carried out according to the pharmacopeia standards.

Result(s): Macroscopic and microscopic analyses allowed for *Clinacanthus nutans* (Burm. f.) Lindau to be readily distinguished through special features. Chemical profiling revealed a few chemical compounds available to become standard marker other than schaftoside. The chromatographic fingerprint provided alternative analytical tool for quality control and act as an approach to express various pattern of chemical ingredients available for future studies.

Conclusion(s): Concisely, the study suggested methods and proposed limits can be used as a reference for the authentication and quality evaluation of the *Clinacanthus nutans* (Burm. f.) Lindau.

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Keywords: *Clinacanthus nutans* (Burm. f.) Lindau, plant authentication, quality control, schaftoside

017

Formulation of oil-in-water nanoemulsion containing *Jasminum officinale* and *Anthemis nobilis* L. essential oils as repellent against *Aedes aegypti*

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Purpose: Essential oils can be a better alternative to synthetic repellents, however their efficacy reduces relatively fast due to high volatility. Formulation of nanoemulsion is one of the approaches used to improve the longevity and efficiency of essential oils. The aim of this work was to formulate the nanoemulsion containing jasmine (*Jasminum officinale*) and chamomile (*Anthemis nobilis* L.) essential oils and to evaluate their repellent activity against *Aedes aegypti*.

Methods: Essential oils from the flowers of jasmine and chamomile were characterised by gas chromatography-mass spectrometry. Oil-in-water nanoemulsions were synthesised using ultrasonic homogenisation technique and characterised with respect to ternary phase behaviour, droplet size, zeta potential and stability. The repellent activity of the nanoemulsions was evaluated against *Ae*. *aegypti* using rat model.

Result(s): Oxygenated monoterpenes and diterpenes constituted 31.14% and 21.20% of jasmine essential oil, respectively. The major constituents of the chamomile essential oil are oxygenated monoterpenes (84.79%). Formulation nJC1 consists of v/v, 20% jasmine and chamomile essential oils, 7.5% water, 5% Montanov™ 82, and 37.5% glycerol while formulation nJC2 comprised of 20% jasmine and chamomile essential oils, 5% water, 5% Montanov™ 82 and 40% glycerol. The droplet size of nJC1 and nJC2 were 264.2 nm and 291.4 nm, respectively and their nanosizes were confirmed by transmission electron microscopy images. nJC1 and nJC2 exhibited 81.02% and 72.69% repellency for 480 minutes, respectively which was higher than 10% DEET.

Conclusion(s): Formulation of *J*. *officinale* and *A*. *nobilis* L. essential oils into nanoemulsion substantially increased their repellent efficacy and protection time against *Ae*. *aegypti* mosquitoes.

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Acknowledgement: We gratefully acknowledge the Research University (RU) Grant (1001/CIPPT/812121) from Universiti Sains Malaysia for funding this project.

Keywords: essential oil, repellents, nanoemulsion

023

Acute toxicity study of aqueous extract of *Aquilaria Malaccensis* leaves in Sprague Dawley rats

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Purpose: *Aquilaria malaccensis* (AM) or commonly known as 'gaharu' is a species of *Aquilaria* genus and belongs to Thymelaeaceae family. It is widely distributed in Malaysia, Indonesia and Borneo Island. It was scientifically proven as antioxidant, aphrodisiac and tranquilizer. Traditionally, its leaves was used to relieve bruise. However, there is no existing study concerning on safety assessment of AM leaves extract. The OECD Guideline 420 with fixed dose was used as study design.

Methods: The extract powder was lyophilized and constituted by dissolving in distilled water. This acute toxicity study was comprised of two phases; sighting and main study. As for sighting study, female Sprague Dawley rats were orally administered with single doses of 5, 50, 300 and 2000 mg/kg and observed for 24 hours and prolonged until 14 days. The main study was conducted using the highest dose with no mortality in sighting study.

Result(s): The acute oral dose of the AM extract up to 2000 mg/kg did not produce mortality or significant changes in the body weight, food and water consumption. Behavioral patterns were recorded as normal throughout 14 days of observation. The relative weights of the internal organs were normal. Further, histological examination revealed normal morphology of the kidney, heart and liver.

Conclusion(s): The acute toxicity test of aqueous extract of AM leaves suggested that a 2000 mg/kg dose of extract is devoid of any adverse effects in rats and further toxicity studies are required before this plant should be considered as safe in herbal medicines used.

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Acknowledgement: Add acknowledgement here if required, for example, the funding body of the research work.

Keywords: *Aquilaria malaccensis*, toxicity, aqueous extract

024

The safety of herbal extracts in post-radiotherapy head and neck cancer patients

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Purpose: Western medical practitioners mainly concern about herbal-induced hepatotoxicity and nephrotoxicity, and thus bottleneck their referral to complementary herbal treatment. This study aimed to evaluate the safety of herbal extracts in relieving post-radiotherapy dry mouth among head and neck cancer patients.

Methods: Head and neck cancer patients in NCI were included in this 2 arms observational study. Treatment group maintained their oncology follow-up and received 6 months of tailored adjunct herbal extracts which prescribed based on Chinese medicine diagnosis from T&CM Unit, NCI. Control group patients continued their regular oncology follow-up in Oncology Clinic, NCI. Routine full blood count, renal profile and liver function test were monitored at baseline, 1st, 3rd and 6th month post-recruitment. The abnormal lab tests were graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The data from 1/6/2016 till 30/9/2017 was analyzed.

Results: The included 42 patients were in treatment group (n=28) and control group (n=14). The herbal extract prescriptions involved 26 herbal formulas and 90 single herbs (Mean= 8.58±1.29 g/dose). The herbs were prescribed orally twice daily. No reported incidence of grade 3 bone marrow suppression, liver or kidney injury in treatment group patients throughout study period. 5 patients defaulted their herbal treatment due to secondary bleeding tumor (n=2) or advanced disease (n=3) and later deceased. There was no statistically significant change in lab tests series between two groups.

Conclusion: Evidence-based use of herbal extract supports its safety as adjunct treatment in post-radiotherapy head and neck cancer patients.

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Keywords: safety, herbal medicine, neoplasms

025

Ethanolic Extract of Propolis From *Geniotrogona throracica* sp. of Stingless Bees Inhibited the Formation of THP-1 Derived Macrophage Foam Cells by Suppressing TNFα and IL-1β Secretion

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Purpose: The formation of macrophage derived foam cells is a hallmark of the early stage of atherogenesis. Propolis is a complex resinous obtained from the buds and exudates of plants which demonstrates various therapeutic potential. Many studies showed that propolis extracted from honey bees may inhibit atherosclerosis but no studies have been performed using propolis extracted from Indo-Malayan stingless bee. Hence, the current study was carried out to investigate the effect of propolis from *Geniotrogona thoracica sp*. on the formation of foam cells.

Methods: Propolis from three different colonies of *Geniotrogona thoracica sp.* were collected and extracted with 80% ethanol. The volatile and non volatile compounds in the propolis were analysed by GC-MS and HPLC respectively. The optimal concentrations of propolis extracts (0-200 μg/ml) to treat THP-1 derived macrophages were determined by using PrestoBlue®. THP-1 cells were differentiated into macrophages using 100 ng/ml of PMA for four days. Then, THP-1 derived macrophages were treated with 80 μg/ml of oxidized LDL (oxLDL) and 20 μg/ml of propolis extract at 6, 24 and 48 hours. The morphological appearance of foam cells was determined by Oil Red O staining while the amount of cholesteryl ester present in the cells were quantified by using Cholestryl Ester Quantitation Assay Kit. IL-1β and TNFα secreted by treated cells were measured by ELISA.

Result(s): Propolis from colony 2 was selected as it contained more abundance of bioactive compounds based on HPLC and GCMS analysis. Selected propolis was shown to have EC_{90} at 20 μg/ml and this concentration was used for further cells treatment. Morphological observation showed that lipid droplets were reduced in the cytosol of propolis and oxLDL-treated THP-1 derived macrophages. Similarly, cholesteryl ester contents were significantly reduced in propolis and oxLDL-treated THP-1 derived macrophages at all time points. In addition, propolis was also significantly reduced TNFα and IL-1β secretion in supernatant of oxLDL-treated THP-1 derived macrophages.

Conclusion(s): Ethanolic extracts of propolis inhibited the formation of THP-1 derived macrophage foam cells by suppressing TNFα and IL-1β secretion which normally up-regulated in the atherosclerotic plaques.

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Keywords: Propolis, Geniotrogona throracica, foam cells

027

Mechanisms of cytotoxicity induced by *Garcinia atroviridis* essential oils and 2-Deoxy-D glucose (2-DG) in Panc-1 human pancreatic cancer cell line.

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Purpose: *G.atroviridis* possesses strong antioxidant content, good antimicrobial and anti-inflammatory properties. 2-DG is a glucose analog and is currently undergoing clinical trials as promising anticancer drug. In this study, we aimed to elucidate the mechanisms of cytotoxicity induced by *G.atroviridis* essential oils from leaf (EO-L) and bark (EO-B) on Panc-1 cells. 2-DG was also used to study the combination effects with *G.atroviridis* essential oils on the cell proliferation, mitochondrial membrane potential and cell cycle distribution of Panc-1 cells.

Methods: The cytotoxicity of EO-L, EO-B and 2-DG was evaluated and compared using 3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay and IC_{50} values were established. The cytotoxic effects of EO-L, 2-DG and combination treatment were further evaluated on the mitochondrial membrane potential using JC-1 assay kit (Cayman, USA) by fluorescence microplate reader. The cytotoxic effects of EO-L, 2-DG and combination treatment were also evaluated on the cell cycle distribution of Panc-1 using BD CycleTestTM by flow cytometry. The combination index (CI) values were further calculated and isobologram was plotted using Compusyn software for synergy quantification analysis between EO-L and 2-DG.

Results: Cytotoxicity assay showed higher inhibitory effect in cells treated with EO-L when compared to EO-B. Based on the IC50 value, EO-L was further analysed for downstream mechanisms of action. Both EO-L and 2-DG exhibited synergism effect. EO-L, 2-DG and combination treatment had induced significant ($p < 0.05$) depolarization in mitochondrial membrane potential when compared to control. Cell cycle analysis revealed that EO-L and 2-DG alone had induced cell cycle arrest at G2/M and G1 phases, respectively, whilst combination treatment caused a G1 phase arrest in Panc-1 cell line.

Conclusions: In conclusion, EO-L either alone or in combination with 2-DG, exhibited potential anticancer properties in vitro. Downstream analyses involving mRNA expression by Real-Time PCR is currently underway to elucidate the molecular mechanism of cytotoxicity induced by EO-L and 2-DG.

Acknowledgement: This study was supported by Research University Grant from Universiti Sains Malaysia (1001.CIPPT.812197).

Keywords: cytotoxicity, EO-L, 2-DG, mitochondrial membrane potential assay, cell cycle analysis.

028

Biosynthesis and characterisation of silver nanoparticles using leaf extract of *Garcinia atroviridis* as phyto-reducer of silver ions.

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Purpose: Silver nanoparticles (AgNPs) synthesis procedures assisted by green chemistry using leaf extract of *Garcinia atroviridis* for reducing silver ions was applied in this study. This study focused on the biosynthesis, optimisation and characterisation of the AgNPs.

Methods: Various reaction conditions which controlling the shapes and sizes of the synthesised AgNPs such as metal ion concentration, mixing ratio of reactants (ratio of leaf sample to extraction solvent), leaf extract concentration, incubation time and temperature were optimised. The biosynthesised of AgNPs were then characterised by analytical techniques such as ultraviolet-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD).

Results: Optimum yield of AgNPs obtained as follows: Ag^+ concentration = 0.1M; mixing ratio of reactants = 1:4; leaf extract concentration = 10% w/v; incubation time = 72h and temperature = 32°C. The UV-Vis spectra exhibited surface plasmon resonance at the range of 443–459 nm of the biosynthesised AgNPs. The morphological studies by SEM and TEM reveal the spherical shape of biosynthesised AgNPs with size range from 5-60 nm. FTIR analysis confirmed the occurrence of hydroxyl, carbonyl and carboxyl functional groups in the *G. atroviridis* leaf extract, which were responsible as the bioreductant agent participated in the stabilisation of AgNPs formation. The XRD diffractogram which corresponded to (111), (200), (220) and (311) crystalline planes were further validated that AgNPs was face-centered-cubic in shape.

Conclusion: A simple green method for the preparation of AgNPs using the leaf extract of *Garcinia atroviridis* as potential bioreducer and several parameters have been optimised, characterised and established.

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Keywords: *Garcinia atroviridis*, biosynthesised silver nanoparticles, optimisation and characterisation studies

045

Phytochemical constituents, α-Glucosidase inhibitory activity and preliminary acute toxicity studies of the ethanol-water extracts of *Salacca zalacca* fruits in rats

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Purpose: To evaluate the phytochemical constituent screening, α-glucosidase inhibitory activity and *in vivo* preliminary toxicity study of ethanol and water *S. zalacca* fruit extracts.

Methods: The fresh fruit was collected, cleaned, peeled, and dried using freeze dryer. The flesh powder was extracted using solvent with different concentration of ethanol and water (100, 80, 60, 40, 20, 0%). All extracts were tested for α-glucosidase inhibitory activity and phytochemical constituents were evaluated using different chemical methods. The extract showing highest α-glucosidase inhibitory activity was tested for acute toxicity studies according to the up and down method. A dose (0.15, 0.48, 1.54, 4.91 and 5.00 g/ kg body weight) of the extract was used in 15 healthy male rats. The rats were observed for 4 h first day, then daily for 14 days.

Result: The highest yield was determined for the extract of 80% concentration, whereas the highest α-glucosidase inhibitory activity (IC_{50} 13.43±2.43 µg/mL), total phenolic content (TPC) (14.12+1.19 µg AAE/g) resulted for the extract of 60% concentration. The phytochemical screening showed the presence of saponins, carbohydrates and cardiac glycoside while alkaloids and flavonoids were not detected in all the extract. The five doses caused neither visible signs of toxicity nor mortality. The LD_{50} is above 5000 mg/kg.

Conclusion: The ethanol and water extract possessed some active principles having α-glucosidase inhibitory activity and is safe following single oral administration dose but not via the intraperitoneal route.

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Keywords: Salak fruit, acute toxicity, α-Glucosidase inhibitory activity

046

Determination of antioxidant content and antioxidant activity of seeds extracts from *Archidendron jiringa* (Jering) and *Archidendron bubalinum* (Kerdas)

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Purpose: The aim of this study was to determine the antioxidant content and antioxidant activity of *Archidendron jiringa* and *Archidendron bubalinum* seeds.

Methods: *A. jiringa* and *A. bubalinum* seeds were extracted using hot aqueous, cold aqueous and 70% ethanol. Five different antioxidant assays, namely, 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), β-carotene bleaching and phosphomolybdenum were deployed to gauge the antioxidant activity. Folin-Ciocalteu and aluminium chloride colorimetric assays were used to determine the antioxidant content for total phenolic content (TPC) and total flavonoid content (TFC), respectively.

Result(s): 70% ethanol extract of *A. bubalinum* and *A. jiringa* seeds exhibited the highest TPC and TFC, respectively. The 70% ethanol extract of *A. jiringa* seeds exhibited the greatest antioxidant activity using DPPH, FRAP and phosphomolybdenum assays. All extracts of *A. jiringa* showed higher activity in ABTS assay when compared to *A. bubalinum* extracts. In addition, the aqueous extracts of both plants showed the greatest antioxidant activity when compared to 70% ethanol using β-carotene bleaching assay. In particular, hot aqueous extract of *A. jiringa* seeds (79.09%) exhibited the strongest antioxidant activity, followed by cold and hot aqueous extract of *A. bubalinum* (71.00% and 70.19%, respectively).

Conclusion(s): The best solvent for crude extraction of *A. bubalinum* and *A. jiringa* seeds was 70% ethanol for TPC, TFC, DPPH, FRAP and phosphomolybdenum whilst both hot and cold aqueous extracts showed highest antioxidant activities using ABTS and β-carotene assays. *A. bubalinum* and *A. jiringa* seeds have potential as natural antioxidant agents.

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Keywords: *Archidendron jiringa*, *Archidendron bubalinum*, extraction, antioxidant activity, total phenolic content, total flavonoid content

047

Inhibitory activity of various extracts of Selaput tunggul (*Mikania micrantha)* against pancreatic lipase, lipoprotein lipase, and HMG-CoA reductase *in vitro*

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Purpose: This study was aimed to investigate the inhibitory activity of various extracts of the leaves and stems of *Mikania micrantha* against lipases, *i.e*., pancreatic lipase (PL), lipoprotein lipase (LPL), and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase *in vitro*.

Methods: The inhibitory activity of hot water, cold water, 70% ethanol and ethyl acetate extracts of the leaves and stems of *M. micrantha* against the enzymes were determined spectrophotometrically using PL, LPL, and HMG-CoA reductase inhibition assays.

Result(s): The highest inhibition on the PL activity was exhibited by ethanol stems (ETS) extract followed by hot water leaves (HWL) and ethanol leaves (ETL) extracts (IC_{50} = 4.49±2.50 μg/mL, 4.56±0.07 μg/mL, and 8.02±1.56 μg/mL, respectively) which showed no significant ($p > 0.05$) difference between Orlistat (IC_{50}=0.31±0.01 μg/mL) as the control drug. For LPL, ethanol leaves (ETL) extract showed the highest inhibitory activity (IC_{50}=1.42±0.48 μg/mL), however, no significant difference was found among all extracts and Orlistat (IC_{50}=1.98±1.22 μg/mL). ETL also showed the highest inhibitory activity against HMG-CoA reductase where the percentage inhibition was 50.1±3.44% at 1 mg/mL, and no significant difference was found among other extracts except hot water stems (HWS) extract. HWS showed the least inhibitory activity against PL, LPL, and HMG-CoA reductase.

Conclusion(s): The present results demonstrated that *M. micrantha* extracts had anti-lipases and anti-HMG-CoA reductase properties, where ethanol extracts stand out as the best solvent. These enzymes inhibition implies the inhibition of dietary fat, plasma triglyceride and cholesterol synthesis which might have a great potential for prevention of hypercholesterolemia.

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Keywords: HMG-CoA reductase, hypercholesterolemia, lipoprotein lipase, medicinal plant, pancreatic lipase

049

Cytotoxicity and antiviral effect of *Carica Papaya Linn* against dengue virus

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Purpose: Active phytochemicals against dengue virus without having damaging effect on the host have been continually explored for safe and cost effective approach. *Carica Papaya Linn* leaves (CPL) are used as a traditional herb to treat dengue, in this study we focused to evaluate the cytotoxicity and antiviral effect of CPL against dengue virus.

Methods:
Crude extract (CE) and ethyl acetate fraction (EAF) were used for the current study. The total polyphenol content (TPC) was evaluated via Folin Ciocalteau method. MTT assay was performed to determine the maximal nontoxic dose (MNTD) of the CE and EAF and later screened for its antiviral activity by different time of addition of extract and dengue virus (pre and simultaneous) with Focus Forming Unit Reduction Assay.

Result(s):
The TPC of the CE and EAF were 12.07±0.002 mg per GAE/g dry extract and 36.6±0.008 mg per GAE/g dry extract respectively. The MNTDs observed on Vero cells were 500µg/ml for CE and EAF. EAF showed strongest inhibitory activity with 64 % reduction of foci (RF %) compared to CE at 33 RF% at 500µg/ml for simultaneous treatment of extract and virus. The pretreatment of the extract had weak antiviral activity with CE and EAF showing 27 RF% and 8.6% RF% at 500µg/ml of treatment respectively.

Conclusion(s): CE and EAF had no cytotoxic effect on Vero cells at the highest concentration tested. EAF exhibits good antiviral property against dengue *in vitro* with reduction of dengue infectivity in Vero cells, possibly due to its high polyphenol content.

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Acknowledgement: Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education (MOHE)

Keywords: *Carica Papaya* Leaves, Antiviral, Dengue

050

The induction of ER stress by green tea epigallocatechin-3-gallate (EGCG) in the colorectal cancer line HT-29.

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Purpose: Green tea has long been known as a timeless healthy beverage and the application of green tea has now been extended to food and pharmaceutical products. Taking into account Malaysians unhealthy eating habit, this has risen the number of colorectal cancer cases nationwide. This study attempts to combat the colorectal cancer incidences by inducing endoplasmic reticulum (ER) stress in colorectal cancer cell lines, HT-29. Being the most abundant and bioactive compound in green tea, the potential of EGCG has been further examined as ER stress inducing agent in our study.

Methods: The expressions of ER stress sensor proteins were determined by using Western blotting methods. The HT-29 cell lines were first treated with 88.1μM of EGCG (based on IC50 value from MTT results) at three different time points; 24h, 48h and 72h before the protein lysates were extracted. The protein lysates were also taken from control and positive control samples. The positive control drug used in this study is deferoxamine (DFO).

Result(s): Western blotting results showed the expression of two of ER stress main sensor proteins, Inositol-Requiring Enzyme 1 α (IRE1α) and Protein Kinase-like ER kinase (PERK) and as well as its downstream targets; eif2α and ATF4.

Conclusion(s): Green tea EGCG has demonstrated its potential as an ER stress inducer in colorectal cancer in our study. This ER stress induction by EGCG can potentially further induce cells apoptosis and therefore, EGCG could be an ideal candidate as pharmacological agent to treat cancer.

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Keywords: Green tea, Epigallocatechin-3-gallate, Colorectal cancer

054

Metabolomics approach to investigate the ergogenic effect of *Morinda citrifolia* l. xxtract in obese sprague dawley rats

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Purpose: Natural products are getting much acceptance as ergogenic aid, in enhancing physical performance, not only among the athletes but also the general population. Obese person mostly has reduced desire and ability to exercise; resulting in difficulty for obese person to reduce weight and fat in the body. Thus, they need to boost their energy production so that they can be more active and healthier. In this study, *Morinda citrifolia* L (MLE) believed to possess ergogenic property was used to evaluate its effect on obese animal model using H-NMR based metabolomic.

Methods: The rats were fed with High Fat Diet (HFD) for 12 weeks for obese development. Once become obese, all the rats were undergoing endurance exercise every two weeks for 8 weeks together with treatment. Forced Swimming Test (FST) was used in this study as endurance exercise. The time of exhaustion was recorded for each rat. Three different dosages of MLE were 50mg/kg, 100mg/kg and 200mg/kg of body weight was used together with two different positive control were 5mg/kg Caffeine and 100mg/kg Green Tea. Blood and urine were collected at week 0, 4 and 8 for metabolomic study.

Results: From this study showed that feeding the rats at a dose of 200mg/kg body weight MLE significantly prolonged the exhaustive swimming time of the rats, and alter metabolites present in their serum and urine. Discriminating metabolites involved were the product of various metabolic pathways, including carbohydrate, energy metabolism, and lipids metabolism. Treatment with 200mg/kg body weight MLE resulted in significantly improvement in the metabolic perturbations where the proximity of this obese exercise treatment group to the normal exercise group in the OPLS-DA score plot.

Conclusion: This study reports on the potential ergogenic property of MLE based on the metabolic perturbation in exercise obese rats.

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Keywords: ergogenic, MLE, obesity, exercise, H-NMR

055

A 90-day oral toxicity study of a Nigerian vegetable *Gongronema latifolium* in *Sprague Dawley* rats

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Purpose: The leaves of *Gongronema latifolium* Benth. (Apocynaceae) are consumed in Nigeria as spices and treatment for diabetes mellitus. In our laboratory, we previously reported on the use of an ethanolic extract of the leaves, GLES, in diabetic rats. This article evaluates the possible toxic effects of long-term use of GLES in male and female *Sprague-Dawley* rats.

Methods: GLES was given by oral gavage at 250, 500 and 1000 mg/kg consecutively for 90 days. Toxicity indices were measured afterwards, including body weight, organ weight and relevant biochemical and haematological parameters. Histopathological assessment of the key organs involved in xenobiotic metabolism and excretion - liver and kidneys - was conducted.

Result(s): GLES did not exert a marked impact on general haematological parameters. In addition, nephrotoxicity was absent as indicated by relative kidney weight, histology and serum creatinine levels. Serum triglycerides, total cholesterol and low-density lipoprotein levels were decreased in the male rats along with depletion in WAT paired retroperitoneal fat depots ($p < 0.05$). However, negative gender-specific alterations were observed with the highest dose. The liver was significantly enlarged in both sexes ($p < 0.05$). Adverse risk was evident in the female rats due to marked body weight gain and cerebrum weight decrease. Histopathological studies suggested adverse effects on the liver in the male rats.

Conclusion(s): Further research is needed to reach more guarded conclusions with regards to the safety of long-term high doses of GLES.

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Keywords: Diabetes Mellitus, *Gongronema latifolium*, Subchronic Toxicity

059

Bioactive glass doped with *Acmella oleracea* extract promotes the viability and proliferation of dental pulp stem cell (DPSC).

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Purpose: Bioactive glass (BG) is a biomaterial able to promote bone regeneration and creation of interfacial bonding between the materials and host's tissues. *Acmella oleracea* (AO), locally known as *Subang Nenek* is a medicinal herb traditionally used as a toothache medicament due to its strong antibacterial, antiseptic properties and anti-inflammatory activity. Therefore, this study aims to develop BG combined with AO extracts in promoting dental tissue regeneration.

Methods: The sol-gel BG 45S5 powder was synthesized with particle size less than 38µm. AO extracts was prepared using ethanol extraction method. Sol-gel BG conditioned medium doped with AO extracts at various concentrations were prepared and exposed to dental pulp stem cells (DPSC), and the cells response were evaluated using Alamar Blue assay at specified time frame.

Result(s): AO- and BG-conditioned medium promoted DPSC viability. However, an increase in DPSC cell viability was observed in BG-AO-conditioned medium at the ratio of 1mg/ml BG with 50, 100 and 250 µg/ml AO in comparison with AO alone. BG-AO-conditioned medium at dose of 25µg/ml supported greater DPSC viability compared to other dosage. All BG-AO dosage combination revealed continuous cell proliferation over the observation period.

Conclusion(s): Combination of BG-AO demonstrated a positive effect on the proliferation and viability of DPSC. Thus, it may help to promote dental and hard tissue regeneration.

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Keywords: Bioactive glass, Sol-gel, *Acmella oleraceae*

060

The modulation of cytokines secretion by *Clinacanthus nutans* extracts in J774.2 macrophages

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Purpose: *Clinacanthus nutans* plant from Acanthaceae family, is well-known as 'Belalai gajah' and Sabah Snake Grass in Malaysia. *C.nutans* extracts have been reported to exhibit anti-inflammatory, analgesic, antioxidant, anti-viral and anti-bacterial properties. However, the effects of *C.nutans* extract on macrophage activation have not been elucidated yet. Thus, in this study, the regulation of cytokines production by *C.nutans* extracts in J774.2 mouse macrophages is studied to investigate the immunoregulatory role of *C.nutans* extracts on macrophage activation.

Methods: *C.nutans* leaves were extracted using different polarities of solvents to prepare ethanol (EtOH), aqueous (AQ) and ethanol-aqueous (EtOH-AQ) extracts of *C.nutans*. J774.2 cells were treated with 1-1000 µg/ml of extracts and incubated for 24, 48 and 72 hours and the cytotoxicity effect of these extracts on J774.2 cells was determined using Presto-blue assay. The Th1/Th2/Th17 cytokines secretion in 48-hours extract-treated and LPS-treated macrophages was assessed using multiplexed cytokine bead-based assay and the data were analyzed using BD Facs Divaa. The median fluorescence intensity readings was used to generate the standard curve and interpolate the results.

Results: All the three extracts tested do not exhibit any cytotoxic effect towards J774.2 macrophages within the extract concentration range tested. 500 µg/ml EtOH-AQ extract significantly inhibited LPS-induced IL-6, IL-1β and IL-12p40 but not TNF cytokine secretion, whereas 32 µg/ml EtOH extract significantly induced IL-10 cytokine secretion in the presence of LPS in J774.2 macrophages. Meanwhile, IL-2, IL-4 and IL-17A cytokines secretion were below detectable range.

Conclusion(s): *C.nutans* extracts might have displayed their anti-inflammatory properties by inducing IL-10 cytokine to suppress pro-inflammatory cytokines secreted during infection to resolve inflammation.

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Keywords*:* *Clinacanthus nutans*, macrophage, cytokines

062

Mechanisms of action for cytotoxicity of *Crinum asiaticum* leaf methanol extract.

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Purpose: The objective of this study is to investigate the mechanisms of action for the cytotoxicity (anti-cancer potential) of *Crinum asiaticum* leaf methanol extract (CALME).

Methods: The mechanisms of cytotoxicity were studied by clonogenicity study, determination of nuclear condensation, mitochondrial membrane potential changes, and the effects on 10-major cancer pathways, wnt (TCF/LEF), notch (RBP-Jκ), p53/DNA damage (p53), TGFβ(SMAD2/3/4), cell cycle/pRb-E2F(E2F/DP1), NFκB, myc/Max, hypoxia(HIF1A), MAPK/ERK(Elk-1/SRF) and MAPK/JNK(AP-1).

Result(s): The extract previously showed high selectivity for MCF-7 (breast cancer), compared to VERO (African green monkey kidney medulla), and marginally selective for MCF-7, compared to EAhy-926 (hybrid of human endothelial and lung cancer) cells. The clonogenicity study on MCF-7 cells indicated CALME to be cytotoxic, and not cytostatic. The late apoptotic morphological changes in cells were also detected; CALME caused DNA condensation and disrupted mitochondrial membrane potential. CALME was found to induce apoptosis in MCF-7 cells, either by down-regulating pro-proliferative genes (hypoxia and MAPK/JNK), or by up-regulating tumour suppressor genes (p53, TGF-β, pRb-E2F, and NF-κB).

Conclusion(s): The main mechanism of action for cytotoxicity of CALME is apoptosis via the above-mentioned pathways. The results of this study clearly highlight the anti-cancer potential of CALME towards breast cancer.

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Keywords: *Crinum asiaticum,* cytotoxicity, apoptosis, breast cancer.

064

Immunomodulatory activity of *Moringa oleifera* leaves ethanol extract on normal lymphocytes and leukaemic cell lines

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Purpose: The present study aimed to investigate the *in vitro* immunomodulatory effect of *Moringa oleifera* leaves' ethanol extract on healthy peripheral blood mononuclear cells (PBMCs) and leukaemic cell lines.

Methods: Healthy donors were used as a source of primary lymphocytes while Jurkat and BV173 cells were utilised as transformed cell lines of T cells and B cells, respectively. The cytotoxicity of aqueous and ethanolic extracts of *Moringa oleifera* leaves on the cells was determined using MTT assay. The immunomodulatory effect was evaluated through cell proliferation assays, cell cycle analysis and apoptosis assays. The anti-tumour potential of the extract on Jurkat and BV173 cells was further explored via global secretome and apoptotic proteins proteome arrays.

Result(s): From the cytotoxicity analysis, 70% ethanol *Moringa oleifera* leaves extract exerted a dose-dependent stimulatory effect on PBMCs with an EC50 of 28±3μg/mL as well as cytotoxic effects on BV173 (IC50 = 125±6μg/mL) and Jurkat cells (IC50 = 262±3μg/mL). The extract enhanced the viability and proliferation of PBMCs by committing the cells into the cell cycle and reducing apoptosis while exerting anti-proliferative effects, cell cycle arrest and apoptosis in tumour cell lines. Also, the extract induced overexpression of pro-apoptotic cytokines and proteins but suppressed the expression of angiogenic factors and pro-survival proteins in tumour cell lines.

Conclusion(s): *Moringa oleifera* ethanol extract has immunostimulatory properties on normal lymphocytes and anti-tumour activity on leukemic cell lines. These abilities can be exploited in developing herbal supplements that strengthen the immune system to support aged and immunocompromised individuals as well as serve as adjuvants in therapies against blood cancers.

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Keywords: *Moringa oleifera*, Immunomodulation, Anticancer

066

Chemometric-based analytical study of *Clinacanthus nutans* leaf extract and fractions on cardiac stem cell activities

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Purpose: This study assessed the metabolite variation of crude aqueous *Clinacanthus nutans* (*C. nutans*) leaf extract together with its fractions and evaluated their effects on cardiac stem cells (CSCs) activities via chemometric approach.

Methods: Crude *C. nutans* leaf extract (CE), aqueous fraction (AF), aqueous-ethanol fraction (AEF) and ethanol fraction (EF) were subjected to CSCs cytotoxicity and proliferation assays. Spectrometric data were obtained from liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS). Raw data were normalised before analysing with principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) to reveal the distribution and discrimination of *C. nutans* samples. Partial least square (PLS) was applied to study on the correlation between metabolite contents and CSCs bioactivities.

Result(s): Aqueous fraction (AF) exhibited the highest CSCs activities compared to AEF and EF, attaining 162.59 % of viability and increased of cell number up to 9542 at 1000 μgmL^{-1} ($p < 0.05$). Metabolites such as stachydrine, allyl mercaptan, butyramide, 2-methylpiperidine, diethanolamine, xestoaminol c, A-type proanthocyanidin, schaftoside, and their derivatives were putatively identified in AF. CV-ANOVA ($p < 0.05$) significantly proved that PLS is reliable in demonstrating the relationship between metabolite variation and activities.

Conclusion(s): Chemometric approach is useful in characterising the metabolite variations of different *C. nutans* samples correlated to different activities. Bioactivities result suggested that metabolites in AF such as stachydrine, allyl mercaptan, a-type proanthocyanidin and schaftoside could be the potential alternative regimen for CSCs development.

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Keywords: *Clinacanthus nutans,* LC-Q-TOF-MS, cardiac stem cells, metabolomics

067

Antioxidant and Antiproliferative Activities of *Clinacanthus nutans* and *Dracaena sanderiana* leaves extracts

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Purpose: The objective of the current study was to evaluate the antioxidant and cytotoxic activities of *D. sanderiana* (DS) and *C. nutans* (CN) extracts.

Methods: The total polyphenolic contents (TPC), total flavonoid contents (TFC) and 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) of the extracts were evaluated using spectrophotometric procedures. The cytotoxic activity of the extracts on three cancer cell lines; human breast (MCF7), colon (HT29) and pancreatic carcinoma (Panc1) was determined using the MTT assay.

Result(s): The highest total phenolic and flavonoid content for CN were observed in chloroform extract (10.9660 ± 0.36 mg GAE/g extract, 70.7259 ± 12.22 mg QE/g Extract) and petroleum ether extract of DS (16.64 ± 0.71 mg GAE/g extract, 223.32 ± 8.30 mg QE/g Extract). The methanol extract of both plants showed the highest levels of DPPH radical-scavenging activity (IC_{50} = 2.605 mg/mL for CN and 1.37 mg/mL for DS). All extracts showed reduction in cell viability with dose dependent response. Aqueous extract of CN exhibited the highest toxicity against HT29 and Panc1 while chloroform extract of DS showed the highest toxicity against MCF7 with IC_{50} values of 60 µg/mL, 94 µg/mL and 15 µg/mL, respectively.

Conclusion(s): Overall, this work provides scientific support regarding bio-activities of these plants extracts and discover potential applications for disease treatment in future.

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Keywords: TFC, TPC, DPPH, Cytotoxicity, *D. sanderiana*, *C. nutans*

071

Characterization of phytochemicals and antioxidant activities of three edible ferns from Sarawak, Malaysia

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Purpose: Ferns are wild plants which are usually used by local indigenous people of Sarawak for food, ornamental and medicinal purposes. Among the ferns, three edible ferns, namely *Diplazium esculentum* (DE), *Nephrolepis biserrata* (NB) and *Stenochlaena palustris* (SP) were examined for their extraction yields, phytochemicals, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AOA).

Methods: Four solvents, depending on different polarities, n-hexane, ethyl acetate, 95% ethanol and water were used for extracting of active constituents. Phytochemical analysis was done by using standard procedures as described by Harbone (1965). For AOA, three assay methods, Diphenyl-picrylhydrazyl (DPPH) radical scavenging activity assay, β- carotene bleaching (BCB) assay and ferric reducing-antioxidant power (FRAP) assay were employed.

Result(s): Extraction yields increased with increasing polarity of solvents. Phytochemical analysis of all three ferns in different solvents revealed the presence of phenols and flavonoids whereas alkaloids and reducing sugars were absent. Saponins and tannins were absent in hexane extracts. Tannins were also absent in aqueous extracts. Cardiac glycosides were present in all extracts except in aqueous extracts. There is no difference in the presence of phytochemicals in the three ferns. Result of TPC using Folin-Ciocalteu reagent (FCR) showed that aqueous extracts had the highest amounts, following the order NB > DE > SP. The similar result was obtained for TFC using aluminium calorie method. Generally, the result of AOA by three assays: aqueous extracts had highest antioxidant activity while n-hexane extracts had lowest activity. Among the three ferns, NB expressed the highest activity. Overall, AOA of three ferns using three assays showed that these ferns have low AOA.

Conclusion(s): We can conclude that active constituents which give AOA are water soluble and there is correlation between TPC, TFC and AOA in these ferns.

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Keywords: phytochemicals 1, antioxidant activity 2, edible ferns 3, *Diplazium esculentum* 4, *Nephrolepis biserrata* 5, *Stenochlaena palustris* 6

072

Evidence-based herbal medicine for arthritis : Insight from herbs used in Siddha medicine

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Purpose: The Siddha system of medicine is centuries old and is still being practiced today in India, Sri Lanka, Malaysia and other countries for a multitude of diseases including arthritis. However, the efficacy of these treatments has not been examined with respect to arthritis. Thus, we aim ascertain the extent to which Siddha herbs used for arthritis are backed by scientific research.

Methods: PubMed, SpringerLink, ScienceDirect and Cochrane databases were searched. References of key articles were also hand searched. The articles were retrieved and those that fulfilled the inclusion criteria were examined.

Result(s): *In-vitro* studies revealed several herbs that may be useful in the treatment of arthritis. Notably, administration of *Cardiospermum halicacabum* resulted in cartilage regeneration in complete Freund's adjuvant (CFA)-induced arthritic rat model. *C. halicacabum* displayed dose dependent anti inflammatory effect by inhibiting TNF α and nitric oxide. *Cocculus hirsutus* and *Indigofera tinctoria* also exhibited significant anti-arthritic activity in a CFA rat model. *Ocimum grattissimum* may be useful for the management of arthritis as it down regulated TNF α, IL-2, and IL-16. Clinical trials of Siddha formulations revealed promising results with improvements in the symptoms of arthritis including pain, stiffness, and swelling.

Conclusion(s): Evidence suggests that herb used in Siddha medicine may be useful in the management of arthritis. Though the traditional usage of the herbs for arthritis appears to be validated, there is a need for further studies to provide conclusive evidence. Nonetheless, the potential for the development of these herbs into anti-arthritic agents is undeniable.

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Keywords: Arthritis, herbs, Siddha, Evidence-based medicine, herbal medicine

Pre-conference: Abstracts for Poster Presentation

P004

Effect of *Phaleria macrocarpa* (Mahkota dewa) on fertility in male adult rats

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Purpose: PM has been claimed to improve sexual strength in man. Andropause occurs after middle age due to low testosterone level, affecting sexual strength and libido. The potential of PM for improving sexual strength in males has not yet been fully explored.

Methods: A comparative study on the effects of PM on the fertility in sixty Sprague Dawley adult male rats (7 weeks old) weighing 200-250g was conducted. A two by five experimental design with two supplementation periods (3 and 7 weeks) and 5 different doses of PM extract (0, 24, 48, 240mg PM aqueous extract/kg bw and 80mg of commercial PM product/kg bw) were used.

Result(s): The results of the study were analyzed using SPSS showed that the mean testes size was similar ($p>0.05$) among treatment groups, ranging from 1.3 to 1.8cm. The mean sperm count and spermatogonia cells were significantly highest ($p<0.05$) in rats treated with 240 mg/ kg (455 cells/ml and 87 cells), followed by 48 mg/kg (393 cells/ml and 70 cells), commercial product (375 cells/ml and 69 cells), 24 mg/ kg dose (306 cells/ml and 53 cells) and untreated rats (192 cells/ml and 34 cells) respectively. Body weight was significantly highest ($p<0.05$) in rats treated with 240 mg/kg (301g), followed by 48 mg/kg (291g), 24 mg/kg (291g), commercial product (268g) and untreated rats (223g).

Conclusion(s): The effect was dose and time dependent. The results showed that *PM* supplementation significantly increased the fertility of rats and the effect was dose and time dependent. The study suggests that *PM* offers as an attractive and alternative ways for improving the fertility in man.

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Acknowledgement: Universiti Putra Malaysia Funding.

Keywords: *Phaleria macrocarpa*, fertility, medicinal plant

P005

Nutritional composition, total phenolic content, total flavonoid content and antioxidant activities of diferent parts of *Crescentia cujete*.

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Purpose: Calabash tree or locally known as 'labu kayu' (*Crescentia cujete* L.) was traditionally used to treat various types of diseases and ailments. This study aimed to investigate the nutritional composition, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of different parts of *C. cujete* namely immature fruit (FY), mature fruit (FM), bark (B) and leaves (L).

Methods: Nutritional composition was determined using standard AOAC method while TPC and TFC were determined using Folin-Ciocalteu and aluminium trichloride assay, respectively. Antioxidant activities were assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability and Ferric Reducing Antioxidant Power (FRAP) assay.

Result(s): The predominant macronutrients in FY were moisture and fat while bark had the highest ash and fiber compared to the other parts. The highest amount of TPC and TFC were discovered in FY extract. Antioxidant activities of the samples were decrease in the following order: L > B > FY > FM.

Conclusion(s): The leaves of *C. cujete* exhibited excellent natural antioxidant and may be beneficial to human nutrition. Further analyses on phytochemical profiles of *C. cujete* can be conducted.

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Acknowledgement: We would like to thank Universiti Putra Malaysia for financial support

Keywords: *Crescentia cujete*, phenolics, antioxidant activity

P006

Effect of *Ocimum sanctum (*Tulsi) aqueous leaf extract on prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) of human plasma

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Purpose: Conventional anticoagulant therapy is the mainstay of medical treatment for deep vein thrombosis disorders. However, there are many complications associated with these agents such as bleeding. Hence, the search for novel anticoagulant derived from natural substances such as plants origin is in high demand nowadays. *Ocimum sanctum* (O. sanctum) also known as *Ocimum tenuiform* (OT), tulsi or holy basil from the family of Lamiaceae has been widely used for thousands of years in Ayurveda and Unani systems to cure or prevent a number of illnesses such as headache, malaria, ulcers, bronchitis, cough, flu, sore throat and asthma. The objective is to investigate the effect of *O. sanctum* (Tulsi) aqueous leaf extract on prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) in human plasma.

Methods: Coagulation activity of *O. sanctum* was measured via PT, APTT and TT assay in citrated plasma collected from thirty six healthy regular blood donors. The plasma was tested against different concentrations of *O. sanctum* aqueous extract as follows: 0.1mg/ml, 0.5 mg/ml and 1.0 mg/ml.

Result(s): Shows the aqueous extract of *O. sanctum* prolonged the PT and APTT assays ($p<0.05$) but had no effect on TT assay ($p>0.05$). The gas chromatography-mass spectrometry (GC-MS) analysis had identified the linolenic acid at 1-10% of ethanol and aqueous concentration at different retention time which was responsible for the coagulation activities of *O. sanctum* in human plasma.

Conclusion(s): This study suggests that *O. sanctum* does affect coagulation activity in human plasma and can be potentially used as naturally derived anticoagulant products in the future.

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Keywords: *Ocimum sanctum*, linolenic acid, anticoagulant, GCMS, coagulation assays

P009

Propolis extracts from Malaysian *Trigona apicalis, Trigona itama and Trigona thoracica* were capable of inhibiting the expression of adhesion molecules on stimulated endothelial cells

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Purpose: There have been tremendous number of medicinal claims on the effects of propolis belonging to the stingless bee, but little is known about its role in the modulation of adhesion molecules, which are important pro-inflammatory mediators. Thus, the present study investigated the effects of propolis extracts from three different stingless bees species on the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) on Human Umbilical Vein Endothelial Cells, HUVEC EA.hy926.

Methods: Cultured HUVEC cell lines were stimulated with Tumor Necrosis Factor- α (TNF-α) for 6 hours, in order to induce ICAM-1 and VCAM-1, respectively. Endothelial surface membrane expression of ICAM-1 (CD54) and VCAM-1 (CD106) were examined by flow cytometry, and the cell viability by trypan blue exclusion assay.

Results: Flow cytometry analysis determined the expression of ICAM-1 and VCAM-1 after stimulation of TNF-α on HUVEC. The result shows that all propolis samples revealed strong inhibition activities and propolis from *Trigona thoracica* was found to be the most potent extract against the expression of both ICAM-1 and VCAM-1.

Conclusion: As natural modulators of pro-inflammatory mediators, stingless bee propolis extract may have the potential as a source of new anti-inflammatory agent and provide lead compounds for therapeutic improvement.

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Keywords: Adhesion molecules, HUVEC, Propolis, Stingless bee, TNF-α

P010

The antioxidant activities and GC-MS analysis of *Clinacanthus nutans* (Acanthaceae) in northern regions of Peninsular Malaysia

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Purpose: *Clinacanthus nutans* is a valuable medicinal plant which has gained more attention in the last few years mainly because of its pharmacological properties. Therefore, a study had been conducted to determine antioxidant activities and phytochemical compounds of *C. nutans* at northern regions of Peninsular Malaysia.

Methods: *C. nutans* leaves were collected from eight locations and macerated with 80 % methanol. Then, *C. nutans* extract were subjected to phytochemical screening using 2,2 diphenyl-2-picrylhydrazyl hydrate (DPPH) assay method and Gas Chromatography-Mass Spectrometry (GC-MS).

Result(s): The phytochemical study revealed that *C. nutans* extracts in location KKK (Kuala Ketil, Kedah, Malaysia) with neutral pH sandy clay soil exhibited high antioxidant activities (54.3 TEAC/100g) compared to other locations. In addition, GC-MS analysis results revealed distinct differences in the content of the phytochemical compounds with a total of 20 compounds above 80% match to the chemicals in the National Institute Standard and Technology library (NIST). The results revealed that environmental factors such as light intensity, temperature and soil characteristics of eight different locations were responsible for variations of antioxidants activities and GC-MS analysis in *C. nutans.*

Conclusion(s): In conclusion, variability among *C. nutans* compounds in different locations will provide baseline data for future breeding programs for commercial cultivation of *C. nutans*.

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Keywords: Antioxidant, *C. nutans*, flavonoid, phenolic, phytochemistry

P016

Medicinal plants used and the methods of application during the traditional postpartum care

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Purpose: This study explored the use of medicinal plants by the Malay postpartum practitioners (MPP) and the methods of application during the traditional postpartum care.

Methods: Data were collected from four states in Northern Malaysia, using semi-structured in-depth interviews to document the preparation and plants used during the traditional postpartum care. Thirteen traditional MPP were selected using purposive and snowball sampling. Twelve other key informers were among the MPP's clients who received the traditional postpartum care.

Result(s): Various medicinal plants were used during the traditional postpartum care, and they were applied in the process of a) *Bertungku* (abdominal hot compression) b) *Bertangas basah* (herbal steam) c) *Bertangas kering* (herbal sauna) d) *Mandian herba* (Herbal bath) and e) *Berbengkung* (abdominal wrap). Most plants are usually cultivated in the gardens and often used as spices.

Conclusion(s): This study demonstrates that many plants are beneficial for physical recovery after childbirth. The knowledge of traditional medicine is still utilized among the Malay community in Malaysia and plays a significant role in postpartum care.

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Keywords: medicinal herbs, traditional postpartum care, Malay postpartum practitioner

P018

Manilkara zapota (L.) P. Royen leaf water extract induces apoptosis mediated by reactive oxygen species and JNK1 pathway in HepG2 human hepatocellular carcinoma cells

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Purpose: Our present study is designed to identify the anticancer properties of *Manilkara zapota* leaf water extract in HepG2 cells.

Methods: Apoptosis and intracellular reactive oxygen species (ROS) activities were analysed using Annexin V-propidium iodide staining and dichlorodihydrofluorescein diacetate, respectively, by NovoCyte Flow Cytometer. Caspase-3 and -8 activities were evaluated by colorimetric assay. The c-Jun N-terminal kinase 1 (*JNK1*) mRNA expression was evaluated by quantitative real-time PCR.

Result(s): We found that treatment with *Manilkara zapota* leaf water extract significantly increased the total percentage of apoptotic HepG2 cells ($p<0.05$) in comparison to untreated cells at 72 h of incubation period. Treatment with *Manilkara zapota* leaf water extract increased intracellular ROS level and caspase-3 and -8 activities. Moreover, incubation with *Manilkara zapota* leaf water extract for 72 h resulted in the transcriptional upregulation of *JNK1* mRNA expression.

Conclusion(s): These findings suggest that *Manilkara zapota* leaf water extract has noteworthy apoptotic potentials via the mechanism involving *JNK1* pathway.

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Keywords: c-Jun N-terminal kinases, liver cancer, signaling pathway

P019

In Vivo and *In Silico* Toxicity Assessment of Hexane rhizome extract from Black Turmeric (Curcuma caesia Roxb).

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Purpose: Black Turmeric from zingeberacea family is commonly used food preservative spice. Many medical properties are associated to this spice. Small molecules present in the plant are responsible for its various medicinal properties. Some of these natural products may be toxic. Understanding the toxicity of HRE will provide useful information that may act as a reference for nutraceutical developments.

Methods: The methodology focuses on developing an integrated *in vivo* and *in silico* approach for natural product drug discovery that combines bioactivity screening in zebrafish embryos and rapid computational toxicity predictions.

Result(s): Hexane Rhizome Extract (HRE) of *Curcuma Caesia* Roxb (CC) (1µg – 5 µg) was administered to screen the toxicity in zebrafish embryos. Only higher concentrations of 5 µg showed considerable mortality. In the *in silico* prediction, 80% of the 20 small molecules identified in HRE were found to be mutagenic and carcinogenic.

Conclusion(s): These results suggest that the combination of zebrafish bioassays with predictive toxicity methods is an effective strategy for the researchers to design and conduct preclinical studies in a timely and cost-effective way.

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Keywords: *Curcuma caesia* Roxb, Zebra fish, *In silico* Toxicity

P020

Methanolic extract *Clinacanthus nutans* leaves and its antiproliferative effect on MCF-7 and MCF-10A cell lines

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Purpose: To assess the antioxidant (DPPH) properties and antiproliferative effects of *Clinacanthus nutans* (CN) leaves methanol extract on human breast cancer (MCF-7) and human normal breast (MCF-10A) cell lines.

Methods: The extraction of CN leaves methanol extract were conducted. The methanolic extract of CN leaves was obtained by soxhlet extraction following by solving removal step using gen vac. The antioxidant activity was determined using 2, 2-diphenyl-2-picrylhydrazyl (DPPH) and trolox as standard. The antiproliferative assays namely Sulforhodamine B (SRB), (3-(4, 5-dimethylthiazol-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) and Alamar Blue assay were performed on MCF-7 cell treated with CN leaves methanol extract. The effect of CN leaves methanol extract on MCF-10A was determined by SRB.

Result(s): The antioxidant of CN leaves methanol extract showed the increasing of percentage of DPPH scavenging when the concentration of sample increased. The IC_{50} value is 1.10µg/mL of antioxidant activities. In antiproliferative effects result showed in MCF-7 the CN leaves methanol extract in all assays were shown dose dependent however in normal cell lines shown no cytotoxic effects in normal cell lines (MCF-10A).

Conclusion(s): CN leave methanol extract has a potential in drug discovery for cancer. Future investigation of CN extracts on cancer cell line at molecular level are needed to determine the metabolic mechanisms of action.

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Acknowledgement: FRGS grant

Keywords: *Clinacanthus nutans*, methanol extract, antiproliferative

P021

Simple extraction of natural colour from plant materials based on cold infusion technique using Orotropicos - Hydrophilic Extractor 100

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Purpose: Synthetic colourant has been widely used in consumer products even in food and medicine. The use of colourant benefits the products by increasing the attractiveness, creating distinction, stimulate appetite etc. However, some synthetic colourant in the product are found to cause adverse effects to consumers. Thus, in food industry, colourant is categorized as additives which require pre-market approval with national food authority, e.g. United States Food and Drug (USFDA) as to assure the colourant to be marketed is safe for long term use by human in their daily diet. The study is intended to test the capability of a newly formulated substance produced by Orotropicos Sdn. Bhd. named as "Hydrophilic Extractor 100" (HE-100) to extract natural colour (pigments) from plant materials. Some plant pigments are derived from flavonoids, e.g. anthocyanins which is also known for its antioxidant activities.

Methods: Plant materials in dried condition (roselle, chilli and clove) and fresh (dragon fruit, beetroot and pandanus leaf) are selected by convenience as source of natural colours namely red, yellow, brown and green. The fresh plant materials are washed with tap water, skin is peeled and cut into small pieces then the 20 grams of each plant material is submerged in 100 grams of HE-100 for several days in an airtight glass jar at temperature of 25 to 30 ºC. After the infusion period, content in the glass jar is filtered using a sieve to remove the plant materials and collect clean coloured solution. The apparent colour, liquid density, pH and optical density of the coloured solutions were measured to evaluate the capability of the HE-100 to extract the natural colour from the selected plant materials thru cold infusion technique.

Result(s): The colourless HE-100 visually changed to various apparent colour influenced by the original natural colour of the plant materials ranging. The liquid density of the coloured solutions are higher than the density of the HE-100. Whereas for pH, some of the coloured solutions become acidic (pH < 5) compared to the HE-100 which is near neutral. The optical density of the coloured solution exhibited absorbance of 16 – 80 folds higher compared to HE-100.

Conclusion(s): In general, the study showed that the HE-100 has the capability to extract natural plant colours based on the cold infusion technique.

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Keywords: Plant, Natural, Colourant, Extraction, Infusion, Antioxidant.

P022

Phytochemical profiling and anticancer activity of ethanolic and aqueous propolis extracts from Malaysia stingless, *Trigona apicalis*

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Purpose: Propolis is a beehive product forming from various sources of plant. It exists in yellow to dark brown in colour depending on its age and sources. Propolis is well known for its therapeutic properties such as antioxidant and anticancer. The impacts of *T. apicalis* propolis in the forms of ethanolic and aqueous extract towards HeLa cells (viability, toxicity and proliferative characteristics) and their potential bio-activity compounds were studied.

Method: Propolis from *T. apicalis* was extracted with ethanol and aqueous. The *in vitro* cytotoxicity was assessed manually against HeLa cell lines. The extracts were screening by GC- MS to get overview of the possible and potential compound present.

Result(s): Ethanolic extract inhibited 50% (IC_{50}) of cancer cells when treated with 31 µg/mL rather than aqueous extract which needed 120 µg/mL of propolis. Both treatments were proven to be safe as the toxicity study shows no significant changes in normal cell; L929 at suggested IC_{50} dosage while antiproliferative characteristics was shown better in ethanolic than in aqueous extract. Simple screening by GC-MS has listed 1,6-Cyclodecadine,1-methyl-5-methylene-8-(1-methylethyl)-,s- (E, E), 4-(1,3,3- Trimethyl-bicyclo (4.1.0)hept-2-yl)-but 3-en-2-one, Lanosterol and (+)-(z)- Longipinane, Aromadendrine oxide-(2), Androsta-1,4-dien-3-one, 17-hydroxy-17-methyl-, (17. Alpha.)- and 9-Isopropyl-1 methyl- 2-methylene- 5-oxatricyclo (5.4.0.0 (3,8)) undecane.

Conclusion(s): Some of the compounds has been notified by GC-MS library with no valid verifacation on their potential but the positive inhibition on cancerous cells proven the ability of propolis from *T. apicalis* as anticancer agent.

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Keyword: propolis, anticancer activity, GC-MS

P026

Effect of Oil Palm Leaf Extract on Adipocytes Cellularity in Mice

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Purpose: Oil palm leaf extract (OPLE) from *Elaeis Guineensis* leaves contains elevated levels of total polyphenols content compared to green tea, and found in abundance in tropical south east Asia, was candidated to investigate anti-obesity effects *in vivo.* The aims of this current work are investigation of anti-adipogenic effect of OPLE on body weight and adipose cellularity in high fat diet (HFD) animals.

Methods: ACR male mices received either normal diet, high-fat diet or high-fat diet with additional OPLE for 4 weeks. Animales body weight were measured weekly. To display changes in adipocyte cell number, size and area of distribution of adipocytes, H&E staining of adipose tissue selected as a useful tool for the study of adipose tissue. Cellular size, number and cell diameter were recorded with Image J software.

Results: OPLE administration significantly suppressed HFD-induced body weight increases. Histological analysis showed that the number of large adipocytes was lesser in the adipose tissue of the obese mice supplemented with dietary OPLE than in that of the obese control, whereas there were more small adipocytes in the adipose tissue of the obese mice supplemented with dietary OPLE. The diameter of adipose cells in high-fat diet plus OPLE treated group was increased less than that in the group fed the high-fat diet only.

Conclusions: administeration of OPLE in HFD-induced obesity mice showed OPLE held promise as a treatment for reducing obesity.

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Keywords: Obesity, OPLE, Adipose cellularity

P029

The Anti-Inflammatory Effects of Labisia pumila on Postmenopausal Osteoporosis Rat Models

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Purpose: *Labisia pumila var. alata* (LP) has shown potential as an anti-osteoporotic agent in recent years. The crude extract of LP was reported to reverse trabecular microarchitecture changes induced by ovariectomy. The mechanism is still unclear but it may be elucidated by examining the bone molecular pathways. This study aimed to evaluate the pro-inflammatory cytokines expressions to provide a mechanistic overview on how the different LP extracts could prevent degenerative bone changes

Methods: Forty-eight female Sprague-Dawley rats were randomly divided into sham-operated (Sham), ovariectomized control (OVX), ovariectomized and given oestrogen at 64.5μg/kg (ERT), ovariectomized and given LP aqueous extract at 100 mg/kg (LPaq), ovariectomized and given LP methanol extract at 100 mg/kg (LPmet) and ovariectomized and given LP ethanol extract at 100 mg/kg (LPet). All treatments were given daily via oral gavages for nine weeks. Rats were then euthanized and femora dissected out for cytokines expressions analysis.

Result(s): LPaq was found to down-regulate the expression of cytokine IL-1β meanwhile LPet was able to down-regulate TNF-α expression.

Conclusion(s): Aqueous and ethanolic extracts of LP may exert anti-osteoporosis activity via its anti-inflammatory mechanisms due to the presence of bioactive compounds such as flavonoids, beta-carotene, ascorbic acid and phenolic compounds. Further studies are warranted to provide a more detail mechanistic overview on the anti-osteoporotic properties of *Labisia pumila.*

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Keywords: *Labisia pumila*, osteoporosis, postmenopausal, bone, inflammation, cytokine

P030

Evaluation of acute toxicity induced by supercritical carbon dioxide extract of *Canarium Odontophyllum* (CO) Miq. pulp oil in Sprague Dawley rats

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Purpose: *Canarium odontophyllum* (CO) Miq. is a fruit from genus Canarium in the family Burseraceae. The extracted fat of this fruit has been proven scientifically for their natural antioxidant available with good fatty acid composition. However, toxicity data of the CO pulp oil using carbon dioxide supercritical fluid extraction (SCO_2) is still lacking.

Methods: This study investigated acute toxicity of CO pulp oil using SCO_2 on Sprague Dawley rats at a dose of 5000 mg/kg body weight (BW) as limit test at one dose level. The study includes control and treatment group, consisting of 5 male and 5 female rats each. BW, food and water consumption by rats were recorded throughout the 14-day study period. Observation were monitored after dose administration for general behavior and toxic signs during the study period. At the end of the study period, biochemical parameters and relative weights of the organs (liver, kidney, spleen, heart, stomach, brain); were measured.

Result(s): Single oral dose of the CO pulp oil extract did not affect intake of food and water; relative organ weight; biochemical parameters (liver, kidney and lipid profile test); did not show significant changes in the histology of vital organs. Overall, there were neither signs of toxicity nor deaths recorded during the study period.

Conclusion(s): Therefore CO pulp oil extract did not show any signs of toxicity and safe to rats at the highest dose and can be considered for further investigation for its therapeutic efficacy in larger animal model.

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Keywords: acute toxicity, dabai oil, supercritical extraction, OECD

P031

Effect of Extraction Techniques and Ratios on Phenolic Levels of Herbal Mixture

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Purpose: Preparation on herbal mixture from the traditional knowledge have been used for over centuries to improve and maintain health condition. The composition of these herbal mixture is usually determine from the knowledge that develop over generation, long before the availability of modern medicine. Nonetheless, lack of scientific evaluations on regard to their bioactives as a mixture and their pharmacological effects have yet to be reported. Therefore, the purpose of this study are 1) to determine the effect ratio of herbal mixture (containing ginger, garlic, honey, apple cider vinegar, and lemon juice) and 2) to determine the effect of processing techniques (blending and juicing) on extracting polyphenols.

Methods: Raw ingredients such as garlic, ginger, lemon and apple cider were be used as the base for this polyphenol rich mixture (PRM). These base was either blended (sample to water (w/v), 1:1) using blender or juiced using juicer. The mixture was simmered (90ºC) to ½ (rA) or ¾ (rB) of the total volume and cooled down before being added with honey in 1:1 ratio. The mixture were tested for pH, total phenolic and total flavonoid content.

Result(s): Both of juice samples in rA and rB ratio have the lower acidity compare to blended samples. Total phenolic (TPC) and total flavonoid content (TFC) also showed a significant difference (p <0.05) between juiced and blended samples. Ratio rB have the highest TPC and TFC (p <0.05) compared to rA ratio.

Conclusion(s): In conclusion, juiced and ratio rB samples have the highest TPC and TFC values and lower acidity compare to the blended samples. These results indicate that extraction techniquesb and ratio influences the phytochemical levels in the herbal mixtures.

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Keywords: Extraction techniques, Ratio, Polyphenols

P032

Beneficial effect of defatted dabai pulp (Canarium odontophyllum) in hypercholesterolemia induced SPF Sprague-Dawley rats.

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Purpose: The food industries are often compensated by the fruit and vegetable residues that are often discarded. Ironically, fruit and vegetable waste still contain large amount of bioactive compound. Defatted dabai pulp is a novel fruit residue which are plausible to be investigated. This study aims to investigate the effect of defatted dabai (*Canarium odontophyllum*) pulp in hypercholesterolemia induced rats

Methods: Male specific pathogen free (SPF) Sprague-Dawley rats were fed with high cholesterol diet for 4 weeks to induce hypercholesterolemia. The rats were subsequently administered with defatted dabai pulp where 2% of fiber source was replaced with defatted dabai pulp for another 4 weeks. Change in body weight and biochemistry profile were measured.

Result(s): Rats fed with high cholesterol diet showed elevation of body weight gain by 64% and significant increase in total cholesterol level compared with rats consumed normal basal diet ($p<0.05$). Interestingly, treatment of defatted dabai pulp in high cholesterol rats led to lower bodyweight. Additionally, administration of defatted dabai pulp also showed reducing trends in total cholesterol and triglyceride levels.

Conclusion(s): These findings demonstrated that defatted dabai pulp contained potential factors contributing to cholesterol lowering effect and indicate its potential application towards formulation of a new nutritious, healthy and natural functional food ingredients.

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Keywords: *Canarium odontophyllum,* defatted dabai, hypercholesterolemia rats

P033

Moringga oleifera ethanol extract suppresses migration of MCF-7 cells

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Purpose: To evaluate the anti-migration effects of *M.oleifera* leaf extract on breast cancer cell line, MCF-7.

Methods: Ethanol extract was prepared by Ultrasound Assisted Sequential Extraction (UASE). Confluent monolayers of MCF-7 cells were plated in 6-well plates. Concentration at IC_{50} value and double IC_{50} value of the sample were used. At time intervals of 12, 24 and 48 the images were photographed and qualitative assessment was done by observing the distances between sides of the scratch under an inverted microscope (×20 magnification).

Result(s): M.oleifera ethanol extract showed significant inhibition of MCF7 cell migration. At IC_{50} concentration (25µg/mL) the sample caused significant inhibition in the closure of the wound. The sample at higher concentrations which is double IC_{50} concentration (50µg/mL) demonstrated dislodgement of monolayer of MCF-7 cells (indicated by the arrows) with almost complete inhibition of migration.

Conclusion(s): The current results suggested that the ethanolic extract confer the anti-migration activity more specifically towards the MCF-7 cells through non-cytotoxic pathways.

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Keywords:*Moringga oleifera*, ethanol extract, anti-migration

P034

The antioxidant activities of *Carica papaya*, C*itrullus lanatus* and *Punica granatum* juices

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Purpose: The concern on wellness healthcare using plants/fruits juices has been increasing recently, leading to great interest in exploring their antioxidant efficacies and phytochemical contents. Therefore, we aim to investigate the antioxidant capacities, total flavonoids and phenolic contents of selected juices like *Carica papaya* (papaya), *Citrullus lanatus* (watermelon) and *Punica granatum* (pomegranate).

Methods: Three parts of the plants were selected (unless stated otherwise), mainly leaves, peels and flesh of the fruits. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) and ferric reduction activity potential (FRAP) methods were used to examine their antioxidant activities. Gallic acid was used as the standard.

Result(s): Pomegranate peel juice showed the highest antioxidant activity (lowest IC_{50} 0.004 g/mL), followed by papaya leaves juice (0.081g/mL), pomegranate seed juice (0.168 g/mL), papaya fruit juice (0.278g/mL), papaya peel juice (0.600g/mL) and watermelon fruit juice (0.977 g/mL) in DPPH assay. In FRAP tests, pomegranate peel juice showed the highest antioxidant activity with 1.963 $mMFeSO_4$/g, followed by pomegranate seed juice at 1.785 $mMFeSO_4$/g, watermelon fruit juice at 1.454 $mMFeSO_4$/g, papaya leaves juice at 1.298 $mMFeSO_4$/g, papaya fruits juice at 0.293 $mMFeSO_4$/g and papaya peel juice 0.275 $mMFeSO_4$/g. Likewise in FRAP, ABTS assay exhibited pomegranate peel juice as the highest antioxidant activity with lowest IC_{50} at 0.00641 g/mL and decreasing in the order of pomegranate seed juice (0.0380 g/mL) > watermelon fruit juice (0.3234 g/mL) > papaya leaves juice (0.949 g/mL) > papaya fruit juice (1.421 g/mL) > papaya peel juice (2.786 g/mL). Papaya juices were then chosen to determine the total phenolic and flavonoids contents. Highest amount of total phenolic was found in papaya leaves juice at 2.282 mgGAE/g, followed by papaya fruit juice (1.796 mgGAE/g) and papaya peel juice (0.651 mgGAE/g). Same trend was observed for total flavonoids content.

Conclusion(s): Overall, results indicated that pomegranate peel juice possessed the highest antioxidant activity among all the juices, which may provide a good source of antioxidants for wellness healthcare.

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Keywords: *Antioxidant*, *Carica papaya*, *Citrullus lanatus*, *Punica granatum, TPC, TF*

P035

Non-targeted analysis of spatial metabolite composition of Cinderella weed (*Synedrella nodiflora*): Oven dry versus freeze dry

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Purpose:
The aim of this study is to distinguish the metabolic variation between the freeze and oven-dried *Synedrella nodiflora* using HRMS-based metabolomics in combination with multivariate data analysis (MVDA).

Methods:
Briefly, fresh *S.nodiflora* underwent freeze dry and oven dry separately and the powdered using blender. Dried sample were extracted using modified Folch extraction protocol with 1% sodium chloride added. Following, UHPLC-qTof-MS was utilized to conduct non-targeted analysis on both polar and semi polar extracts. Multivariable analysis was carried out using Metaboanalyst 3.0 to distinguish the differences between different drying procedures on different organs of the *S.nodiflora*.

Result(s) & discussion:
MVDA demonstrated that there were significant differences in sample dried with oven- or freeze-dry approach in different organs. From base peak chromatogram, more peaks were observable in roots extracts in oven-dried approach; while, flower was more significant using freeze-dring approach. Such result revealed that different dehydration process tend to affect the overall phytochemical compositions in different plant organs. Further identification of the metabolites will reveal the metabolome degradation during dehydration.

Conclusion(s):
Metabolite composition of plant extracts were significantly different using different dehydration processes. The understanding of the degradation of metabolites (including secondary metabolites) will benefit researchers to determine the most appropriate dehydration method for their research.

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P036

Extraction, formulation and evaluation for antimicrobial activity of *Senna alata* leaves

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Purpose: In present years, there has been a viable enthusiasm in researching and developing new antimicrobial agents from various sources to fight microbial resistance. Emerging evidence suggests that phytoconstitiuents of Senna Alata has many pharmacological effects, includes, anti-inflammatory, antioxidant, anticancer, antimicrobial and antifungal activities. Senna Alata (L) is a herbal plant where pure phytoconstitiuents and extracts offer many opportunities to develop new drug due to its chemical diversity and various pharmacological activity. And are widely used for prevention, and treatment of various health conditions in tropical regions for over 1000 years as traditional herbal medicine. The aim of study was to prepare leaf extracts of Senna Alata, formulate and evaluate antimicrobial activity against Dermatophytes.

Method: Soxhlet Extraction was carried out using aqueous 80% ethanol and various molecular dispersions were formulated using carrier and evaluated for antimicrobial activity using disc diffusion method.

Results: Increased antimicrobial activity is seen with formulated extract compared to pure extract and also the crude leaf powder.

Conclusion: In conclusion suitable formulation development of extract with right combination of excipients provides enhanced antimicrobial activity compared to non-formulated pure extract, Senna Alata leaf extract could be potential source against active antimicrobial agents and effective therapy can be achieved by formulating into suitable drug delivery system.

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Key words: Senna Alata extract, Antimicrobial activity and Dermatophytes.

P037

Phytochemical and antioxidant activities of propolis extract of Malaysian Stingless Bee, *Geniotrigona thoracica*

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Purpose: To determine phytochemical and antioxidant activities of the ethanolic propolis extracts from Malaysian stingless bee, *Geniotrigona thoracica*.

Method: Total phenolic content (TPC) and total flavonoid content (TFC) were determined by using Follin-Ciollcateu reagent and aluminium chloride respectively. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was also performed to measure the antioxidant activity of the propolis extract.

Result: Total phenolic content found in propolis ethanolic extract (PEE) was 221.569 ± 0.02 mg GAE/g, meanwhile, total flavonoid content was 214.575 ± 0.05 mg QE/g. The EC_{50} (effective concentration of sample to reduce 50% of DPPH assay) of the PEE was 0.0483 ± 0.002 mg/ml. Statistical analysis showed that TPC and TFC were significantly correlated with DPPH, where TPC was negatively correlated (-0.749, $p<0.05$), meanwhile, the TFC was strongly correlated (-0.909, $p<0.01$) with EC_{50} of DPPH.

Conclusion: The correlation between TPC and TFC towards DPPH showed that the antioxidant properties of the propolis might be contributed by the phenolic and flavonoid compounds. This study also showed that propolis from Malaysian *Geniotrigona thoracica* exhibited a potent antioxidant and can be used as a source of natural antioxidants to promote human health.

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Keywords: TPC, TFC, anti-oxidant, propolis, *Geniotrigona thoracica*.

P038

Toxicity study of *Physalis minima* extract on rat

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Purpose: To determine the toxic hazard category of *P. minima* leaves extraction by oral and to investigate the toxicological effect of P. minima leaves extraction on SD rat's organ.

Method: Six rats were orally treated with *P. minima* extraction and observed closely for 14-day. The animal body weight, food consumption and water intake were recorded daily.

Result: From the results, no moribund, severe pain/distress or mortality were recorded during the study. All rats showed no significant changes in feed consumption, water intake and body weight. Gross examination of internal organs and histopathological examination of tissues exhibited normal appearance.

Conclusion: In conclusion, *P. minima* extract was safe without causing any adverse effects and organ damage in female SD rats.

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Keywords: *Physalis minima,* toxicity, oral gavage

P039

High throughput analysis: Revealing metabolite distribution in different parts of *Synedrella nodiflora*

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Purpose:

Synedrella nodiflora is a medicinal plant that widely used by natives to overcome rheumatisms and other complications. During traditional medicine practices, whole plant undergoes extraction. Such approach potentially enhance the effectiveness of the nature, bioactive compounds as certain compounds tend to accumulate at specific part of the plant. In current study, we are aimed apply high-throughput, mass spectrometry-based metabolomics combination with chemometric analysis to compare the metabolites variances between organs.

Methodology:

UHPLC-qTof-MS was utilized to conduct non-targeted analysis on both polar and semi polar plant extracts to localize metabolites distribution in different plant organs. Multivariable analysis using Metaboanalyst 3.0 to distinguish of the differences between the plants. Compounds-pathway analysis using Global Nature Product Social-Networking (GNPS) allow us to understanding the distribution and interaction of metabolites (primary or secondary) in different organ of the entire plant at better point of view.

Results and Discussion :

Multivariable analysis, Principle component analysis, revealed that different organs possess different metabolites. GNPS network visualization revealed that only specific metabolites (compounds) can be identified from parts of the plant. These metabolites found to be metabolized, biotransform and migrate to the other plant organs. Evaluation of the metabolite variance between plant organs definitely will enhance the collection of the nature product, which cumulate at specific organs.

Conclusion

High-throughput, untargeted profiling coupling with chemometric analysis and GNPS enables the discovery and enhances collection of new compound. Besides, GNPS enables us to understand the distribution, biotransformation of the compounds from organs to organs within the plant.

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Keyword: High-throughput, Multivariable analysis, GNPS

P040

Antioxidant activities of honey produced by different species of stingless bee

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Purpose: Honey has been used since ancient times for its nutritional as well as curative properties. There are several bioactive compounds that are present in honey has display antioxidant capacity such as phenolic acid, flavonoids and enzyme glucose oxidase to prevent disease associate with oxidative stress[1]. This study was carry out to determine antioxidant activity of honey extracts from three species of Malaysian stingless bee, *Tetrigona apicalis, Heterotrigona itama*, and *Geniotrigona thoracica*.

Methods: Honey extracts were evaluated for antioxidant activities by using Folin-Ciocalteu (TPC), total flavonoids content (TFC), DPPH and $ABTS^+$ assay.

Result(s): In DPPH assay, honey extract of *T. apicalis* showed the most potent with IC_{50} of 65.583± 0.01 mg/mL, followed by *H. itama* (86.375± 0.01 mg/mL) and *G. thoracica* (98.250± 0.02 mg/mL). $ABTS^+$ assay showed that *T. apicalis* has the highest antioxidant activities, (IC_{50} of 6.727± 0.05 mg/mL) followed by *H. itama* (22.198± 0.06 mg/mL) and *G. thoracica* (24.836± 0.04 mg/mL). In Folin-Ciocalteu assay, *T. apicalis* honey extract showed the highest amount of phenolic content (932.78± 0.05 µM GAE/ g) followed by *G. thoracica* (898.818± 0.07 µM GAE/g) and *H. itama* (827.939± 0.06 µM GAE/g). The concentration of flavonoids in this study showed that *T. apicalis* is the highest (445.111± 0.02 µg QE/g) followed by *H. itama* (317.333± 0.01 µg QE/g) and the *G. thoracica* (212.889± 0.04 µg QE/g) of extract.

Conclusion(s): Honey extract from *T. apicalis* showed the most potent antioxidant when compared to the other two species. It also possesses the highest total phenolic and flavonoid contents compared to the other species of stingless bee.

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Keywords: *Trigona sp.*, Honey, Antioxidant

P041

Antibacterial Activity Of The Essential Oils Of *Orthosiphon Stamineus* Benth And *Ficus Deltoidea* Jack Against Pathogenic Oral Bacteria

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Purpose: In this study, the antibacterial activity of the herbal oils of *Orthosiphon stamineus* Benth and *Ficus deltoidea* Jack was evaluated against invasive oral pathogens namely *Enterococcus faecalis, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum* and also the Streptococcus spp. (*Streptococcus mutans, S. mitis, S. salivarius).*

Methods: Chemical compositions of both oils were analysed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The antibacterial activities of the oils and their major constituents were investigated using the broth microdilution method (minimal inhibitory concentration, MIC and minimal bactericidal concentration, MBC) for susceptibility test, anti-adhesion, anti-biofilm, checkerboard and time-kill assays. Physiological changes of the bacterial cells after exposure to the oils were observed under the field emission scanning electron microscope (FESEM).

Result(s): Our results showed that *O. stamineus* and *F. deltoidea* oils mainly consist of sesquiterpenoids (44.6% and 60.9%, respectively) and β-caryophyllene (26.3% and 36.3%, respectively). Other compounds present in *O. stamineus* were α-humulene (5.06%) and eugenol (8.1%) while α-humulene (5.5%) and germacrene D (7.68%) was dominant in *F. deltoidea*. The oils of both plants showed moderate to strong inhibition against all tested bacteria grown in suspension medium at 0.63-2.5 mg/ml (MIC and MBC). However, neither showed any inhibition on all tested monospecies biofilms. The time-kill assay showed that combination of both oils with amoxicillin at concentrations of 1x and 2x MIC values demonstrated additive antibacterial effect. The SEM study showed that both oils produced significant alterations on the cells of Gram negative bacteria as they became pleomorphic and lysed.

Conclusion(s): Our study showed that both oils possessed antibacterial properties against the pathogenic oral bacteria and may have caused disturbances of membrane structure or cell wall of the bacteria. The antibacterial activity may be enhanced by the presence of their active constituents like β-caryophyllene, α-humulene, eugenol and germacrene D.

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Keywords: *Orthosiphon stamineus*; *Ficus deltoidea*; antibacterial; oral; biofilm

P042

Comparison of Antioxidant activity, total phenolic content and total flavonoid content between methanolic extract of *Phyllanthus niruri* and *Phyllanthus urinaria*.

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Purpose: We aimed to determine the antioxidant activity, total phenolic content and total flavonoid content of *Phyllanthus niruri* and *Phyllanthus urinaria* methanolic extracts.

Methods: The antioxidant activities were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,20- azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. The chemical contents of the *Phyllanthus niruri* and *Phyllanthus urinaria* were analyzed using total phenolic content (TPC) and total flavonoid content (TFC) assays.

Results: The antioxidant activity by using 1,1-diphenyl-2-picrydydrazyl (DPPH) assay showed that *Phyllanthus urinaria* had lower EC_{50} (15.8 ± 0.01 µg/mL) compared to *Phyllanthus niruri* (29.3 ± 0.01 µg/mL). ABTS assay also shows similar pattern of result where the EC_{50} of *Phyllanthus urinaria* (11.2 ± 0.01 µg/mL) was lower than *Phyllanthus niruri* (26.0 ± 0.02 µg/mL). *Phyllanthus urinaria* also showed higher TPC and TFC value compared to *Phyllanthus niruri*. There was a positive correlation between antioxidant activity and total phenolic and total flavonoid content.

Conclusion(s): Methanolic extract of *Phyllanthus urinaria* showed higher antioxidant activity, TFC and TPC compared to *Phyllantus niruri. Phyllanthus urinaria* should be further characterized and tested for its biological activity.

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Keywords: Phenolic, flavonoid, DPPH, ABTS

P043

The Antifungal Effects of Honey from *Koompassia Excelsa* against *Candida Albicans* : An *in-vitro* Study

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Purpose: *Koompasia excela* or known by Malaysians as *Tualang* is a tree that is commonly found in Peninsular Malaysia. Honey produced by bees that build their hives on this tree is known as *Tualang Honey*. Many literatures suggested that there a few types of honey presented with antimicrobial properties. However, up to the present time, there is no literature present on the antifungal activities of tualang honey. The objective of this study is to identify the antifungal effect of Tualang honey against *Candida albicans*.

Methods: Tualang honey was obtained from the Federal Agriculturural Marketing Authority (FAMA) and samples of Tualang honey were prepared into several concentrations which were 100%, 80%, 60%, 40% and 20%. Honey was diluted with distilled water according to the concentrations before they were sent for gamma irradiation for sterilization. Each concentration were placed on a disc and tested on *Candida albicans* colony. An antifungal agent (Nystatin) disc acts as positive control and plain disc containing sterile distilled water as negative control. Zone of inhibition were to be observed after 24 hours.a total of five samples from each concentration were prepared and tested on *Candida albicans*.

Result(s): After 24hours of disc placement, results showed that there was no inhibition zone detected in the Candida albicans colony surrounding the multiple honey concentrations as well as the negative control. Inhibition zone was only present surrounding the positive control disc which as Nystatin antifungal agent.

Conclusion(s): With the limitation of this study, there is no antifungal effect detected in Tualang honey against *Candida albicans*.

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Keywords: *Tualang honey*, antifungal, *Candida albicans*

P044

Antimicrobial activity of *Aloe vera* extract in irreversible hydrocolloid impression material : An *in vitro* study.

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Purpose: Most dental impressions that are sent to the dental laboratories show the presence of numerous pathogenic microorganisms. Research has shown that the commercial methods of disinfecting impression (spraying and immersion) are not effective and also has some effect on the dimensional accuracy of the resultant cast. Thus, the urge to find an impression that is self-disinfected is needed in order to overcome the problems. *Staphylococcus aureus* is one of the microorganisms found to cause healthcare-associated bacterial infections in dentistry. *Candida albicans* is a common pathogen in human with the ability to endure the condition in oral mucosal surface and cause diseases to the host when condition is more favourable. The aim of this study is to determine the antimicrobial activity of *Aloe vera* extract mixed with alginate against *Staphylococcus aureus* and *Candida albicans*.

Methods: Three different specimen groups are prepared i.e. alginate mixed with distilled water, alginate mixed with 0.12% Chlorhexidine and alginate mixed with *Aloe vera* extract. The samples were prepared under sterile condition and placed in different petri dishes that are inoculated with *Staphylococcus aureus* and *Candida albicans*. After incubation in aerobiosis, inhibition of the microbial growth was measured and the results were interpreted. The data was analysed with SPSS 22.0 by using One-way ANOVA test.

Results: The result showed that *Aloe vera* extract mixed with alginate has significantly inhibited *Staphylococcus aureus* and *Candida albicans*.

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Keywords: Aloe vera, Alginate, antimicrobial

P048

Instant Coffee Effect on Gastric Cell Line: The Cytotoxicity Analysis

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Purpose: The purpose of this study was to investigate the effect of bioactive compounds; caffeine, cholorogenic acid and its isomers (3-caffeoylquinic acid, 4-caffeoylquinic acid and 5-caffeoylquinic acid) in instant coffee on cell viability of human parietal carcinoma cell lines (HGT-1).

Methods: The human gastric carcinoma (HGT-1) cell line was treated separately with seven different types of instant coffee samples (regular, low sugar, low fat and low sugar, white coffee; low acid, decaffeinated and instant black coffee as control) with concentration ranging from 15.625-1000 µg/ml for 24 hours. The treated cells were then subjected to MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay to study the sample's cytotoxicity effect on the cell's viability. The results obtained were used to calculate the cell's viability (%).

Result(s): The MTT assay results showed that seven different types of instant coffee at different concentration have different cell's viability. 16 µg/ml of regular instant coffee leads to the highest percentage of cell viability (141.00% ± 78.60), as for 1000 µg/ml of decaffeinated instant coffee leads to lowest percentage of cell viability (11.00% ± 3.00).

Conclusion(s): Overall, this study showed that different types of instant coffee have different cytotoxicity effect towards human gastric carcinoma (HGT-1) cell line.

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Keywords: HGT-1 Cells, Cytotoxicity, 3-in-1 Instant Coffee, Chlorogenic acid, Caffeine

P056

Potential of Silvestrol from Borneo, for Treatment of a Prevalent Cancer in Sarawak

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Purpose: Silvestrol is a compound from the plant genus *Aglaia* (family Meliaceae) found primarily in the tropical forests of Asia. There is an exceptionally high incidence rate of nasopharyngeal cancer (NPC) in the population of Sarawak, particularly in the Bidayuh ethnic community. The failure of conventional treatments, made worse by associated toxicities, incited us to assess silvestrol as a new therapeutic strategy for the management of NPC to improve treatment outcome.

Methods: Silvestrol was isolated from the plant *Aglaia stellatopilosa*, which is exclusive to Borneo. Silvestrol was first evaluated as a single agent for anti-cancer properties in two cell lines used as cell models for NPC. This was followed by silvestrol with a potent RNA Pol I inhibitor to determine enhancement of anti-cancer effects and finally, validation of that enhanced effect, if any.

Result(s): NPC cells were clearly inhibited by silvestrol. There was a prominent synergistic interaction between silvestrol and an RNA Pol I inhibitor, corroborated by several *in vitro* cell based assays, including cell proliferation and viability, morphological observation and flow cytometry.

Conclusion(s): Synergistic drug combination as a treatment strategy may make use of multiple agents with different modes of action, which may treat a disease more effectively, reduce the dose of each individual agent used, consequently reducing toxicity whilst maintaining efficacy. The exhibited synergism between silvestrol and an RNA Pol I inhibitor as anti-cancer agents showed that this combination could be explored as a prospective chemotherapeutic strategy for treatment of NPC.

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Keywords: *Aglaia*, silvestrol, synergistic

P057

Screening compounds in dichloromethane (DCM) fraction of *Clinacanthus nutans* leaves by Liquid Chromatography–Time-of-Flight Mass Spectrometry (LCTOFMS)

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Purpose: DCM fraction of *C. nutans* was screen for compound identification by Liquid Chromatography–Time-of-Flight Mass Spectrometry (LCTOFMS) as previous work showed it gave the least IC50 value when treated with HeLa cells.

Methods: Chromatographic separation of *C. nutans* DCM fraction was performed on a Waters Alliance 2795 liquid chromatography system using an Eclipse XDB-C18 column. The mass spectrometer was operating in the negative ion mode. Chemical formulas which were generated based on the mass accuracy of the peak by MassLynx 4.1 software was then used to search for putative compound by using several publicly available databases and literature searches of *C. nutans*.

Results and discussions: About 38 compounds were tentatively identified where the major compounds belonged to the class of flavonoids (12) followed by terpenes (8), fatty acids (6), phenolic acids (5), sugar alcohols (2), phenolic glycosides (2), ketone (1), prostaglandin (1) and organooxygen (1). Of these, four compounds have been reported to present in *C. nutans*, involving 6,8-Di-C-alpha-L-arabinopyranosylapigenin, 3',7-Dimethoxy-3-hydroxyflavone, 4-hydroxybenzoic acid and chlorogenic acid. These compounds have been reported to exerted antioxidant and DNA-protective activities.

Conclusions: The identified phytochemicals in *C. nutans* DCM fraction belonged mainly to the classes of flavonoids and this was supported by the highest total flavonoids content (TFC) as determined before. All of these might contribute to the finding of DCM fraction as promoting antiproliferative activity among HeLa cells.

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Keywords: *Clinacanthus nutans*, DCM fraction, LCTOFMS

P058

Investigating proteomic and gene expression regulation by thymoquinone in lipid-loaded MCF7 breast cancer cell

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Purpose: Thymoquinone (TQ) is one of the most studied active compounds due to its medicinal potentials. Recently, oxidized low-density lipoprotein (oxLDL) were found to be increased among breast cancer and prostate cancer patients [1, 2]. Therefore, this study aims to determine the effect of TQ towards oxLDL using breast cancer cell model.

Methods: MCF7 cells were treated with 10 μg/ml of oxLDL for 72 hours, followed by treatment with 20 μM TQ at 24, 48, and 72 hours. Localization and expression of target proteins were studied through immunofluorescence staining and Western Blot, respectively. Relative gene expression analysis was performed with qRT-PCR method.

Result(s): Bright immunofluorescence staining was noted of Bcl-2 expression in oxLDL-laden MCF7 cells at the cytoplasm and nucleus. The FASN and LDLR expression were localized at cytoplasm of the oxLDL-laden MCF7 cells. Native MCF7 cells did not exhibit expression of these proteins. Western Blot showed increased expression of NFκB, Bcl-2 and LDLR in these cells compared to native MCF7. In contrast, FASN exhibited lower expression compared to native cells. This could be due to availability of extracellular oxLDL and reduced *de novo* lipid synthesis. qRT-PCR analysis demonstrated down regulation of *EGLN1* gene in oxLDL-laden MCF7 cells, thus indicating cell proliferation in line with higher expression in Bcl-2 and NFκB. Similarly, *VAMP4* gene was downregulated which may indicate presence of mature granules in oxLDL-laden MCF7 cells. Treatment with TQ inhibited expression of Bcl-2, LDLR and FASN but induced *VAMP4* gene expression.

Conclusion(s): This study suggests that thymoquinone may regulate breast cancer growth exerted by oxLDL.

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Keywords: Oxidized low-density lipoprotein, LDLR, Bcl-2, breast cancer, thymoquinone, FASN, NFκB, VAMP4

P061

Chemical profiles of methanolic extracts from microalgae, *Nannochloropsis* sp.

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Purpose: This study was aimed to determine the total phenolic content, total flavonoid content and antioxidant activity, of *Nannochloropsis* sp. methanolic extract.

Methods: Chemical contents of *Nannochloropsis* sp. were measured using total phenolic content and total flavonoid content and represented as gallic acid equivalent (GAE) and quercetin equivalent (QE) respectively. For antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure the antioxidant activity and the result was presented as EC_{50} (the efficient concentration required to decrease the initial DPPH concentration by 50%).

Result(s): For chemical content analysis, *Nannochloropsis* sp. showed high TPC and TFC value with 58.43 ± 0.85 mg GAE/g DW and 79.87 ± 0.12 mg QE/g DW respectively. In antioxidant measurement, *Nannochloropsis* sp. showed greater percentage of DPPH inhibition with EC_{50} which was at 0.195 ± 0.007 mg/mL.

Conclusion(s): Methanolic extract of *Nannochloropsis* sp. showed high TPC, TFC and antioxidant activity, which is useful in health promoting effects.

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Keywords: Antioxidant, Microalgae, TPC, TFC, *Nannochloropsis*

P063

Cytotoxicity and Gas chromatography-mass spectrometry (GC-MS) analysis of ethanol leaves extract of *Gynura procumbens* and its fractions

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Purpose: *Gynura procumbens* (GP) is a medicinal plant that possessed various interesting pharmacological activities. The aim of this study was to determine the cytotoxicity activity and to identify the bioactive phytochemicals of ethanol leaves extract as well as hexane, chloroform, ethyl acetate and aqueous fractions of GP using GC-MS.

Methods: The cytotoxicity activity of the GP ethanol extract and its fractions-treated RAW 264.7 cells was evaluated *in vitro* by PrestoBlue® cell viability assay. GC-MS analysis was performed on GP ethanol extract and its fractions using AGILENT (GC 6890N/ MS 5673i) GC-MS equipment.

Result(s): The GP ethanol extract and its fractions exhibited weak cytotoxic activity with inhibitory concentration at an average of 90% at 24, 48 and 72 hours incubation respectively. Furthermore, GP extract and fractions showed high RAW264.7 cell proliferation instead of cell death. Meanwhile, the qualitative determination of the different biologically active phytochemicals of GP revealed different types of high and low molecular weight volatile constituents with varying quantities. The crude extract and fractions contained mainly a triterpene compound–squalene and a plasticizer compound–1, 2-benzenedicarboxylic acid, mono [2-ethylhexyl] ester. No compounds were reported in aqueous fraction of GP.

Conclusion(s): The GP leaves extract and fractions from different solvents are not having any toxic effects and possess diverse biologically active volatile compounds, which warrant various biological and pharmacological activities.

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Keywords *Gynura procumbens*, Cytotoxicity, GC-MS

P065

Liquid chromatograhy time-of-flight mass spectrometry (LC-TOF/MS) analysis of *Clinacanthus nutans* (Acanthaceae) from different locations in the northern region of Peninsular Malaysia.

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Purpose: Determination of chemical constituents in the leaves of *Clinacanthus nutans* from six different locations in the northern region of Peninsular Malaysia using liquid chromatography coupled with time of flight mass spectrometry analysis (LC-TOF/MS).

Methods: The C. *nutans* leaves were collected from Teluk Kumbar (PTK), Sungai Petani (KSP), Perlis (SBP), Batu Maung (PBM), Kuala Ketil (KKK) and Pongsu Seribu (PPS). The fresh blended leaves were macerated in 80% methanol:water and extracted after 24 hours at room temperature. The extraction was repeated three times. The extracts were prepared at the concentration of 1 mg/mL and filtered prior the separation using C18 column at the flow rate of 0.7 mL/min. The mobile phase used consisted of 0.1% formic acid and acetonitrile. The LC-TOF/MS analysis was performed in the negative ionization mode with the scan range of m/z 100-1000.

Results: The results indicated the differences in the chemical constituents of the leaves of *C. nutans* from six different locations. Based on the MS and the literature data, six compounds detected in *C. nutans* extract were determined as vitexin, isovitexin, shaftoside, orientin, isoorientin and 6,8-apigenin-C-α-L-pyranarabinoside

Conclusions: This data is useful for further analysis of the compounds by tandem mass spectrometry and NMR. Based on the new drug discovery in the future, the data also might contribute to *C. nutans* planting strategy for herbs.

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Keywords: *Clinacanthus nutans*, chemical constituents, LC-TOF/MS.

P068

Chemical profiles of three different extracts from fresh leaves of *Goniothalamus umbrosus*

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Purpose: This study was aimed to determine the total phenolic content and total flavonoid content of *Goniothalamus umbrosus* fresh leaves extracts in three different solvents; Hexane, Chloroform and Dichloromethane.

Methods: Chemical contents of *Goniothalamus umbrosus* fresh leaves were measured using two assays which are total phenolic content (TPC) and total flavonoid content (TFC). For TPC, gallic acid was used as a standard and the data were represented as gallic acid equivalent (GAE). While for TFC, quercetin was used as a standard and the data were represented as a quercetin equivalent (QE).

Result(s): For chemical content analysis, *Goniothalamus umbrosus* fresh leaves showed higher TPC value in Dichloromethane compare to Chloroform and Hexane with 262.00 ± 0.21, 129.26 ± 0.06 and 73.96 ± 0.03 mg GAE/g DW respectively. While in TFC, Chloroform recorded highest value compare to Dichloromethane and Hexane with value 61.81 ± 0.07, 61.66 ± 0.03 and 51.71 ± 0.05 mg QE/g DW respectively.

Conclusion(s): Dichloromethane extract and Chloroform extract of *Goniothalamus umbrosus* fresh leaves showed highest total phenolic content and total flavonoid content, which is useful in health promoting effects.

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Acknowledgement: Fundamental Research Grant Scheme 203/CIPPT/6711340

Keywords: Fresh Leave, TPC, TFC, *Goniothalamus umbrosus*

P069

Synthesis and Characterization of Thymoquinone PLGA-Nanoparticles and its Cytotoxicity against Resistant ER-Positive Breast Cancer Cell

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Purpose: Thymoquinone (TQ) is an active compound derived from *Nigella sativa* seed extract and reported to exhibit various medicinal benefits. Therefore, aim of this study was to determine modulatory effects of Thymoquinone-PLGA nanoparticles towards the developed drug resistant breast cancer cells.

Methods: Polymeric drug nanoparticles were prepared by emulsion solvent evaporation method. The polymeric drug nanoparticles consist of thymoquinone and Poly (D,L Lactide-co-glycolide) (PLGA) with the addition of Pluronics F127 and Pluronics F68 as stabilizers. The prepared TQ-PLGA nanoparticles were characterized by Fourier Transform Infrared spectroscopy (FTIR), entrapment efficiency, particle size, the morphology investigation by transmission electron microscop (TEM) and *in vitro* drug release study. Development of drug resistant MCF7 cells were done using pulse method. Initially, dose-dependant assay was performed to determine the IC_{50} value of Tamoxifen. After six to seven cycles of drug induction, the P-glycoprotein (P-gp) expression was determined to confirm resistant using flow cytometry.

Result(s): FTIR study indicated that there was no interaction between thymoquinone and polymer. The entrapment efficiency obtained was higher than 90% in both formulations of TQ-PLGA. The average size TQ-PLGA with Pluronics F127 nanoparticles was larger than formulation with Pluronic F68 ($p < 0.05$). TEM analysis showed spherical shaped discrete particles without aggregation with zeta potential value was -15.6 mV and -19.9 mV in TQ-PLGA with Pluronics F127 and Pluronics F68, respectively with 0.2 polydispersity index (PDI). *In vitro* release studies were carried out and showed drug release for up to 6 days. MCF 7 cells developed resistance to Tamoxifen at 16 μM with 26% of P-gp expression. Cytotoxic study indicated drug resistant cells to be more sensitive to TQ-PLGA encapsulated with Pluronics F127 with IC_{50} value at 78 μg/ml after 72 hours treatment.

Conclusion(s): This study suggests that formulation of TQ-PLGA with Pluronics F127 may be a suitable candidate for the sustained delivery of drugs against the resistant breast cancer cells.

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Keywords: TQ-PLGA, Pluronics, Resistant Breast Cancer Cell

P070

Investigating effects of lipids and *Catharanthus roseus* nanoparticles on tamoxifen-resistant UACC732 breast cancer cell line

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Purpose: To determine role of lipids and regulation of *Catharanthus roseus (C. roseus)* nanoparticles in UACC732 tamoxifen-resistant breast cancer cell proliferation.

Methods: Development of tamoxifen-resistance UACC732 cells was done using pulse method. Fold resistance was determined based on comparison of half maximal inhibitory concentration (IC_{50}) of resistant cells and parent cells. Both cell lines were treated with 10-100 µg/ml of oxidized low-density lipoprotein (oxLDL) and very low-density lipoprotein (VLDL) for 72 hours, followed by cell viability analysis using MTS assay. Meanwhile *C. roseus* PLGA-PEG nanoparticles were synthesised using solvent displacement method and characterized by UV-VIS, FTIR, TEM, Zeta Potential and percentage of encapsulation efficiency. Cytotoxicity of the synthesized nanoparticles were compared between tamoxifen-resistant and parent UACC732 cells.

Result(s): The fold-resistance of tamoxifen-resistant UACC732 cells reached 1.37-fold. MTS assay showed the IC_{50} of oxLDL for parent and tamoxifen-resistant UACC732 cells was 30.9 µg/ml and 73.8 µg/ml, respectively. Meanwhile for VLDL treatment, both cells showed high percentage of cell viability, thus indicating that cell proliferation was induced by VLDL. UV-VIS absorption showed that the *C. roseus* PLGA-PEG nanoparticles produced peak of 220 nm. FTIR results indicated encapsulation of *C. roseus* based on the presence of 3327 cm^{-1}, 1637 cm^{-1}, and 1066 cm^{-1} peaks. These nanoparticles were spherical with less than 200 nm and encapsulation efficiency was 63%. Cytotoxicity assay showed IC_{50} of *C. roseus* nanoparticles was 97.0 µg/ml in parent cells and 62.7 µg/ml in tamoxifen-resistant cells.

Conclusion(s): Tamoxifen-resistant UACC732 cells were less sensitive towards oxLDL compared to parent cells. However, both types of cells proliferated in the presence of VLDL. This suggest that lipids mainly VLDL could induce breast cancer cells proliferation compared to oxLDL. However, this needs to be further investigated. Tamoxifen-resistant UACC732 cells was more sensitive towards *C. roseus* PLGA-PEG nanoparticles treatment compared to parent cells.

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Keywords: *Catharanthus Roseus,* drug resistant cell, oxidized LDL, VLDL

P073

Evaluation of antiangiogenic and antitumor properties of *Anogeissus leiocarpus*

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Purpose: *Anogeissus leiocarpus* is a traditional medicinal plant with strong antioxidant and hypoglycemic properties. This study was aimed to investigate the antiangiogenic and cytotoxic effects of eight extracts from *Anogeissus leiocarpa* (leaves and bark). Eventually, the most active extract was subjected to a series of *in vitro* and *in vivo* studies to elucidate the mechanism of action.

Methods: In order to confirm the effect of the extract on motility of human endothelial cells, cell migration assay was conducted. In addition, VEGF suppressive effect of the extract was assessed in endothelial cells. Finally, the antitumor effect of the extract was evaluated using *in vivo* human tumor xenograft model.

Results: Results of the present study indicated that, hexane extract of the stem bark of *A. leiocarpus* was found as the most active extract on inhibition of sprouting of microvessels (89.56%). Additionally, ethanol extract of the leaves exerted high antiangiogenic (inhibition 82.12%) in rat aortic ring assay. Hexane extract of the stem bark displayed significant inhibitory effect on endothelial cells proliferation (76.87 %) while ethanol extract of the leaves was save on HUVEC cell lines (inhibition 5.80%). The two extracts inhibited HUVEC migration by 87.57 and 65.23% respectively. The extracts demonstrated significant inhibition of VEGF levels (45.32 and 30.52 % respectively) in treated endothelial cells. Finally the extracts exhibited potent anti-tumorigneic effect in anthymic mice with $\Delta T/\Delta C$ =8.43 and 12.54% at doses 400 and 200mg/kg, respectively.

Conclusion: These results may provide novel guidelines towards improved strategies using *Anogeissus leiocarpus* extracts based on the suppression of angiogenesis to curb the growth of tumors. The plant can be used as promising candidate for anti-neoplastic drug development.

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Key words: Medicinal plants, Antitumor *in vivo*, antiangiogenic *Anogeissus leiocarpus*

Conference: Abstracts for Oral Presentation

S3

Inhibition of CYP3A by antimalarial piperaquine and its metabolites in human liver microsomes with *in vitro* to *in vivo* extrapolation

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Purpose: The potential of the antimalarial piperaquine (PQ) and its metabolites to inhibit human cytochrome P450 (CYP3A) was investigated in pooled human liver microsomes.

Methods: CYP3A activity was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as the rate of 1'-hydroxymidazolam formation. Physiologically based pharmacokinetic (PBPK) was applied to predict the drug-drug interaction, using midazolam as CYP3A substrate.

Result(s): Piperaquine (PQ) was found to be a reversible, potent inhibitor of CYP3A with the following parameter estimates (%CV): IC_{50} = 0.76 µM (29), K_i = 0.68 µM (29). In addition, PQ acted as a time-dependent inhibitor (TDI) with IC_{50} declining to 0.32 µM (28) during 30 min pre-incubation. TDI estimates were k_{inact} = 0.024 min^{-1} (30) and K_I = 1.63 µM (17). Metabolite M2 was a highly potent reversible inhibitor with estimated IC_{50} and K_i values of 0.057 µM (17) and 0.043 µM (3), respectively. M1 and M5 metabolites did not show any inhibitory properties within the limits of assay used. Average (95th percentile) simulated *in vivo* area under the curves (AUCs) of midazolam increased 2.2-fold (3.7-fold) on the third which is the last day of PQ dosing while for its metabolite M2, AUCs of midazolam increased 7.7-fold (23-fold).

Conclusion(s): Simulations of the clinical situation suggested that the inhibitory effect of PQ and metabolite M2 may persist for several days or weeks after treatment initiation.

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Keywords: piperaquine, CYP inhibition, drug-drug interaction

S5

Simplified ligature-induced periodontitis model in rats for better diagnosis

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Purpose: Ligature placement around the cervical position of molar teeth has been used for many years as an experimental model for periodontitis inducement in animal subjects. However, the common experimental animals such as mice and rats have tiny molar teeth which are located farthest to the rear, hence difficult to assess and rendered the model complicated. In this report, we present simple and straightforward method for ligating incisor teeth as an alternative ligature-induced periodontitis model that could enhance diagnosis of the disease.

Methods: Twelve *Sprague Dawley* rats were used for the study. The rats were grouped into two, intact rats (control) and periodontitis model rats (test). After acclimatization for two weeks, the test group were anaesthetized. Then a sterile, 3/0 non-absorbable silk thread was placed around the cervical position of the lower incisors. The rats were euthanized after two weeks of the ligature and the mandibles were removed, excised and defleshed by using 2 M sodium hydroxide (NaOH), and then washed, air dried and examined by stereoscope for morphometric study.

Result(s): Inflammation of the periodontium was apparently observed from 3rd day of the induction, with swollen and change in the gingival colour from pinkish to dark red. Shrinkage of the gum mass and plaque accumulation was also observed around the ligated teeth from the 5th day. By the 14th day, all the clinical features became more apparent. Morphometric analysis showed a significant difference in the alveolar bone loss between control and test group ($p < 0.05$).

Conclusion(s): The present study demonstrates that this modified ligature-induced model could be a simple alternative to the existing model and could enhance the diagnosis of periodontitis in rats.

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Acknowledgement: Universiti Sains Malaysia Fellowship Scheme.

Keywords: Ligature-induced periodontitis, periodontitis, alveolar bone loss, *Sprague Dawley* rats

S6

Proliferation reduction and migration inhibition of IL-21- and Clusterin-silenced in colorectal cancer cells

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Purpose: This study evaluates the effects of IL-21 and Clusterin, which were detected in the serum samples of colorectal cancer patients infected with *Schistosoma mansoni*, in a variety of cellular activities.

Methods: Silencing IL-21 and Clusterin in colorectal cancer cells were performed, whereby the reduction of cell proliferation was determined using LDH assay, whereas the apoptosis induction and cell cycle arrest were detected using Flow cytometry. All mRNA expressions in specific gene-silenced cancer cells were detected by Real-time PCR, whereas the inhibition of cell migration was investigated by Wound healing assay.

Result(s): The results found a significant reduction in the proliferation, whereby the mRNA expressions of proliferation markers, e.g. Ki67, PCNA and TGFα were detected significantly reducing in the IL-21- and Clusterin-silenced cancer cells, though additional CCL5 was found to be significantly induced in the Clusterin-silenced Caco2 cells. However, no apoptosis induction and cell cycle arrest were detected, which could be further explained by the down-regulation of Caspase-9 and Caspase-3 in the specific gene-silenced cancer cells. The inhibition of cell migration was also observed in the IL-21- and Clusterin-silenced cancer cells, which was likely associated with FGF4 and BNIP3. Other than those molecular targets, IL-17 and IL-13 may also play the roles in the IL-21-silenced cancer cells.

Conclusion(s): This study provides useful information of IL-21 and Clusterin in colorectal cancer cells whereby these genes could be potential targets to aid cost-effective therapeutic agents for the treatment of *S. mansoni* colorectal cancer patients in the third world countries.

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Keywords: Infectious disease, colorectal cancer, interleukin-21, Clusterin, cell proliferation, cell migration

S7

In silico docking of phage-displayed peptides targeting *Plasmodium vivax* apical membrane antigen 1 (PvAMA1)

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Purpose: *Plasmodium* is an obligate intracellular parasite that rely on the ability in host cell penetration and replication. Apical membrane antigen 1 (AMA1) is one of the most well characterized malarial surface antigen for parasite-host cell invasion and served as a pivotal vaccine candidate and potent antimalarial drug target. The present study aimed to predict the binding sites of three previously selected phage-displayed dodecapeptides, i.e., PdV1 (DLTFTVNPLSKA), PdV2 (WHWSWWNPNQLT), and PdV3 (TSVSYINNRHNL) with affinity to refolded recombinant *P. vivax* AMA1 (rPvAMA1).

Methods: The binding sites of the PdV1, PdV2, and PdV3 peptides to the native PvAMA1 (PDB ID: 1W8K) were predicted *in silico* using CABs-dock web server (http://biocomp.chem.uw.edu.pl/CABSdock). The pairs of peptide/receptor residues with 3.5 Å contact cutoff were then mapped to the native amino acid residues of PvAMA1 (1W8K) and rPvAMA1.

Results: The simulation models of protein-peptide docking indicated that the PdV1 and PdV3 peptides were mapped to the similar regions, mainly at domains II and III with sharing 12 similar binding sites, whereas PdV2 peptide was mapped solely to the DI of PvAMA1.

Conclusion: Phage display technique was used to screen the phage clones that bind to the rPvAMA1. CABS-dock is a free web server for the flexible docking of peptides to target protein without prior knowledge of the binding site and also allow prediction of complex arrangements close to the native structure. A greater understanding of the molecular interaction between the parasite and its host would assist in the development of new therapeutics and most importantly, a vaccine for long term sustainable reduction in the global burden of malaria.

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Keywords: *Plasmodium vivax*, apical membrane antigen, phage display, *in silico* peptide docking.

C1

Audit of surgical antibiotic prophylaxis for obstetric and gynaecologic procedures in a tertiary hospital in Nigeria

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Purpose: Surgical site infections are the most common hospital acquired infections affecting surgical patients. Surgical antibiotic prophylaxis prevents these infections. The efficacy of antibiotic prophylaxis is dependent on appropriate administration. The objective of this study was to evaluate adherence to guideline recommendations for timing and duration of surgical antibiotic prophylaxis in Obstetrics and Gynaecologic procedures.

Methods: This is a 10 weeks prospective study conducted in the Department of Obstetrics and Gynaecology in a Nigerian tertiary hospital. Women undergoing surgical procedures, with no established or suspected infection, were included. Data was collected through review of patient medication chart, medical, nursing and anaesthetic records.

Result(s): There were 90 patients who underwent 95 procedures. The mean age of patients was 30.8 (± 8.3) years. Preoperative antibiotic prophylaxis was administered in 50.5% of the procedures. Appropriate duration of antibiotic prophylaxis was observed in 17 (17.9%) procedures. Post-operative antibiotic regime was prescribed in 82.1% of the procedures consisting of 48 to 72 hours intravenous regime followed by 5 to 10 days oral course.

Conclusion(s): Adherence to guideline recommendations for optimal timing and duration of surgical antibiotic prophylaxis is poor among Obstetricians and Gynaecologists. The findings of this study underline the need for antibiotic stewardship interventions.

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Keywords: antibiotic prophylaxis, gynaecology, obstetrics

C3

Is needle exchange programme still relevant in the advent of Methadone Maintanence Therapy?

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Purpose: Opioid dependence carries a high cost to society by resulting in social problems and transmission of diseases such as HIV and Hepatitis C. Harm reduction practices done in Malaysia are Methadone Maintenance Therapy (MMT) and needle exchange programme. For MMT, relapse during treatment hinders successful outcome of reducing transmission of blood-borne infections. This study aims to determine the prevalence of relapse in those receiving MMT and study the harmful practices done during the relapses with their perceived causes.

Methods: This is a mixed-method cross-sectional study done from June-July 2016. All MMT clients in primary care therapy centres in Kuala Nerus district, Terengganu, Malaysia who fulfilled the inclusion and exclusion criteria and consented were included. A semi-structured questionnaire was filled via face-to-face interview and reference to the case records was done. 122 questionnaires were assessed.

Result(s): Current relapse, defined as any episode of intake of heroine for the past one month after a period of abstinence was 34.4%. Of those relapsed, majority (78.6%) had Hepatitis C, 19.0% had HIV. Majority (97.6%) deny sharing needles during relapse. All attributed this due to ease of accessibility of needle exchange programme, 50.8% still shared needle-washing containers despite non-sharing needles, all unaware that this may spread infections. Those who shared needles had both HIV and hepatitis C, could not get any needle despite exchange programme nearby.

Conclusion(s): To achieve aim of harm reduction, MMT and needle exchange programme need to go hand to minimize the transmission of blood-borne diseases during opioid re-injecting behaviour.

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Acknowledgement: Short-term research grant from Universiti Sultan Zainal Abidin, Malaysia

Keywords: Methadone Maintenance Therapy, needle exchange, blood-borne infections

C5

Bacteriostatic properties of heterostructure TiO_2/ZnO nanocomposite against *Staphylococcus aureus* and *Escherichia coli* with potential biomedical applications

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Purpose: Hospital-acquired infections (HAIs) are responsible for over 40% of cases in acute-care hospitals and commonly associated with catheters-associated urinary tract infections (CAUTIs). Current nanotechnology approach focuses on improving the aseptic procedures for medical devices and manage the HAIs risk. TiO_2 and ZnO nanocomposite have been widely reported independently, to have a photocatalytic killing potential. The present study evaluates the antibacterial activity of heterojunction between TiO_2 and ZnO nanocomposite on two types of bacterial pathogens model including methicillin-susceptible *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

Methods: The antibacterial screening test on bare nanocomposite (powder form) was done under dark and light conditions according to Clinical Laboratory Standards Institute (CLSI) guidelines MO2-A11. Further investigation of nanocomposite incorporated with linear low-density polyethylene (LLDPE) were studied following the American standard test method (ASTM) E2149 for biomaterial product with immobilized antimicrobial agents under dynamic contact condition.

Result(s): The bacterial strains used in this study have shown strong biofilm production capability. Based on the antibacterial results obtained, the bacterial inhibition zone for TiO_2/ZnO was observed approximately between 8-15 mm in diameter, corresponding to the samples concentration from 100 μg/mL to 1000 μg/mL. The ASTM E-2149 results revealed that the LLDPE with TiO_2/ZnO showed bacteriostatic activity toward *S.aureus* and *E.coli*, respectively.

Conclusion(s): Findings from this study highlights the impregnation of TiO_2/ZnO nanocomposite on the surface of biomedical appliances could become a promising bacteriostatic and/or bactericidal agent to combat HAIs.

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Keywords: titanium and zinc oxide photocatalyst, hospital-acquired infections, antibacterial activity, bacteriostatic activity, biomedical product, biomaterial.

C6

Evaluation of a recombinant *Toxoplasma gondii* epitope-based antigen as potential vaccine candidate

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Purpose: Toxoplasmosis is a highly prevalent zoonotic disease caused by the obligate intracellular protozoan parasite *Toxoplasma gondii.* Given the high distribution of the disease, as well as the lack of effective drugs, development of effective vaccines for controlling toxoplasmosis is of high priority. In this study, the immunogenicity of a recombinant multiepitope antigen (USM.TOXO1) was evaluated in BALB/c mice.

Methods: The mice received three subcutaneous injection of 10 µg/ml USM.TOXO1 antigen. Subsequently, the IgG antibody, IgG subclass, IFN-γ and IL-4 production was evaluated using ELISA.

Result(s): The results showed that USM.TOXO1 is sufficiently potent to induce significant humoral and cellular immune responses. It is elicited a mixed of Th1/Th2 response polarized toward the IgG1 antibody isotype. While the cytokine analysis revealed a significant release of IFN-γ.

Conclusion(s): This study indicated that USM.TOXO1 is a potential vaccine candidate. The strategy of using multi-epitope antigens seems to be highly promising in the development of effective vaccine candidates that would generate lasting protective immune responses against *T. gondii*. Furthermore, it could be an important approach in investigating the improvement of the disease vaccination in the future.

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Keywords: *Toxoplasma gondii*, multiepitope antigen, BALB/c mice

C7

Identification of phages-bearing peptide that bind specifically to murine norovirus 1 (MNV-1) NS6 protease using phage display technique

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Purpose: Norovirus is one of the leading causes of gastroenteritis worldwide. The importance of the enzymatic activity possess by the norovirus NS6 protease has made it as an attractive target for developing antiviral drug. In this study, we identified several peptide phages that bind to the active site mutant (C139A)-recombinant MNV-1 NS6 using phage display technique. Small peptides could subsequently be further developed as a novel inhibitor against norovirus NS6 and their efficiency to act as antiviral therapy could be further assessed.

Methods: Initially, C139A NS6 was cloned, expressed and purified. Then, Ph.D-7™ Phage Display Peptide Library (NEB) was used in bio-panning assay for 6 rounds against C139A NS6. In those bio-panning, bound phages were eluted using general elution buffer. A total of 98 phage clones were selected and peptide sequences were identified. The binding interaction of peptides towards C139A NS6 and wildtype NS6 protease were assessed through docking analysis.

Results: The C139A NS6 was successfully cloned, expressed and purified. The sequence analysis from 6 rounds of panning showed similar peptide sequences in a few selected phage clones. The identified peptide sequences are ADARYKS, QTEKNPL and NSKLVLG. Docking analysis showed that none of the identified peptide sequences bind to the amino acid residue at position 139 of the NS6 protein.

Conclusion: Peptide phages with binding activity towards the C139A NS6 have been identified via bio-panning from the library. Further analysis on specificity, affinity and inhibitory effects are warranted.

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Keywords: phage display, murine norovirus 1, NS6 protease

C16

In silico molecular docking study of caffeic acid derivatives as potential efflux pump inhibitor(s) of *Pseudomonas aeruginosa*

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Purpose: The prevalence of multidrug resistant (MDR) *Pseudomonas aeruginosa* in hospital-acquired infections has rendered their treatment a challenging task. One of the major factors that contribute to this problem is the expression of multiple efflux pumps. The use of efflux pump inhibitor (EPI) is a promising approach in combating this problem. In this study, the potential of caffeic acid derivatives (CAD) as EPI(s) in *P. aeruginosa* was accessed using *in-silico* molecular docking.

Methods: Initially, twenty compounds of CAD with various biological activities were compiled from the literature. Their chemical structures were drawn, optimized and filtered based on the Lipinski's rule of five. The proteins, MexB (PDB ID: 3W9J) and MexY (homology model) from MexAB-OprM and MexXY-OprM efflux systems, respectively were selected as the target proteins. Molecular docking of these proteins and CAD was performed using Autodock4.2.

Result(s): Nine out of twenty selected CAD that met the Lipinski's rules of five were employed for docking study. Two CAD that showed most favorable free energy of binding (FEB) towards the drug targets (MexB and MexY) were caffeic acid phenethyl ester, CAPE (-7.28 and -7.40 kcal/mol) and caffeic acid phenethyl amide, CAPA (-7.60 and -7.14 kcal/mol). Some essential interactions were formed between these CAD and the active residues in the target proteins MexB (Val139, Lys151, Phe178, Gly179, Ile277, Tyr327, Phe610, Val612, Phe628 and Met630) and MexY (Asp133).

Conclusion(s): These results indicated that inhibitors CAPE and CAPA would be potent inhibitors for the MexAB-OprM and MexXY-OprM *P. aeruginosa* efflux pump systems.

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Keywords: *Pseudomonas aeruginosa*, efflux pump inhibitor, caffeic acid derivatives, molecular docking

C17

Integrase strand transfer inhibitors (INSTI) resistance among HIV-1 patients in Malaysia: A preliminary data

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Purpose: Raltegravir is the first FDA approved integrase strand transfer inhibitor (INSTI) which acts to block the activity of integrase enzyme of HIV-1. Its therapeutic benefits in treating HIV-1 infection with a combination with HIV-1 reverse-transcriptase inhibitors (both nucleoside analogues and nonnucleoside inhibitors) and HIV-1 protease inhibitors have been proven. This study aimed to determine the most common drug resistance mutation (DRM) towards INSTI and the most predominant HIV-1 subtype for this study population.

Methods: 22 plasma samples from treated HIV-1 adult patients who have demonstrated high level resistance patterns towards first line anti-retroviral therapy (ARV) or are currently treated with INSTI with evidence of virological failure were included in this study. The in-house assay was based on the protocol from the Burnett Institute, Melbourne and used to sequence the integrase region of the *pol* gene. Results were submitted to the Stanford HIV-1 Drug Resistance Database to yield associated DRMs and for subtype analysis.

Result(s): HIV-1 viral load for all samples ranged from 3.32–5.68 log copies/mL. Of 22 samples, 17 samples were amplified and sequenced successfully. DRMs were detected in 5 patients (22.7%). Major resistance mutations towards INSTI were G140A and Q148R which have caused high level resistance towards Raltegravir, the only INSTI available in Malaysia. Predominant HIV-1 subtypes detected in this study was CRF_01 AE (88.2%).

Conclusion(s): The detection of DRMs demonstrated the importance of genotypic resistance test in the management of HIV-1 patients as DRMs can alter patient's susceptibility towards ARV drugs.

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Keywords: integrase strand transfer inhibitor, drug resistance mutation, Malaysia

C18

Observation of vancomycin minimum inhibitory concentration (MIC) creep among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from surgical sites infected patients at a Clinical Training Centre, Malaysia

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Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) causing skin and soft tissue infections is one of the most common nosocomial infections among post-surgical patients. Vancomycin, the mainstay of treatment, when its minimum inhibitory concentration (MIC) breakpoint is 2 ug/mL (MIC creep) is considered to be reduced in susceptibility and may be associated with treatment failures. Here we describe the MIC of our MRSA isolates and the clinical impact.

Methods: A retrospective data collection of post-surgical patients of variety conditions admitted to the Clinical Training Centre Sungai Buloh public section from 2016-2017 with MRSA isolated from their microbiological specimens and a documented MIC to vancomycin were analyzed. The specimens consist of pus swab, mediastinal fluid, sternal bone, and tissue.

Result(s): A total of 34 MRSA were isolated from 8 patients. There were 18, 4, 3, and 4 MRSA with MICs (ug/mL) of ≤ 0.5, 1, 1.5 and 2 respectively. The MRSA with MICs of 2 ug/mL were observed from three different patients and one of them showed a vancomycin creep from 0.5 ug/mL which grew from wound swabs to 2.0 ug/mL which grew sternal bone and mediastinal fluid. All patients were successfully treated for their infections.

Conclusion(s): Vancomycin creep has been observed in our clinical training setting. This phenomenon, despite did not result in therapeutic failure, underscores the need of surveillance for MRSA MIC and vigilant infection control measures to prevent further spread of such infection.

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Keywords: Methicillin-resistant *Staphylococcus aureus*, vancomycin creep, surgical site infections

C20

Preliminary study on prevalence of latent tuberculosis infection (LTBI) and its associated factors among diabetic patients in selected health clinics in Terengganu

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Purpose: *Mycobacterium tuberculosis* infection causes significant morbidity and mortality worldwide. Diabetic patient is recognized as in immunocompromised state, has major risk to carry LTBI. An effective strategy for reducing the transmission, morbidity, and mortality of active disease among diabetic patients is through the identification of LTBI. This study aimed to describe the prevalence of LTBI and its associated risk factors among diabetic patients.

Methods: This cross-sectional study involving diabetic patients was conducted at two health clinics in Terengganu. Participants (n=133) were administered to Tuberculin Skin Test (TST) and interview session was done to obtain the socio demographic data and clinical data. Simple and multivariate logistic regression was tested for the association between independent variable and dependent outcomes (associated factors of LTBI and LTBI status).

Result(s): Out of 133 patients 63.2% were females and 36.1% were males. This study revealed that the risk factors for LTBI were having history of TB contact (p= 0.01, OR=1.76), and smoking status (p=0.004, OR= 2.573)

Conclusion(s): Active screening, infection control measure and glucose controls are recommended for reducing the risk of LTBI and reactivation of LTBI.

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Keywords: latent tuberculosis infection, tuberculin skin test, diabetes mellitus

C2

Development, characterization and *in vivo* evaluation of triclosan loaded nanoparticles against periodontal infection

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Purpose: Every nine out of ten people around the globe may risk contracting periodontal infection due to its high prevalence. In Malaysia, up to 94% of the adult population can be affected by this disease, hence the urgent need for effective treatment options to address this pandemic. The objective of this investigation was to develop, optimize, characterize, and evaluate nanoparticles (NPs) containing triclosan (TCS) for local treatment of periodontal infection.

Methods: NPs was developed by solvent displacement method and then optimized by Design-Expert® software version 10 using Resolution IV design. The optimized NPs was characterized with respect to their particle size, zeta potential, entrapment efficiency (EE) and morphology by scanning electron microscopy (SEM). Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) were employed to demonstrate the encapsulation/entrapment of TCS in the NPs. The *in-vivo* study was conducted by using *Sprague-Dawley* rats to evaluate the efficacy of the developed NPs.

Results: The optimized TCS-loaded NPs exhibited particle sizes of 140.7±8 nm, polydispersity index (PDI) of 0.092±0.05, zeta potential of -30.1±-7 mV and EE of 78±6%. SEM showed that TCS-loaded NPs exhibited solid structures mostly in round shape. Encapsulation/entrapment of TCS in the NPs was confirmed by FTIR, XRD and DSC. Morphometric analysis for ligature-induced periodontitis in rats showed pronounce gain of alveolar bone in the group treated with the TCS-loaded NPs.

Conclusion: The developed NPs loaded with antimicrobial TCS could be a promising alternative to the conventional formulation in the treatment of periodontal infection.

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Acknowledgement: Universiti Sains Malaysia Fellowship Scheme.

Keywords: periodontal infection, triclosan nanoparticle, ligature-induced periodontitis

S11

Zika NS5 protein interactomics reveal possible role played by RPS20 in pathogenesis

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Purpose: Host cellular machinery plays a vital role in the survival of Zika virus. The outcome of infection is determined by complex host-Zika interactions with a large number of altered transcriptional and translational rates, and functional kinetics of participating genes. To date, the first-hand information on the molecular changes in the host induced by the virus to promote its replication and also the pathways triggered in the host that result in immunity and or clearance of the viral infection are still lacking. Having insights into the host's responses to viruses would help define targets for therapeutic intervention.

Methods: We performed whole genome yeast two hybrid studies to map the interaction between Zika NS5 and human liver cDNA library. Through this initiative, we uncovered novel host factors which contribute to the survival of this virus. Particularly, we were interested in RSP20, which may be involved in Zika virus pathogenesis.

Result(s): We confirmed the interaction between Zika NS5 and RSP20 in both yeast and mammalian cells. Cellular localization studies using confocal microscopy showed that Zika NS5 and RSP20 proteins co-localize predominantly in the nucleus at early infection Furthermore, siRNA knockdown of RSP20 in 293T cells resulted about nearly 10-fold decrease in virus.

Conclusion(s): Taken together, these results could provide new clues for further studies of the mechanism of Zika virus pathogenesis.

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Keywords: Yeast two hybrid, Zika virus, non-structural protein 5, Interactomics, RSP20

S12

Non-coding RNA profiles of DENV1-infected *Ae. albopictus* cells

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Purpose: Non-coding RNAs (ncRNAs) are crucial in many biological processes such as development, immunity, and infection. Unlike *Ae. aegypti*, ncRNA profiles of *Ae. albopictus* are not well-characterized and little is known about their biological functions. Here, we comprehensively characterized ncRNAs in *Ae. albopictus* cells (C6/36) following DENV1 infection. In this study, we only focus on three classes of ncRNAs – micro-RNA (miRNA), PIWI-interacting RNA (piRNA), and long intergenic non-coding RNA (lincRNA), primarily because they were shown to be involved in virus infection.

Methods: C6/36 cells were infected with DENV1 at multiplicity of infection of 0.5. After 3 days post-infection, total RNA was extracted. RNA samples were sent for Illumina next-generation sequencing. miRNA discovery was analyzed using mirDeep2 software, while analysis on piRNA was done using proTRAC. A specific pipeline was developed to identify and characterize lincRNA.

Result(s): A total of 363 miRNAs were discovered. Following DENV1 infection, most miRNAs were downregulated. piRNAs in C6/36 cells exhibit canonical characteristic of piRNAs (5'U bias and ping-pong signature). Upon DENV1 infection, piRNA expression was unchanged. It was found that certain predicted piRNA clusters only appeared following DENV1-infection; thereby, they were categorized as DENV1-specific clusters. We found a total of 27,190 lincRNA transcripts in C6/36 cells, and they were differentially expressed upon DENV-infection.

Conclusion(s): DENV1 infection results in the rewiring the transcriptional pattern of ncRNAs in C6/36 cells. Further functional assays are necessary to dissect the mechanisms underlying this observation.

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Keywords: miRNA, piRNA, *Aedes albopictus*, dengue virus

C19

Multiplex CRISPR/Cas9 for specific and effective elimination of prevalent HIV strain in Southeast Asia

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Purpose: Although HIV was first discovered about 30 years ago, a cure has yet to be discovered. The challenges include the persistence of latent reservoirs, immune system exhaustion & viral escape. We designed a multiplex CRISPR/Cas9 (mCRISPR/Cas9) system to knockdown the structural genes, regulatory genes and accessory genes of HIV simultaneously as a multi-pronged approach to overcome all the problems typically seen in HIV cure. Our mCRISPR/Cas9 system was also designed to work across strains AE, B and C, the three most common strains of HIV found in the world. Our preliminary data showed a decrease in viral load even at 24 hours after treatment. Next, we will be employing this method against peripheral blood mononuclear cells (PBMC) from infected patients to determine the effect of mCRISPR/Cas9 against latent reservoir and immune system recovery.

Methods: Sequences of *Pol, Gag, Rev, Tat* and *Vif* were cloned into CRISPR/Cas9 system. In-vitro functionality of the mCRISPR/Cas9 system was determined by treating ACH-2 and HEK 293 cells with mCRISPR/Cas9 to assess the HIV-1 viral load and cytotoxicity respectively. Samples were collected at days 1, 3 & 5 post-transfection to perform p24 ELISA for viral load determination and FACS analysis respectively.

Result(s): Decrease in p24 antigen expression was observed in mCRISPR/Cas9 treated ACH-2 cells suggesting HIV-1 viral load was decreased. Increase in treated HEK 293 cells in cytotoxicity assay suggests the safety of the mCRISPR/Cas9 in human cells.

Conclusion(s): mCRISPR/Cas9 promises an efficient, specific & timely elimination of HIV latent reservoirs for potential functional cure.

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Acknowledgement: Fundamental Research Grant Scheme, 203/CIPPT/6711440

Keywords: Clustered regularly interspaced short palindromic repeat/Cas9, human immunodeficiency virus, Functional cure

S16

Brewers' rice ameliorates oxidative stress in azoxymethane-induced colon carcinogenesis in rats

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Purpose: To investigate the chemopreventive potential and mechanistic action of brewers' rice during colon carcinogenesis in male Sprague-Dawley rats.

Methods: Male Sprague–Dawley rats were randomly divided into five groups: (G1) normal; (G2) azoxymethane (AOM) alone; and (G3), (G4), and (G5), which were AOM fed with 10%, 20%, and 40% (w/w) of brewers' rice, respectively. Rats in group 2 to 5 were injected intraperitoneally with AOM (15 mg/kg body weight) once weekly for two weeks. Colon tumor incidence and multiplicity was assessed by H&E staining. The apoptosis-inducing activity was analyzed using a TUNEL assay. The expressions and protein levels of β-catenin, cyclin D1, and c-myc were evaluated by immunohistochemical staining and western blotting, respectively.

Result(s): Overall analyses revealed that brewers' rice reduced colon tumor incidence and multiplicity. The results from immunohistochemistry and western blotting analyses showed that brewers' rice decreased the expression of β-catenin, cyclin D1, and c-myc in a dose-dependent manner.

Conclusion(s): Our data suggested that brewers' rice can inhibit cell proliferation and induce apoptosis via the Wnt signaling pathway and holds great promise in the field of chemoprevention as a dietary agent.

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Keywords: beta-catenin, colorectal cancer, Wnt signaling

S17

Alleviation of cancer induced by AFB1 via probiotic intervention

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Purpose: Aflatoxin B1 (AFB1) plays an etiological role in the development of liver cancer, gastrointestinal pain, as well as affects the growth and development in livestock and humans being. In this study, *Lactobacillus casei* Shirota (Lcs) was employed as an aflatoxin-reducing microorganism in both *in vitro* and *in vivo*.

Methods: In this study, the AFB1 removal capacity was measured using ELISA. By using scanning electron microscope, the structural changes of AFB1-bound Lcs was evaluated. This study also investigated the effects of Lcs in the small intestine, colon, and liver of rats exposed to AFB1 using haematoxylin and eosin (H&E) stained histology.

Result(s): The AFB1 binding study indicated that the amount of AFB1 removed was concentration-dependent. The Langmuir isotherm, a theoretical model was applied to evaluate the binding efficiency of live cells, heat-treated cells, and cell wall. The results indicated that the interacting force was the strongest between the AFB1 and microorganism cell wall. After incubated with AFB1, scanning electron microscopic image obtained revealed that the lactobacillus cells became curved and their surface became rougher in comparison to control indicates strong binding occurred between the bacterial cell wall and AFB1. After 4 weeks treatment of AFB1, large carcinoma and lymphocytes accumulation can be observed in the small intestine and colon of AFB1 treated group based on histological analysis. AFB1 is commonly linked to liver cancer, however the H&E stained liver in this study did not showed any changes.

Conclusion(s): The cell wall is the binding site of AFB1 where the binding efficiency is the highest. The structure of lactobacillus was altered by AFB1 indicates the strong binding between AFB1 and the cell wall of Lcs. The results demonstrated the negative effects of AFB1 towards small intestine and colon, while such effects can be greatly reversed by probiotic treatment.

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Keywords: aflatoxin, lactobacillus, carcinogenic

S18

Evaluation of efficacy of the recombinant Newcastle disease virus (rAF-IL12) as therapeutic cancer vaccine in colon cancer-challenged BALB/c mice

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Purpose: Newcastle Disease Virus (NDV) has been known to have the oncolytic effect towards cancer cells but its high pathogenicity is still a major concern. However, the use of bioinformatics and genetic engineering have paved its way in manipulating the viral genome, thus addressing the aforementioned issue as well as increasing the specificity and efficacy of this virus strain. In this study, the effects of recombinant NDV that expresses interleukin 12 (rAF-IL12) together with parental NDV (AF2240-i) are determined towards the colon cancer-challenged BALB/c mice.

Methods: The mice were separated into 4 groups (PBS (negative control), AF2240-I, rAF, and normal) whereby the first 3 groups were injected with colon cancer cell, CT26. The treatment was given intra-tumorally according to their group once the tumor mass reaching a size of 0.5cm whereas in the normal group, no treatment was given. The treatment was given twice a week for 28 days (i.e: 8 times in total). The tumor burden was recorded once a week and at day-29 the mice were sacrificed for further analysis.

Result(s): Treatment with rAF-IL12 reduced the tumor burden of the mice compared in the other two treatment groups. Based on the immunophenotyping assay, the rAF-IL12 could increase/restore the level of CD4 and CD8 T-cell population to the level of the normal group. Level of cytokines such as IL-2, IL-12, IL-6, and IFN-y were also increased in the rAF-IL12 treatment group.

Conclusion(s): rAF-IL12 is effective in treating colon cancer and has the potential to be used as therapeutic cancer vaccine in the near future.

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Acknowledgement: This research work was supported by the Ministry of Science, Technology, and Innovation (MOSTI).

Keywords: colon cancer, Newcastle disease virus, rAF-IL-12, BALB/c mice

S19

Safety, stability and efficacy of recombinant Newcastle disease virus expressing human interleukin 12 (rAF-il12) as an anti-breast cancer vaccine

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Purpose: Newcastle Disease Virus (NDV) is an avian paramyxovirus, which has been demonstrated with significant oncolytic activity against cancer. However, the mechanism underlying its anti-tumor effect is largely unresolved. Today, genetically modified viruses coding for immunomodulatory agents, such as cytokines or chemokines, have come into focus. Such engineered viruses can promote an efficient anti-tumour immune response.

Methods: In this project, the safety and stability of a recombinant NDV (rAF) expressing human interleukin (IL) 12 in comparison to the parental NDV AF2240 will be studied. The safety of the newly developed rAF-IL12 will be tested *via* mean death time (MDT) and intracerebral pathogenicity index (ICPI). In addition, rAF-IL12 virus will be passaged in specific pathogen free (SPF) eggs up to passage 10 and will be tested *via* hemagglutination assay, MDT, ICPI, and Enzyme-Linked Immunosorbent Assay (ELISA) to ensure the stability of the virus throughout each passage. This study will also include *in vitro* cytotoxic studies on breast cancer MDA-MB-231, MCF-7 and 4T1 cell line to further prove the functionality of rAF-IL12 as an anti-cancer agent.

Result(s): From the proposed study, it is expected that the IL12 will be successfully expressed in the rAF construct and proven to be safe by only causing cytotoxic effects towards cancer cell lines while not affecting normal cell lines and remains stable up to passage 10.

Conclusion(s): IL12 will significantly enhance the anti-cancer immune response of the rAF in breast cancer cell lines compared to the parental virus, NDV AF2240 and serve as a therapeutic cancer vaccine in the near future.

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Keywords: Newcastle disease virus, anti-cancer, human IL-12.

S20

The implication of Aflatoxin B1 and Ochratoxin A exposure on tumor retated genes in normal and cancerous breast cells

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Purpose: Mycotoxin contamination of food commodities caused by fungal strains such as *Aspergillus* and *Penicillium* is common in countries with tropical weather. Mycotoxins have been reported to cause severe food poisoning, liver damage and carcinogenic to kidney and liver cells. This study aimed at evaluating the effect of mycotoxins on the expression of cancer-related genes which potentially could increase the risks of breast cancer.

Methods: Stock solutions of 100 ug/ml of Aflatoxin B1 and Ochratoxin A were prepared using Dimethyl sulfoxide (DMSO) as a solvent to treat Michigan Cancer Foundation-7 (MCF7) and Michigan Cancer Foundation-10A (MCF10A), human cancerous and normal breast cell lines respectively in a concentration ranges from 1-6 ug/ml. Cells were seeded at a density of 3 x 10^5 and once they reached 90% confluency, cells were treated with a designated concentration of mycotoxins and incubated for 48 hours prior to cytotoxicity assay. The optimal concentration of both toxins was used to treat both cells for 48 hours before the RNA was extracted to conduct gene expression analysis using real-time quantitative polymerase chain reaction (RT qPCR) for the selected 3 oncogenes, 3 tumor suppressing genes, 5 apoptosis genes and 4 cell cycle genes.

Results: Treatment of MCF7 with AB1 and OTA caused growth arrest with an upregulation of p35 and Cmyc expression as defense mechanism. Treatment of MCF10A with AB1 and OTA caused growth arrest which could make cells malignant and increase the risk of breast cancer by downregulating BRCA1 and BRCA2.

Conclusion(s): The exposure to both toxins caused changes in gene expression within the MCF7 and the MCF10A tumor-related genes.

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Keywords: Mycotoxins, Aflatoxin B1, Ochratoxin A, Breast cancer.

Conference: Abstracts for Poster Presentation

P1

Synergistic effects investigation of chamomile and lavender essential oils for antibacterial activity

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Purpose: Essential oils have been used for extensive applications of variety of wellness throughout documented history. With massive advancement in science, essential oils today have undergone numerous refining methods to give an improved effect.

Methods: Determination of the antibacterial activities alone and in combination of lavender and chamomile oils was carried out in this study. Three laboratory strains of cultured bacterial (*Pseudomonas aeruginosa,* ATCC 27858; *Staphylococcus aureus,* ATCC 6538 and *Escherichia coli* ATCC), were used for analysis. The stock cultures were maintained at −20ºC and subjected to sub-culturing onto Tryptone Soya agar and incubated at 37ºC for 24 hours. The fractional inhibitory concentration index (FIC) was used in determining the essential oils effect. The FIC was calculated by dividing the minimum inhibitory concentration value of the combined essential oils with the MIC value of each essential oil placed in the combination. The ΣFIC was calculated by adding these two values.

Result(s): Lavender oil showed the greatest antimicrobial effect, with the lowest MIC values of 2mg/mL for both *Pseudomonas aeruginosa* and *Escherichia coli* and 4mg/mL for *Staphylococcus aureus* pathogens compared to chamomile oil when used individually. The combination of these essential oils in various ratios indicated synergistic effect for all the nine ratios analysed.

Conclusion(s): The minimum inhibitory concentration analysis indicated that these oils have favorable antimicrobial interactions when in combination, that are 100% and 70.4% synergistic and additive effects for the oils selected and this will offer potential for the common use of combining oils in achieving a greater therapeutic result.

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Keywords: essential oils, antibacterial, inhibitory

P2

Neutralizing FGF4 protein in conditioned medium of IL21-silenced HCT116 cells restores the invasiveness of the colorectal cancer cells

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Purpose: The interleukin-21 (IL21) protein was found to be expressed at an elevated level in clinical samples of colorectal cancer patients with a parasitic infection that were collected from Sudan in our previous study. As such, our study aimed to correlate this protein with the cell proliferation and cell migration in HT29 and HCT116.

Methods: The IL21 gene in HT29 and HCT116 cells were first silenced by siRNA technology then it was correlated to cell proliferation and cell migration using LDH cytotoxicity and Wound healing assays, respectively. The mechanisms associated with gene and protein expressions were also performed using Real-time PCR and ELISA in the present study.

Result(s): Our results demonstrated that silencing the IL21 gene in HCT116 cells increased the cytotoxic level and fibroblast growth factor-4 (FGF4) mRNA expression in the cancer cells. Moreover, gene silencing reduced the aggressiveness of cancer cells compared to non-silenced cancer cells. These events were not found in IL21-silenced HT29 cells. Neutralizing FGF4 in conditioned medium of IL21-silenced HCT116 cells further increased the cytotoxic level and restored the aggressiveness of HCT116 cells in the culture compared to the silencing the IL21 gene alone.

Conclusion(s): Our results indicate the importance of both silencing the IL21 gene and co-expression of the FGF4 gene in HCT116 cells and pave the way for the discovery of important factors to be used as biomarkers for the design of drugs or cost-effective supplements to effectively treat the patients having infectious diseases and HCT116 cells of colorectal cancer simultaneously.

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Keywords: Infectious disease, colorectal cancer, IL21, FGF4, cell proliferation, cell migration

P3

Computational identification and experimental validation of 18 novel sRNAs in *Leptospira Interrogans* serovar Lai

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Purpose: To identify small non-coding RNA (sRNA) by integrated computational-based bacterial sRNA prediction pipeline and to validate their expression. The potential association of the sRNAs with physiological pathways of *Leptospira interrogans* serovar Lai were predicted *in silico*.

Methods: Genome sequences of 8 *Leptospira* spp. were retrieved for whole-genome sequence alignment to mark the conserved regions. nocoRNAc along with SIPHT and sRNAscanner were selected to identify potential intergenic-derived sRNAs. The common sRNAs signatures, such as structural conservation, thermodynamic stability, presence of upstream promoters and downstream rho-independent termination signals, were used as the basis for identification. Expressions of these sRNA candidates were then confirmed by reverse transcription-PCR (RT-PCR) and their potential targets predicted using CopraRNA software.

Result(s): A total of 31 sRNA candidates were detected in *Leptospira interrogans* serovar Lai, among which the expressions of 18 candidates have been validated by RT-PCR. Four of the validated sRNAs were conserved among Leptospira *spp*. Two conserved sRNAs were predicted to be involved in heavy metal metabolism and haemolysin production.

Conclusion(s): Computational-based bacterial sRNA prediction pipeline has successfully predicted and experimentally validated 18 sRNA novel candidates from pathogenic *Leptospira interrogans* serovar Lai. Their potential roles in regulatory network of *Leptospira spp*. will be further evaluated. The computational-based pipeline has proven to be an alternative and cost-effective approach compared to labor intensive conventional experimental sRNA discovery method.

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Keywords: sRNA, nocoRNAc, SIPHT, sRNAscanner, *Leptospira Interrogans* serovar Lai

P4

Presence of *bla*$_{TEM}$ and *bla*$_{OXA-10}$ in clinical isolates of *Alcaligenes faecalis* from a hospital in Kuala Lumpur

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Purpose: Production of extended-spectrum β-lactamase (ESBL) in drug-resistant organisms represents a challenge to our current antimicrobial armamentarium. ESBL-producing gram-negative bacteria have been described particularly in *Enterobacteriaceae* and non-fermenting gram-negative bacteria, including *A. faecalis* which is the only *Alcaligenes* species of clinical importance. It is commonly found in soil, water and hospital environments and is an opportunistic pathogen causing infection in patients with severe underlying disease. In this study, we investigated the presence and diversity of ESBL producing *A. faecalis*.

Methods: Seven archived clinical isolates of *Alcaligenes*-like species obtained from patients with systemic lupus erythematosus (1), diabetic ulcer (1) and urinary tract infections (5) at the tertiary University Hospital, University of Malaya, Kuala Lumpur. They were identified with DNA amplification, followed by sequencing of the 16S rRNA gene. All isolates were screened for genes encoding for β-lactamases TEM, OXA, SHV, CTM-X, PER-1, VIM and GES by PCR, using primers as described in published studies.

Result(s): A phylogenetic tree constructed with partial 16S rRNA gene sequences demonstrated that all isolates were grouped into a cluster with the reference *A. faecalis*. PCR screening showed evidence of the presence of *bla*$_{TEM}$ (7/7) and *bla*$_{OXA-10}$ (4/7) genes. Their identity was confirmed by subsequent sequencing of these determinants. A combination of both genes was present in 4 isolates.

Conclusion(s): All 7 *A. faecalis* isolates were found to have at least one ESBL gene and this probably contributed to their drug resistance.

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Keywords: *Alcaligenes faecalis,* Antibiotic susceptibility, Extended-spectrum beta-lactamase genes

P5

In silico sequence analysis, homology modeling and function annotation of putative subtilisin-like serine protease from *Cryptosporidium parvum*

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Purpose: This study predicted three-dimensional (3D) protein structure of *Cryptosporidium parvum* subtilisin-like serine protease (*Cp*SUB) using homology modelling as the approach of 3D structure prediction.

Methods: *Cp*SUB is an extracellular protein comprising of 1324 amino acid residues. Its amino acid sequence was computed to determine the physiochemical properties. 3D protein structure was developed by using MODELLER v9.18 software before subjected to model refinement and assessment. The refined model was then computed for functional annotation. Quality assessment analysis of the protein model for *Cp*SUB through its PROCHECK Ramachandran Plot and other reliable protein structure assessment tools indicated that the selected protein model is a reliable model.

Result: The result from functional annotation revealed that it is sorted to cd07473 superfamily or peptidase S8 family domain in subtilisin-like proteins. To our best knowledge, this protein model of *Cp*SUB was first proposed for the modeling of 3D structure despite not having a crystallized protein structure deposited yet in Protein Data Bank (PDB).

Conclusion: Thus, this study may provide understanding to the molecular and structural function of *Cp*SUB. This predicted 3D model may be further studied for wet lab characterization studies and may be used for a prospective therapeutic anti-cryptosporidial agent.

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Keywords: *Cryptosporidium parvum*, homology modeling, MODELLER, PROCHECK, serine protease.

P6

The effect of TLR2 agonist (Pam3csk4) on the phagocytic activity of macrophage in mice immunized with the rBCG expressing the MSP-1C of *Plasmodium falciparum*

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Purpose: Malaria is one of the major public health concerns due to the high morbidity and mortality associated with the disease. BCG is the only vaccine currently available for the prevention of TB. Using recombinant DNA technology, researchers have re-engineered BCG as a vector for developing recombinant vaccines for other diseases, including malaria. Innate immunity plays pivotal role in the early control of malaria infection because it impedes parasite replication and prevent the progression of severe and fatal disease. Macrophages are a major type of phagocytic cell involved in innate immune protection against malaria. The recognition of BCG by macrophage is mediated by the asset of pattern recognition receptors including Toll-like receptors such as TLR2. The study aimed to determine the effect of TLR2 agonist (pam3csk4) on the phagocytic activity of macrophage in mice immunized with the rBCG expressing the MSP-1C of *Plasmodium falciparum.*

Methods: Balb/c mice (n=6) with BCG and rBCG either in the presence or absence of 10 µg/ml TLR2 agonist. After 72 hours of immunization, the mice were sacrificed and peritoneal macrophage were harvested. The phagocytic activity of the macrophages was determined using phagocytic assay.

Result(s): In the presence of TLR-2 agonist, the phagocytic activity of macrophage immunized with rBCG and BCG were shown to be higher than those immunized with only rBCG and BCG. However, in all cases, the phagocytic activity of rBCG was significantly higher compared to those immunized with parent BCG.

Conclusion(s): This data indicates TLR-2 is important for the interaction of BCG and rBCG with the immune system. Therefore TLR-2 might have implications for the development of vaccine.

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Keywords: malaria, TLR-2, rBCG, Macrophage, Pam3sck4

P7

Small regulatory RNA profiling of *Mycobacterium tuberculosis* in response to stress conditions

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Purpose: This study aimed to identify novel *Mycobacterium tuberculosis* (MTB) small regulatory RNAs (sRNAs) and characterize their functional roles in responses to stress.

Methods: MTB was cultured to mid-log (ML) phase and exposed to various stress conditions (iron, antibiotics, starvation and surfactant) that mimic the lung and intracellular conditions for total RNA extraction. sRNA-enriched (<120-nt) cDNA libraries were subsequently constructed from total RNA for RNA-seq. The resulting RNA-seq data were subjected to bioinformatic analyses to identify novel sRNA transcripts. The expression level of each transcript was computed in count-per-million (CPM), from which respective fold-changes relative to ML during stress was calculated to identify upregulated transcripts. Besides, their potential regulatory functions were inferred via gene ontology (GO) terms of respective mRNA targets.

Result(s): A total of 1254 novel transcripts, including 920 antisense, 166 UTR-derived and 168 intergenic transcripts were uncovered. Fold-change analyses detected 478, 286, 412, 394 and 113 upregulated transcripts during iron, isoniazid, kanamycin, starvation and surfactant treatments, respectively. Some of these candidates could regulate responses to oxidative stress, antibiotics, starvation and surface stress (GO term searches), among which, some candidates were found to target anti-sigma factors for sigD, sigH and sigL that regulate responses to starvation, oxidative stress and virulence. Besides, we also detected a transcript antisense to PhoP that regulates responses to oxidative stress and metal uptake.

Conclusion(s): This study has identified abundant sRNA candidates that potentially regulate MTB responses to various environmental stress. Further validation and characterization to construct their regulatory networks in MTB are still on-going.

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Keywords: Small Regulatory RNA, Mycobacterium tuberculosis, RNA-seq, stress responses

P8

Comparison between microscopy and PCR detection of *Giardia lamblia* among school-age children in Khartoum, Sudan

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Purpose: *Giardia lamblia* (*G. lamblia*) is one of the most prevalent human intestinal parasites worldwide. It is considered among the major public health problems in many developing countries, including Sudan. The routine laboratory investigation of *G. lamblia* diagnosis is mainly based on microscopy; however, they typically exhibit low sensitivity. Therefore, this study was aimed to determine the prevalence and the diagnostic efficacy of microscopy and PCR for the detection of *G. lamblia*

Methods: A total of 200 fresh fecal samples were collected form children in Al Kalakla area in the south of Khartoum, Sudan and subjected to microscopic examination. In addition, 25 positive and 25 negative samples by microscope were further tested by PCR.

Result(s): The result showed that out of 200 stool samples 25 were positive with prevalence rate of 12.5% based on microscopic examination. PCR identified the entire microscope positive 100% (25/25), however, 10 out of 25 microscope negative samples were showed positive result by PCR.

Conclusion(s): This study shows higher prevalence of *G. lamblia* among the study participants. As well as higher sensitivity of PCR over microscopy for *G. lamblia* identification

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Keywords: *G. lamblia*, Sudan, polymerase chain reaction

P9

The effect of Dental Unit Waterline(DUWL) flushing on the quality of water in dental training center in Malaysia

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Purpose: This study aimed to assess the efficiency of flushing method of Dental Unit Waterline (DUWL) system in reducing the number of microorganisms.

Methods: Water samples were taken before and after four minutes flushing from air-water syringes system in ten randomly selected dental units in a dental training center. These samples were immediately transferred to the microbiology laboratory in the cool box within 8 hours for the heterotrophic plate count (HPC) test. Data was analyzed using SPSS 22. Paired t-test was used to analyze the number of microbe before and after flushing.

Result(s): The number of colony forming unit (cfu) ranged from 13,000 to 120,000 in unflushed samples and 3,000 to 15,000 in flushed samples. The mean HPC post-flushing is lower than pre-flushing [8360.00(4561.48) vs 63300.00 (44587.12) CFU/ml]. The mean HPC between pre and post flushing is significantly different (P=0.004, 95%CI 22039.52, 87840.48). We are 95% confident that the mean difference of HPC between pre and post flushing will be between 22039.52 and 87840.48 respectively. The coliform count from the control was 140cfu/ml.

Conclusion(s): Flushing method of DUWL system significantly reduced the number of microorganism in the dental unit. However, the level of microorganisms still did not meet the standard guideline by Environmental Protection Agency which is safe drinking water at <500CFU/ml. Presumably, the duration of flushing should be increased and additional chemical treatment of the dental units should be implemented to ensure the safety of patients and dental personnel.

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Keywords: Dental Unit Waterline, microorganism, flushing

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P10

Investigation on the effect of antifungal active alkaloids of *Ruta angustifolia* (L.) Pers. on gene expression in *Candida albicans* using real time PCR

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Purpose: *The pathogenic fungus Candida albicans* produce infections that range from mucosal surface to bloodstream especially in immunosuppressed patient. Due to over-intake of current antifungal drugs, there were increased in drugs resistance, presence of undesirable side effects and re-emergence. Study of antifungal active alkaloids derived from *Ruta angustifolia* (L.) Pers. reinforces the need of finding more efficient and less toxic drugs.

Methods: The extracts of dichloromethane and acetone of *R. angustifolia* were screened for antifungal active alkaloids based on Agar Overlay Assay. The localized active compounds were further isolated by using column chromatography. The isolated compounds were prepared from 0.25 to 500 µg/ml concentrations and tested for its Minimum Inhibitory Concentration (MIC) and minimum fungicidal activity (MFC) before they were analyzed by HPLC and NMR. The effect of the isolated alkaloids on genes level expression of ICL1, PCK1, CDR1, and ERG11 were evaluated by using RT qPCR. Culture of *C. albicans* were treated according to the determined MIC of the alkaloids for 8 hours at 37°C. Then, the RNA of the treated *Candida* was extracted before synthesized into cDNA and investigated with real time-PCR.

Result(s): Antifungal active alkaloids were purified named as arborinine and graveoline. The MIC for each of the compound were 500 and 250 µg/ml respectively. Gene expression level in RT qPCR reaction showed that all compounds were able to down regulates all selected genes.

Conclusion(s): The alkaloids from *Ruta angustifolia* (L.) Pers is effective in reduction of important gene expression which in turn plays a basic role in resistance against the current antifungal agents.

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Keywords: Alkaloids, *Ruta angustifolia*, antifungal.

P11

Identification of nontuberculous mycobacteria: public health and diagnostic implications

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Purpose: Nontuberculous mycobacteria (NTM) have been reported to be continuously increasing and becoming one of the major public health concern. Nevertheless, a proper epidemiological investigation of this notion has been lacking. Correct and timely identification of NTM will affect both treatment and its epidemiology. The study aimed at determining the diversity of NTM in new established clinical training centre in Malaysia.

Methods: A retrospective study was conducted from January 2014 to September 2017. Five hundred and eighty specimens were processed by the Microbiology laboratory for all forms of TB cases and TB-like disease. Identification of all positive isolates was performed by using GenoType Mycobacterium CM kits.

Result(s): Twenty-three of NTM isolates were identified mainly from sputum (18) followed by bronchioalveolar-lavage (2), pus (1), tracheal aspirate (1) and tissue (1). Nineteen NTM isolates were identified using GenoType Mycobacterium CM with four isolates could not be identified to subspecies level. These four NTM isolates were classified as Mycobacterium species only. Identified NTM isolates include M. abscessus (9), M.genavense (5), M. fortuitum (3), M. interjectum (1), and M. intracellulare (1).

Conclusion(s): The species distribution provides resourceful preliminary data for NTM epidemiological evaluation. GenoType Mycobacterium CM represents an appropriate diagnostic identification tool for NTM isolates. However, 16S rRNA gene sequencing should be validated as confirmatory tool. Hence, NTM species identification and distribution are important, as it contributes to the choice of antibiotic due to treatment regimen and response rates differ according to NTM species which makes the management even more challenging.

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Keywords: Nontuberculous mycobacteria, identification, diagnostic

P12

Multidrug resistant tuberculosis: application of preliminary data for surveillance system

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Purpose: Designing treatment and preventive strategies for multidrug resistant tuberculosis (MDR-TB) and forecasting drug demand are challenging without proper data. Yet, available initial evidences could guide the clinician in the selection of appropriate anti-tuberculous regime in MDR-TB cases while waiting for better data. This study was conducted with the aim to determine the rate of MDR-TB in new established clinical training centre in Malaysia.

Methods: A retrospective study was conducted from January 2014 to September 2017. Five hundred and eighty specimens were received and processed by the Microbiology laboratory for all forms of suspected TB cases. The results of identification and susceptibility testing were gathered accordingly.

Result(s): There were sixty-nine of Mycobacterium tuberculosis complex (MTBC) isolated mainly from sputum (31) followed by bronchioalveolar-lavage (24), pleural fluid (5), tracheal aspirate (3), tissue (3), pus (2), and lymph node (1). Of sixty-nine MTBC isolates, sixty-one were susceptible to all tested drugs. There were seven isolates exhibited monoresistant against rifampicin (3), isoniazid (3) and streptomycin (1). One (1.45%) MTBC isolate had MDR-TB detected.

Conclusion(s): The rate of MDR-TB in our setting remains under control and it did serve as preliminary data for further surveillance assessment that includes development of local centralized TB database, transmission assessment, and evaluation of TB control programs. It also provides resourceful information in understanding our local TB epidemiology, monitoring high-risk populations and indirectly contributes to strengthen the national TB policy.

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Keywords: Multidrug resistant, monoresistant, tuberculosis

P13

Five-year review of sputum for Acid-Fast Bacilli (AFB) smear and Mycobacterial culture in Centre of Pathology Diagnostic and Research Laboratories (CPDRL), Universiti Teknologi MARA, Sungai Buloh, Selangor

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Purpose: Acid-fast staining and mycobacterial culture on sputum remain at the core of any diagnostic algorithm of pulmonary tuberculosis.

Methods: This study was conducted to evaluate the detection rate of sputum AFB smear and culture in patients with suspected pulmonary tuberculosis. Retrospective study was carried out. The data of sputum acid fast bacilli (AFB) smears and the details of the *Mycobacterium tuberculosis* culture sensitivity pattern obtained from the Microbiology Laboratory of Faculty of Medicine; UiTM Sungai Buloh from 2012 to July 2017 were revised.

Result(s): About 13.3% patients were AFB smear positive, while the remaining 86.7% patients were AFB smear negative. Among those with AFB smear positive, 90.9% of them were found to be culture positive and only 9.1% patients were culture negative. Those with negative AFB smears, 11.9% of them were found to be culture positive and 88.1% were culture negative. *Mycobacterium tuberculosis* complex was isolated in 67.7% of the positive cultures.

Conclusion(s): Mycobacterial culture reveals higher detection rate compared to microscopy method in our setting.

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Keywords: AFB smear, mycobacterial culture, pulmonary tuberculosis

P14

Systematic optimization of triclosan loaded nanoparticles formulation by Design Expert® for improved local delivery in the treatment of periodontal infection

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Purpose: Triclosan (TCS) is a broad spectrum antimicrobial agent that has been used in the treatment of periodontal infection. The drug has poor aqueous solubility and therefore may prompt permeability problem when applied to oral cavity. The aim of this study was to develop and systematically optimize triclosan loaded nanoparticles (TCS-loaded NPs) formulation for the treatment of periodontal infection.

Methods: Solvent displacement method was used to prepare the nanoparticles (NPs). Resolution IV design and Box–Behnken design of Design-Expert® software, version 10 was used for the pre-optimization screening and complete optimization of TCS-loaded NPs, respectively. Effect of factors that were investigated are drug-polymer ratio, surfactant concentration and stirring speed. Particle size, zeta potential, polydispersity index (PDI) and entrapment efficiency (EE) were the critical quality attributes selected for the study.

Result(s): Desirability function determined by the software for optimized TCS-loaded NPs was 0.771. The observed particle size, PDI, zeta potential and EE of optimized TCS-loaded NPs were found to be 165.8 ± 5.2 nm, 0.013 ± 0.003, -20.6 ± -8 mV and 85.9 ± 4%, respectively. It was found that particle size increases with increase in polymer level and decreases with increase in surfactant concentration and stirring speed. Zeta potential were found to increase with a decrease in surfactant concentration and increase in polymer level. Both surfactant concentration and drug to polymer ratio were found to affect PDI negatively and EE positively.

Conclusion(s): The use of Design-Expert® helped in identifying the critical quality parameters for preparing improved TCS-loaded NPs formulation for delivery of TCS into the periodontal pocket.

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Keywords: Design-Expert®, Resolution IV, Box–Behnken, periodontal infection, triclosan

P15

Drug resistant profile among foreign-born tuberculosis patients in Kuala Lumpur Malaysia

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Purpose: Recent statistic had shown that foreign-born patients make up thirteen percent of all tuberculosis (TB) patients in Malaysia. Not only they added into the incidence rate of TB yearly but also many believed that this group of patients had a significant contribution into the transmission of drug-resistant tuberculosis in the community. However, little is known about the demographic profile of foreign-born drug resistant patients in Malaysia and this has become the aims of this current study.

Methods: This study adopts a retrospective cohort study design and involved laboratory confirmed drug-resistant tuberculosis patients registered in Institute of Respiratory Medicine from 1st January 2009 to 30th June 2013. Data were analysed by using SPSS version 22.0 for Windows.

Result(s): A total of 12799 tuberculosis patients with positive culture of *Mycobacterium tuberculosis* were screened for drug and sensitivity testing in this period. 526 (4.1%) patients were resistant to at least one first-line anti tuberculosis drugs with 38.2% (n=201) of them were foreigners. Out of 201 patients analysed, 67.2% (n=135) of them were mono-resistant, 10.9% polyresistant and 21.9% (n=44) were multi-drug resistant tuberculosis patients. Their mean age was 34.52±11.32 years old. Majority of them were from Myammar (57.7%, n=116), primary TB cases (83.6%, n=168), male (78.1%, n=157), unemployed (36.8%, n=74), and HIV negative (98.0%, n=197).

Conclusion(s): Finding of this study may benefits the clinical expertise in the screening of tuberculosis among foreign-born patients in Malaysia. More emphasis should be given to patients with these characteristics in order to combat the transmission of drug-resistant TB in the community.

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Keywords: Tuberculosis; drug-resistant; Malaysia

P16

Production and purification of RNA-dependent RNA polymerase (NS7) of murine norovirus-1 (MNV-1)

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Purpose: Norovirus is one of the main causes for gastroenteritis worldwide. MNV-1 served as best model to study on norovirus replication. Viral polymerases are typically targeted for antiviral drug development. The main purpose of this study was to produce and purify recombinant wild type (WT) and active site mutant (ΔGDD) NS7 proteins.

Methods: A WT and ΔGDD NS7 encoding genes were cloned into pET26b-Ub expression plasmid. The resulting plasmids (pET26b-Ub-WT NS7 and pET26b-Ub-ΔGDD NS7) were then transformed into BL21 (DE3) cells containing pCG1 plasmid expressing ubiquitin-specific carboxyl-terminal protease. Positive clones were induced using IPTG and NS7 expression was further confirmed through western blot. Expression of WT and ΔGDD NS7 were evaluated based on three variables: cell growths at Abs_{600} between 0.5 (early exponential phase) and 1.0 (mid exponential phase) for IPTG induction, IPTG concentration (0.1mM to 1mM) and temperature of induction (16˚C, 25˚C and 37˚C). Finally, recombinant WT and ΔGDD NS7 were produced in large scale and subjected for purification using immobilized metal affinity chromatography.

Result(s): Cloning of pET26b-Ub-WT and pET26b-Ub-ΔGDD NS7 were successfully achieved and their proteins expression were confirmed through western blot. The intensity of SDS-PAGE bands differ for Abs_{ind} and temperature but no remarkable differences observed for IPTG concentration tested. Overexpression of both WT and ΔGDD NS7 proteins (57kDa) observed in SDS-PAGE for purified protein(s).

Conclusion(s): Genes for recombinant protein of MNV-1 WT and ΔGDD NS7 were successfully cloned, expressed and purified. Optimized conditions for expression of proteins were also determined for large scale production.

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P17

Preliminary toxicity evaluation of Tualang honey in Vero cells for future anti-chikungunya study

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Purpose: Chikungunya, is an acute febrile illness that has been identified worldwide, and is associated with severe debilitating arthralgia and fever, with no treatment or licensed vaccine exists. Tualang honey is the Malaysian multifloral jungle honey. This honey has been use traditionally to cure number of disease and known to have anti-viral effects. In this study, we examined the toxicity of Tualang honey extracts to find a maximum non-toxic dose (MNTD) in African green monkey, Vero cell.

Methods: A total 5g of fresh honey was weighed and diluted in Dulbecco's Modified Eagle Media (DMEM). The honey extract was filtered using 0.22 μm syringe filter. Several concentrations of honey were diluted and start from, 60 mg/ml, 30mg/ml, 15mg/ml, 10mg/ml, 7mg/ml, 6mg/ml, and 5mg/ml was added to confluent Vero cell line in 96 well cell culture plates and incubated for 48 hours. Following this, the MNTD was measured utilizing the XTT cell viability assay (BIOTIUM).

Result(s): The Tualang honey extract were assessed for their toxicity on Vero cell prior to determination of their inhibitory impacts antiviral against chikungunya virus. Cell toxicity was determined by the cell morphology and confirmed by the XTT cell viability assay (BIOTIUM). The honey extract was not toxic to Vero cells at concentration between 20mg/ml to 5 mg/ml.

Conclusion(s): Our finding recommends that honey extract has low toxicity in the Vero cells The MNTD of the Tualang honey extract was 20mg/mL and this concentration will then be utilized in our future antiviral assay.

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Keywords: chikungunya virus, honey, cytotoxicity evaluation.

P18

Production of infectious recombinant murine norovirus 3 (MNV 3) particles for atherogenesis study

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Purpose: Murine norovirus 3 (MNV 3) establishes persistent infection in wild-type mice yet causes gastroenteritis symptoms in immunocompromised mice making it an attractive strain for norovirus study in atherogenesis. Here we employed an established reverse genetics system to generate recombinant MNV 3 for subsequent atherogenesis study *in vitro*.

Methods: MNV 3 recovery was carried out by cDNA based transfection via cationic lipid mediated method. MNV 3 cDNA was transfected into engineered baby hamster kidney cells (BSR-T7) cells that were pre-infected with fowlpox virus expressing T7 RNA polymerase (FPV-T7). Virus production was quantitatively measured by 50% tissue culture infective dose (TCID50) in mice microglial cells (BV 2). Single-step virus growth curve was conducted by infecting mice macrophage (RAW 264.7) cells. Detection of virus infection and virus verification was carried out by immunoblotting and polymerase chain reaction (PCR) targeting viral genome.

Result(s): Virus yield obtained after recovery was $1.54x10^3$ TCID50/ml. Single-step virus growth curve showed that the virus reached peak titer of approximately 10^6 TCID50/ml at 24 hours post infection. Viral genome and protein were successfully detected across the timepoints.

Conclusion(s): MNV 3 recovered entirely from cDNA via reverse genetics system showed efficient infectivity in RAW 264.7 and BV 2 cell lines. The generated MNV 3 is currently being used for atherogenesis study.

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Keywords: murine norovirus 3, reverse genetics, transfection