

Taylor's University Graduate Research Symposium 2021

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ABSTRACT

Graduate Research Symposium 2021 (GRS2021) was held virtually via Zoom on the 30th of November 2021. The theme of the symposium was Scientific Research Today: Borderless, Collaborative and Innovative. The symposium aimed to highlight the various types of scientific research being conducted by postgraduates from different international and local institutions to expand participants' field of knowledge as well as encourage networking. GRS2021 covers a wide range of topics, which include natural products, drug discovery and formulation, life sciences, pharmaceutical sciences, medical sciences, food sciences and technology. This special issue exhibits the latest research findings by postgraduates from various scientific background. The emphasis is on studies that will contribute to the current knowledge and understanding in the targeted field of study.

Keywords: *Postgraduates; symposium; biological; medical and pharmaceutical*

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ABSTRACTS

All presented abstracts are listed from Page 4 to Page 76.

Phytochemical compounds and optimisation of extraction method on red *Christia vespertilionis* leaves using GC-MS

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Christia vespertilionis (L. f.) Bakh. f. is well known for treating various contagious diseases. This plant has been recognised among researchers and locals to have anti-inflammatory properties and has thus become popular for treating cancer. Two types of *C. vespertilionis* are acknowledged as green and red type. The green *C. vespertilionis* has been widely studied by many researchers, however just a few have studied the red type. This study was carried out to analyse the phytochemical compounds and optimise the extraction method of red *C. vespertilionis* leaves in different extraction techniques (maceration and Soxhlet extraction) and solvents (methanol and ethanol) using the GC-MS analysis. The components were identified via comparisons as guided by the National Institute of Standards and Technology (NIST). According to four (4) samples of red *C. vespertilionis* leaves using maceration of methanol (RMM), maceration of ethanol (RME), Soxhlet of methanol (RSM) and Soxhlet of ethanol (RSE), seventy-one (71) phytochemical compounds were identified. Eleven (11) major phytochemical compounds (> 4 % of peak area) identified are: acetic acid, butyl ester; 1-Butanol, 3-methyl-, acetate; Heptanoic acid, propyl ester; Hexanoic acid, 3-oxo-, ethyl ester; Phenol, 3,5-bis(1,1-dimethylethyl)-; 1-Octadecene; 4-O- Methylmannose; .alpha.-d-Mannofuranoside, methyl; 2-Undecene, 9-methyl-, (E)-; n-Hexadecanoic acid and 1-Octadecanol. Only seven (7) out of the eleven (11) compounds were reported to have biological activities. Among those samples, RSM was the most effective using correlation coefficient between peak area (%) and real-time (min) with a significant difference at $P < 0.05$.

Keywords: *Christia vespertilionis*; GC-MS and phytochemical compounds

A modified total RNA extraction protocol for secondary metabolite-rich ginger species (Zingiberaceae)

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Measuring the transcripts of messenger RNA provides a better phylogenomic framework for examining the evolution of related plant species besides enabling a deeper understanding on the gene expression in certain cell or tissue type. However, the nature of different plants, some being rich in polysaccharides and polyphenols often hinders the chances of getting high quality and pure RNAs. These are necessary for transcriptomic studies using next generation sequencing technologies (NGS). *Amomum* genus, a secondary-metabolite rich ginger species of Zingiberaceae family is known for its phytochemical properties but limited RNA related work done. Plant RNA extraction protocols developed in the past, and efficiency have been reported to differ depending on the species and plant parts utilized. Therefore, this study aimed to determine the most appropriate RNA extraction protocols for the secondary metabolite rich *Amomum* species using different tissue or parts of the plant. Fresh leaves of four different species (*K. galanga*, *K. parviflora*, *A. testaceum*, and *A. uliginosum*) tested with three different RNA extraction protocol showed modified Machery-Nagel Nucleospin® RNA plant extraction kit and CTAB-LiCl showed better results compared to SDS-LiCl. However, the modified kit gave lower purity based on A260/280 and A260/230 ratios and low concentration for stems, roots, and rhizomes of *Amomum*, less than 50 ng/μl. Further modification to the RNA extraction kit was required to yield good purity and higher concentration to suffice transcriptome work. The findings of this study emphasize the importance of polyvinylpyrrolidone (PVP) and beta-mercaptoethanol (β-ME) in the extraction process, particularly in Zingiberaceae species and plant parts with higher concentration of secondary metabolites such roots and rhizomes of *Amomum*. The time-efficient modified commercial kit technique developed as a result of this study provides pure, high-quality RNA that can be used in transcriptome analysis of *Amomum* and related species.

Keywords: *Amomum*; RNA extraction; CTAB-LiCl, secondary metabolite and tissue type

Nuclear DNA content in Sabah snake grass *Clinacanthus nutans* from different localities and genome stability using optimized flow cytometer method

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Clinacanthus nutans, an economically valuable medicinal plant has been in the limelight lately. The medicinal values such as anti-cancer, anti-bacteria, and anti-viral is much supported by its high content of phytochemical compounds such as vitexin, isovitexin, stigmasterol and lupeol found in *C. nutans*. However, the genome size estimation had not been reported for *C. nutans*, an information that enables better understanding of evolutionary and taxonomical details. This paper reports the estimated genome size of *C. nutans* and compared the genome size from different geographical localities using optimized nuclei isolation protocol. In this study, flow cytometer was used following optimization using three different types of nuclei isolation buffers. Of the three, Tris.MgCl₂, Otto and LBO1, Tris.MgCl₂ plus 1% PVP along with propidium iodide as the staining fluorochrome was found to be the most appropriate nuclei isolation buffer with higher yield and lower CV values. The genome size of *C. nutans* estimated using *Glycine max* cv. Polanka as internal standard revealed 2C DNA of *C. nutans* as 1.75 + 0.006 pg. Plants obtained from six different localities in Peninsular Malaysia. for genome stability showed no significant differences in genome size detected among the *C. nutans* based on locality. Hence indicating, the stability in genome size of materials from different location. The finding of this study highlights the importance of isolation buffer and reports the stable genome size of *C. nutans* for future reference and evolutionary studies.

Key words: *Clinacanthus nutans*; genome size and flow cytometer

The association of leptin receptor Q223R polymorphism with Type 2 diabetes in a Malaysian population

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Type 2 diabetes mellitus is a major global public health problem that leads to increased risk of morbidity and mortality due to its complications. Leptin receptor plays a crucial role in regulating of glucose metabolism and insulin sensitivity. Thus, mutation in the leptin receptor gene might play a role in the pathogenesis of type 2 diabetes. Our study aimed to evaluate the association of Q223R polymorphism in the *LEPR* gene with type 2 diabetes patients among Malaysian population. A case control study was focused on the three major ethnic groups of Malaysian population: Malay, Chinese, and Indian races. The genotyping analysis of *LEPR* Q223R polymorphism was carried out by polymerase chain reaction- restriction fragment length polymorphism in 150 T2DM patients (50 Malays, 50 Chinese, and 50 Indians) and 150 nondiabetic subjects as controls. Serum insulin level was determined using ELISA. The frequency of GG genotype of *LEPR* Q223R variant was significantly lower in T2DM patients as compared to the control group (34% vs. 50%, $\chi^2 = 7.88$, $p = 0.005$). Furthermore, the A allele frequency was significantly higher in patients with T2DM than those with non-diabetes individuals (36.66% and 29%, respectively). In addition, there were markedly elevated serum insulin level, HOMA-IR, and BMI in diabetic patients with GG genotype of this variant as compared to AA, and GA genotypes ($P = 0.004$, $P = 0.036$, and $P = 0.032$, respectively). Our findings demonstrated that a significant association between *LEPR* Q223R polymorphism and type 2 diabetes mellitus among Malaysian subjects. Moreover, the A allele frequency of Q223R variant significantly increases the risk of T2DM in our population. Likewise, the polymorphism of Q223R in the *LEPR* gene is associated with markedly increased serum insulin level, HOMA-IR, and elevated BMI in type 2 diabetes patients.

Keywords: *Leptin receptor; Q223R polymorphism; insulin; type 2 diabetes mellitus and Malaysian population*

Long-term high temperature stress on polar *Chlorella* strains

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The rise in global temperature has been a topic of major concern for the past decades. IPCC reported that global surface temperature between 2011-2020 was 0.99°C higher than the year 1900. Recent years, the annual average sea ice area in the polar regions reached its lowest level and this is particularly concerning to the polar aquatic organisms including microalgae. Therefore, the aim of this study was to investigate the physiological responses content of two polar *Chlorella*, namely *Chlorella vulgaris* UMACC 250 and *Chlorella vulgaris* UMACC 234 exposed to elevating temperatures for 120 days under future warming scenarios. The cultures were exposed to two different temperatures of 8°C (predicted future warming condition) and 12°C (extreme warming condition). The growth and protein analysis were determined every 40 days until the period of 120 days. The cultures were harvested and analysed for growth and protein every 40 days. Both strains response positively when exposed to higher temperature (12°C) for 120 days with slower growth observed at 8°C. Interestingly, highest OD750 and chlorophyll-*a* were obtained when the cultures were grown at 12°C. Highest protein content was obtained at 8°C after exposure for 80 days for both strains of *Chlorella* indicating a higher amount of protein was required for both strains to adapt to the prolonged increased of temperature. Of the two *Chlorella* strains, *Chlorella* UMACC 250 showed increased protein content at 12°C while UMACC 234 showed the opposite trend. The results indicated that both polar *Chlorella* strains were able to survive under projected elevated stress in the future.

Keywords: Acclimation; *Chlorella*; microalgae; polar and temperature

The anti-proliferative role of 15,16-dihydrotanshinone I (DHTS) extracted from *Salvia miltiorrhiza* in autosomal dominant polycystic kidney disease (ADPKD)

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Autosomal dominant polycystic kidney disease (ADPKD) is a kidney disorder caused by the mutation in the polycystin proteins that drives the cystogenesis in renal parenchyma. It affects 1:500 to 1:1000 livebirths globally and one of the major contributors to chronic kidney disease. Current treatments are only symptomatic and the only FDA approved drug treatment for ADPKD, Tolvaptan, a vasopressin-2 receptor (V2R) antagonist, demonstrated serious side effects. The dihydrotanshinone I (DHTS) extracted from *Salvia miltiorrhiza* has been proven to inhibit proliferation in cancers previously. Given that ADPKD also involves cell proliferation, this study repurposed DHTS for ADPKD treatment. The cell viability of ADPKD cells (WT 9-12), and normal kidney cells (HK2) treated with various DHTS concentration were quantified using sulforhodamine B (SRB) cytotoxic assay at 24-, 48-, and 72 hours post treatment. The DHTS concentration(s) that significantly reduced the WT 9-12 cell viability with minimal inhibitory effect on HK2 cells will be further studied with real time cell analyser (RTCA). 24 μ M DHTS concentration was able to significantly reduce the WT 9-12 cell viability with minimal effects on HK2 cells. (RTCA results will be obtained before conference). As DHTS was previously proven to exert anti-proliferative activity on cancer cells by inducing cell cycle arrest to inhibit the proliferation of cancer cells, further analysis with flow cytometry must be performed to study the cell cycle analysis of the DHTS-treated ADPKD cells. DHTS showed promising cytotoxic effect in WT 9-12 cells. Although the side effects of DHTS is yet to be determined, this natural compound can be gentler alternative than tolvaptan for ADPKD treatment.

Keywords: DHTS; cytotoxic; proliferation; viability and autosomal dominant polycystic kidney disease

What's #trending? Bibliometric analysis of entomopathogenic fungi (EF) research over the past two decades

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Historically, studies on entomopathogenic fungi (EF) at the end of the 19th century focused on their taxonomy and its biocontrol applications against agricultural pests. After a hiatus period into the 1960s, the reawakening of interests to study EF has been driven by advances in molecular biology allowing resolution of species nomenclature and a heightened need in bio-prospecting metabolites with pharmaceutical importance. To date, there has not been a systematic bibliometric analysis to identify EF research trends. Here, we have searched a total of 5,693 relevant publications with 2,000 most cited papers between 2000 to 2021 from the Scopus database. Number of papers published shows an upward trend with an average of 271.1 papers published per year. The largest proportion of papers published were in the field of Systematics/Phylogenetics (57.5%), Biocontrol applications (27.5%), and other topics in Pharmaceuticals, Crop Science, Forest management, or Cell Biology (15%). From 2000 to 2021, new-world group (North and South Americas) and old-world group (Europe, Africa/Middle east, Australia/New Zealand, Asia) contributed towards 30% and 70% of the total papers published, respectively. In Asia, China and Thailand represented majority of publications on the phylogeny of their local taxa and EF medicinal properties. Visualization analysis using VOSviewer based on different keywords indicated connectance between major subject areas of interest with applications in terms of funding endeavors, collaboration efforts between researchers from different region of the world, and productivity among specific research group networks. Overall, bibliometric analysis offers tremendous potential in guiding future EF research directions, collaborations, and relevance across multiple disciplines in closing gaps of understanding on EF as a research community.

Keywords: *Entomopathogenic fungi; bibliometric; phylogeny, biocontrol and VOSviewer*

Child sexual predators are amongst us: A review of child sexual predatory behaviours

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In Malaysia, the increasing number of child sexual abuse (CSA), its ramifications, and a lack of knowledge on child sexual predators (CSP) pertaining to paedophilic interest is worrisome. As a feasible proactive crime prevention strategy, a literature review was conducted to determine relevant factors underlying sexually predatory actions. Articles from Google Scholars, ScienceDirect and Springer Link were sourced for materials pertaining to 'child sexual predators', 'criminal thinking', 'forensic awareness', 'paedophilic interest', and 'sexual predators' for the years 2015 to 2020. After applying the exclusion criteria, 71 articles were reviewed. Results were clustered into several factors underlying sexual predatory actions such as paedophilic interest, criminal thinking, decision making, and forensic awareness. This review concluded that various factors were present and combined to sustain and motivate the sexual predatory interest and behaviour among the predators. There is a lack of studies focusing on CSP with paedophilic interest especially in Malaysia, thus more local studies are recommended to be conducted to better understand the prevalence and protect against the danger of sexual predatory behaviour involving children.

Keywords: *Child sexual abuse; criminal thinking; forensic awareness; paedophilic interest and sexual predators*

Deciphering the molecular basis of silver nanoparticle resistance in the critical priority pathogen *Acinetobacter baumannii*

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Our work reports on the ability of the bacterial pathogen *Acinetobacter baumannii* to develop stable resistance to silver nanoparticles (NAg) following prolonged exposure, for the first time. NAg is one of the most valuable antibiotic alternatives in the medical sector, due to its potent and broad-spectrum antimicrobial activity. *A. baumannii* is an ESKAPE pathogen and the WHO has recently listed it as the highest critical priority pathogen requiring novel antimicrobial treatments. Many studies have reported on the strong efficacy of NAg against *A. baumannii*, including multi-drug resistant strains, highlighting greater cell killing activity in comparison to traditional antibiotics. Yet, because of its high commercial value, NAg has been integrated in many consumer products, often without clear microbial targets, and this indiscriminate use has raised concerns for the generation of silver-resistant bacteria. To date, however, no studies have established the potential for NAg resistance development in *A. baumannii*. Following 30-days of continual treatment to increasing NAg doses, we found that a model strain of *A. baumannii* developed stable resistance to the nanoparticle and proliferated at an otherwise toxic silver concentration. Whole genome sequencing revealed the generation of single nucleotide polymorphisms in the resistant strain, which are potentially relevant to its silver defence. Further experimentation is underway to validate and characterise the genotypic and phenotypic changes and subsequently determine their role in the NAg resistance response of *A. baumannii*. Our study also revealed unique adaptation characteristics in the bacterium to cationic silver (Ag⁺). As no new class of antibiotics have been developed in the last 30 years, the efficacy of alternative antimicrobials, like NAg, need to be protected. With these findings, we eventually seek to decode the molecular basis of silver resistance in this bacterium, and in turn help enable the development of strategies to overcome bacterial silver resistance in the future.

Keywords: *Acinetobacter baumannii*; silver nanoparticles; antimicrobial resistance and mutation

Chemoprofiling of Malaysian stingless bee *Geniotrigona thoracica* propolis from different locations by Gas Chromatography-Mass Spectrometry (GC-MS) and combined with multivariate analysis

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Propolis is a complex resin bee product and consists variations of chemical compounds that contributed to the biological and pharmacological activity. Bees use materials resulting from a variety of botanical process in different part of the plant to produce propolis. The geographical origin of the propolis influenced the chemical composition including volatile compounds as the source of collected plants could be different. The present study is to analyze and identify the volatile compounds in Malaysia.n propolis of stingless bee *Geniotrigona throcacia* from different locations by Gas Chromatography-Mass Spectrometry (GC-MS). Discrimination of geographical origins of propolis was observed using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). GC-MS analysis revealed the methanolic extracts of propolis contained 36 different phytochemicals with 8 classes of compounds which were acids, alcohols, diterpenoids, ethers/oxides, phenolic acids, sesquiterpenes, sugars/sugar alcohols/sugar carboxylics and triterpenoids. The most abundant compounds were cycloartenol, 13, 27-cycloursan-3- one, α -amyrin and β -amyrin that detected in all propolis samples indicating the compounds could be the potential marker for propolis from Malaysia. The PCA of GC-MS data contained important volatile compounds showed variability of PC1 and PC2 with 68.56%. PCA's variations in the propolis from different locations arise from variety of chemical compounds detected. The GC-MS fingerprinting of HCA stingless bee's propolis were clustered into three clusters based on the volatile compounds and geographical locations. In conclusion, the findings in this work confirmed the influence of geographical source and chemical composition of propolis. The PCA and HCA could successfully discriminate the propolis samples from different locations in Malaysia.

Keywords: *Stingless bee propolis; GC-MS; volatile profiling, PCA and HCA*

Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease

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Considerable evidence suggests that blockade of the dopamine-3-receptor (D3R) is neuroprotective and reduces inflammation in models of Parkinson's disease (PD). However, to date there are no selective D3R antagonists in the market. Recently, computational analyses have demonstrated that buspirone, an FDA- approved anxiolytic drug with serotonin 1A (Htr1a) agonist activity, also functions as a potent D3R antagonist. To test if buspirone also elicited anti-inflammatory activities via D3R blockage *in vitro*, we generated stable *Drd3^{-/-}* and *Htr1a^{-/-}* BV-2 microglial cell lines using CRISPR-Cas9 technology and then tested the effects of buspirone after lipopolysaccharide (LPS) challenge. We found that buspirone counteracted LPS-induced NO release ($p < 0.001$), IL-1 β ($p < 0.01$) and TNF- α ($p < 0.0001$) gene expression in WT cells, whereas it exerted limited effects in *Drd3^{-/-}* and *Htr1a^{-/-}* microglia. To determine if buspirone elicited neuroprotective effects *in vivo*, C57BL/6 mice were treated with the PD-mimetic rotenone (10mg/kg rotenone i.p.) and received daily injections of either 1, 3, or 10mg/kg buspirone for 21 days. Buspirone treatment successfully mitigated rotenone-induced deficits in locomotor and exploratory behaviours in the Open Field test. Additionally, we found that rotenone caused variable degrees of toxicity across the different brain regions examined (i.e. midbrain, striatum, prefrontal cortex, amygdala, hippocampus and spinal cord) and these effects were ameliorated by buspirone co-treatment. In the midbrain, buspirone successfully restored inflammation and oxidative stress to levels comparable to healthy mice, as shown by a decrease in CD11b ($p < 0.001$), IL-1 β ($p < 0.0001$), SOD1 ($p < 0.001$) and GFAP ($p < 0.01$). The drug also prevented dopaminergic cell loss in the midbrain (TH expression, $p < 0.0001$) and altered the expression of endogenous neurotrophic molecules such as the neuropeptides PACAP and VIP and the neurotrophic factors BDNF and ADNP. In summary, our findings indicate that buspirone attenuates microglial polarization after LPS challenge and can mitigate rotenone-induced neurotoxicity and inflammation *in vivo*.

Keywords: Parkinson's disease; microglia; neuroinflammation and buspirone; rotenone

MicroRNA in human plasma extracellular vesicles during severe malaria – Potential biomarkers for disease severity?

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Extracellular vesicles (EVs) released during malaria infection can transfer their cargo to other cells and contribute to disease pathogenesis, cellular communication, and biological information exchange. Recent malaria studies have suggested that EVs contain potential biomarkers, such as miRNA, allowing differentiation between malaria patients with complication including neurocognitive impairment (NCI) observed in both severe malarial anaemia (SMA) and cerebral malaria (CM). MicroRNA are small non-coding RNAs that can regulate cellular metabolism, differentiation, and development. EVs isolated from human plasma carry highly stable miRNAs. We therefore hypothesise that miRNA signature in EVs vary based on disease severity. MicroRNAs present in EVs were analysed using next generation sequencing (NGS). Paediatric samples were prepared from 6 groups: community controls (n=3), CM patients with full recovery (n=4), CM patients with fatal outcome (n=4), CM patients with NCI (n=4), SMA patients with full recovery (n=4) and SMA patients with NCI (n=4). EVs were purified from ~200 µL of patient plasma collected in Uganda using QIAgen® exoEasy membrane affinity spin column. RNA was then extracted using the exoRNeasy kit. The miRNA library was then prepared with a QIAseq™ miRNA Library Kit and QIAseq™ miRNA 12 Index IL and sequenced using the miSeq system from Illumina. The sequencing output data was then analysed using Qiagen® CLC Genomics Workbench and differentially expressed miRNA identified. The miRNA of interest was then further narrowed down through bioinformatic analysis and confirmed through TaqMan RT-qPCR. 13 miRNAs of interest were found to regulate 13 genes in the malaria KEGG pathway. Comparing the significance of the miRNAs function and how many disease groups the miRNAs represented, 5 miRNAs were selected for RT-qPCR verification. This study shows for the first time the miRNA content of EVs in malaria patients and our results suggest that they could be used as potential markers of disease severity.

Keywords: *microRNA; cerebral malaria; severe malarial anaemia; paediatric and extracellular vesicles*

Effects of air pollutant particles in the pathogenesis of Alzheimer's disease

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Sporadic Alzheimer's disease (AD) occurs in 99% of cases with air pollutants as a major risk factor. The aim of this study was to determine whether externally derived air pollutant particles will exacerbate AD pathogenesis. In this study, we used (i) micro-abraded hot rolled train track iron and iron oxide particles (IRON), (ii) diesel emission particles (DE), and (iii) magnetite nanoparticles (MNPs). To assess the effects of the particles, we exposed 3-month-old wild-type (W/T; C57BL/6) and APP/PS1 mice to one of the aforementioned particles (n=5-6 per group) or saline (vehicle control; n=5), every third day, via intranasal administration [66ug] over 17-weeks. The elevated plus maze test, performed at week 14 to assess anxiety and stress, showed significant increase in (i) W/T exposed to IRON and DE compared to W/T saline, and (ii) APP/PS1 exposed to IRON compared to APP/PS1 saline. In-vivo fluorescent imaging (CRANAD 2 probe) of the brain and thioflavin S staining at endpoint showed an increase in A β load in (i) W/T exposed to DE and MNPs, and (ii) APP/PS1 exposed to IRON, DE and MNPs compared to saline. Neuronal cell counting was completed with cresyl violet staining showed that (i) W/T and (ii) APP/PS1 exposed to MNPs had significantly increased neuronal cell death. At the cellular level, cultured human neuroblastoma cells (SH-SY5Y) exposed to the pollutant particles showed (i) an increase in reactive oxygen species production; (ii) decrease in cell viability and (iii) increased expression of inflammatory markers IL-6, IL-1 β , compared to control. The results of this study establish that exposure to air pollutant particles results in an increase in cerebral A β levels and induced neurological changes via pathways associated with inflammation and oxidative stress in neuronal cells that play a significant role in the onset of AD.

Keywords: *Alzheimer's disease; magnetite; nanoparticles; air pollutant and particulate matter*

Evaluation of chemical properties and *in vivo* assessment of toxicity and cholesterol levels on refined red palm-pressed mesocarp olein

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The refined red palm-pressed mesocarp olein (PPMO) is recovered from palm-pressed mesocarp, which is a by-product of palm oil mill. The utilization of PPMO in food industry is extremely limited with only the applications in cosmetic, pharmaceutical formulation, animal feed formulation and personal care product. Hence, this study aimed to characterize the chemical properties of PPMO and evaluate its *in vivo* toxicity and cholesterol levels in PPMO fed Sprague-Dawley rats. Total carotene content, vitamin E content, fatty acid composition, free fatty acid content, peroxide value, oxidative stability and anisidine values were measured with the comparison of commercial cooking oils, including palm olein, extra virgin olive oil (EVOO) and PPMO:EVOO blend (1:1). The results indicated that the oxidative stability, vitamin E and carotene contents of PPMO are significantly higher than other cooking oils. Besides, the rats were administered with a single dose of 2 g/kg PPMO in 14-day acute toxicity study, and daily dose 2, 1, or 0.5 g/kg PPMO in 28-day sub-chronic toxicity study. The parameters of acute and sub-chronic toxicity studies, including mortality, clinical signs, body and organ weight, hematological and biochemical analyses, pathological and histopathological examinations of rats, suggested that PPMO is non-toxic with an oral LD50 value of up to 2 g/kg. Lastly, the rats were administered with a daily dose of 2 g/kg for PPMO and other cooking oils in 84-day experimental study. The serum total cholesterol (TC), low-density lipoprotein, high-density lipoprotein (HDL) and TC/HDL levels of rats revealed that PPMO, is consistent with other cooking oils, has no adverse effects towards cardiovascular disease. In conclusion, PPMO is safe to be applied in food products due to its non-toxicity, great oxidation stability, high nutritional value and non- detrimental effects on cardiovascular diseases.

Keywords: *oxidation stability; phytonutrients; lethal dose; biochemical analysis and total cholesterol*

Isolation and characterization of effective microorganisms from palm oil sludge for bio-adsorbent degradation in agriculture wastewater treatment

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Biochar produced from organic wastes has gained increasing interest and application as an adsorbent in treating agriculture wastewater. The end product post-wastewater treatment is known as spent bio-adsorbent. This project aims to isolate, identify, and characterize microbes from two palm oil sludge samples, based on their degradative activities towards cellulose and protein, and their abilities in solubilizing phosphate and fixing nitrogen. The isolated microbes, if tested to be of value, are to be incorporated into spent bio-adsorbents for their conversion into useful products. A total of 52 types of bacterial isolates and 12 types of fungal isolates were obtained from the palm oil sludge samples collected from Air Kuning, Perak, and Nibong Tebal, Pulau Pinang. The isolates were screened for their cellulolytic, proteolytic, phosphate-solubilizing, and nitrogen-fixing activities. The enzymatic index for each plate assay was calculated based on the ratio of colony diameter to the clear zone diameter. Among the 52 bacterial isolates, 13 showed positive results for all the plate assays. The highest index among these isolates for cellulolytic activity was 1.915, proteolytic activity 1.528, and phosphate-solubilizing activity 1.712. Meanwhile, three fungal isolates were positive in these assays with the highest cellulolytic index at 2.790, proteolytic index at 1.943, and phosphate-solubilizing index at 1.111. In future, quantitative enzymatic assays will be performed on selected isolates and the metabolic effects of microbial consortia on the biochar and the macronutrients adsorbed will be evaluated. The microbial-treated spent bio-adsorbents are expected to be of value to agriculture as biofertilizers or soil conditioners.

Keywords: *Biochar; palm oil sludge; effective microorganisms; aquaculture wastewater treatment and bio-adsorbent*

Effects of Tempol on adipogenesis and hepatic steatosis

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Weight gain and obesity are major risk factors for conditions such as insulin resistance (IR) to type 2 diabetes. Chronic overnutrition results in excessive systemic lipid levels that leads to an increase in adipose mass and lipid accumulation in the liver. Increasing adipose tissue mass and hepatic steatosis is associated with an increase in inflammation and oxidative stress leading to alteration in glucose/fatty acid metabolism ultimately leading to the development of insulin resistance. Administration of a nitroxide, Tempol, has been shown to result in the prevention of weight gain. This study aimed to assess the effects of Tempol in hepatocytes (HepG2) and adipocytes (3T3L1) exposed to palmitic acid (PA) and in a high-fat diet (HFD) mouse model. To assess the effects of Tempol *in vitro*, HepG2 or 3T3L1 cells were exposed to PA for 24 hours before exposure to Tempol (200mM, 500mM, 1mM) for 5- or 24 hours, respectively. The results showed Tempol reduced PA-induced (i) lipid accumulation (Oil Red O assay), (ii) inflammatory cytokines (RT-qPCR/Western blot) and ROS levels (DCF assay) in both cell types. In HepG2, Tempol was also shown to reduce insulin-mediated glycogenesis (Glycogen assay) whilst in 3T3L1, Tempol reduced PA-induced adipogenesis markers and triglyceride levels. To assess the effects of Tempol *in vivo*, C57BL/6 mice were fed an HFD for a total of 16 weeks. A subset of mice received HFD supplemented with Tempol from the 8th week. Supplementation with Tempol showed improvement in (i) glucose tolerance and insulin sensitivity, reduced (ii) inflammatory cytokines, (ii) hepatic inflammation and (iv) prevented further weight gain. Findings suggest that Tempol plays a protective role in weight gain, an effect associated with the suppression of adipose and hepatic inflammation, oxidative stress, and insulin-mediated glycogenesis in the liver.

Keywords: Tempol; hepatic steatosis; adipogenesis; oxidative stress; inflammation and insulin resistance

Blood meal analysis: Insight into blood feeding preference of *Anopheles* vectors of simian malaria

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Malaysia. had reported zero indigenous human malaria cases since 2018. However, the increasing cases of zoonotic malaria due to *Plasmodium knowlesi* is indeed alarming. The prevalence of malaria is highly influenced by mosquito's host selection behaviors which can be studied using blood meal analysis. Blood feeding and host-seeking behaviors of a mosquito play an imperative role in determining its vectorial capacity in transmitting pathogens. Unfortunately, limited information is available regarding blood feeding behavior of *Anopheles* species in Malaysia. Collection of resting *Anopheles* mosquitoes for blood meal analysis poses a great challenge especially for forest dwelling mosquitoes. Therefore, a laboratory-based study was conducted to evaluate the potential use of mosquitoes caught using human landing catch (HLC) for blood meal analysis, and subsequently to document blood feeding behavior of local *Anopheles* mosquitoes in Peninsular Malaysia. Individual animal-specific PCR assays targeting the mitochondrial cytochrome c oxidase subunit 1 gene was used to amplify the different host bloods from the field-caught *Anopheles* mosquitoes. The laboratory-based experiment from this study revealed that mosquitoes caught using HLC had the potential to be used for blood meal analysis. Time course analysis in the laboratory showed that DNA of the tested vertebrates were detectable in the mosquitoes' abdomen up till 72 hours post-feeding. Overall, 47.4% of 321 field-caught *Anopheles* mosquitoes belonging to six species were positive for vertebrate host DNA in their blood meal. The most frequent blood meal source was human (45.9%) followed by wild boar (27.4%), dog (15.3%) and monkey (7.5%). Interestingly, only *Anopheles cracens* and *Anopheles introlatus* fed on monkey. This study further confirmed that members of the Leucosphyrus Group are the predominant vectors for knowlesi malaria transmission in Peninsular Malaysia. mainly due to their simio-anthropophilic feeding behavior.

Keywords: *Anopheles*; blood meal; *Leucosphyrus* group; Simian malaria and zoonotic

Epidemiology and distribution of *Giardia duodenalis* Genotypes in humans in New South Wales, Australia

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Giardiasis is the most common enteric protozoan infection notifiable in New South Wales (NSW) and surveillance by NSW Health has shown a steady increase in the number of cases reported since 2012. Interestingly, recent state-wide analyses on NSW Health data found persistent spatial clusters of giardiasis in urban neighbourhoods. Nevertheless, the epidemiological significance of the various *Giardia* assemblages are still unclear. Contradictory results have been published regarding geographical distribution and clinical manifestation of the assemblages. In collaboration with tertiary hospitals and private laboratories located in Sydney, Australia, the purpose of this study is to further investigate the molecular epidemiology of giardiasis in NSW. A total of 157 human stool samples were collected and the presence of *Giardia* cysts and co-infections were confirmed by multiplex PCR. Samples were genotyped by sequence analysis of the triosephosphate isomerase (*tpi*) gene and the small subunit rRNA region (SSU rRNA). Genotyping results showed that most samples belong to assemblage B, and only a small percentage (8%) belonged to assemblage A. Surprisingly, mixtures of genotypes A and B in individual isolates were relatively common (29%). Co-infections were observed in 51% of samples with the most common co-infection being *Blastocystis hominis*, followed by *Dientamoeba fragilis*. This study provides new insights into the molecular diversity of this parasite in Sydney, Australia. This can inform enhanced surveillance and prevention strategies in developed metropolitan areas.

Keywords: *Giardiasis; assemblages; genotyping; public health and epidemiology*

Targeting cyst wall in the development of anti-acanthamoebic approaches

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Acanthamoeba castellanii is a free-living parasite commonly associated with a blinding corneal infection termed *Acanthamoeba* keratitis (AK). AK is primarily observed among contact lens wearers. With the increase in contact lens users, *Acanthamoeba* presents a significant concern to public health globally. The effectiveness of conventional therapy relies on prompt diagnosis and aggressive treatment. However, treatment is hindered by the parasite's ability to enclose itself within a hardy shell and remain dormant as a cyst. Using gas chromatography combined with mass spectrometry, we have shown that *Acanthamoeba* cysts contain 1,4-linked glucan, which is likely to be cellulose. We hypothesized that degrading cyst walls is a logical strategy for the effective killing of the parasite. In this study, a range of compounds that may have the potential to target *Acanthamoeba* cyst walls and/or its biosynthesis pathway were assessed for anti-amoebic effects using bioassay-guided testing. Amoebicidal and cysticidal assays were performed to evaluate the effects of the compounds on *Acanthamoeba* trophozoites and cysts, respectively. Our study revealed notable cysticidal effects exhibited by acarbose, indaziflam, terbuthylazine, glimepiride and inositol with MIC₅₀ ranging between 5.3 μ M and 20 μ M. On the other hand, DPP4 inhibitor, terbuthylazine, inositol and quinclorac demonstrated significant cysticidal activity against the trophozoites. Furthermore, these compounds showed limited toxicity on human corneal epithelial cells and human keratinized skin cells. Future study would be to conjugate these compounds with nanoparticles to try to enhance the amoebicidal and cysticidal activities.

Keywords: *Acanthamoeba*; eye infection; keratitis and cysticidal

***In vitro* study on the effects of hypoxia-mimicking agents in hepatocellular carcinoma cell growth**

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Hypoxia, or oxygen deprivation, is a common pathological feature found in hepatocellular carcinoma. Hypoxia-inducible factor is the master transcription factor of oxygen homeostasis, eliciting a wide range of cellular adaptive responses to either enhance oxygen delivery or reduce oxygen consumption. The hypoxia-inducible factor is negatively regulated by two types of oxygen sensors, the prolyl hydroxylases and asparaginyl hydroxylase. Recent advances using small molecule inhibitors to activate the hypoxia pathway has been proven to be effective in treating renal anaemia. Given the heterogeneous tumour microenvironment and pleiotropic nature of hypoxia, hypoxia-inducible factor protein functions as a double-edged sword whereby it could either promote or hinder tumour progression. Although the activation of the hypoxia pathway is effective in the treatment of anaemia, the implications of this pathway in the context of tumour development remain unclear. Therefore, this study aims to investigate the effects of hypoxia pathway activation on hepatocellular carcinoma cell growth. Firstly, *in vitro* hypoxia was established through the utilisation of hypoxia-mimicking agents, deferoxamine and cobalt chloride. HepG2 was used as the cellular model of hepatocellular carcinoma. Effects of hypoxia pathway activation on tumour growth were assessed using the Trypan blue dye exclusion assay. Our results show that activation of the hypoxia pathway using deferoxamine ($\leq 100\mu\text{M}$) leads to decreased HepG2 cell proliferation in a dose- and time-dependent manner. On the other hand, the cobalt chloride demonstrates contrasting responses. Treatment of cobalt chloride at low concentrations ($\leq 12.5\mu\text{M}$) promotes proliferation but at higher concentrations ($12.5\text{-}100\mu\text{M}$), it inhibits HepG2 cell proliferation. These differential effects may be attributed to their distinctive mechanisms of action on the hypoxia pathway. Deferoxamine works as an iron chelator whilst the divalent metal cobalt chloride competes and displaces iron in the oxygen sensors' active site. Taken together, activation of the hypoxia response pathway reduces hepatocellular carcinoma cell growth.

Keywords: Hypoxia; hepatocellular carcinoma; cell biology; small molecule inhibitor and hypoxia-mimicking agents

Antifungal susceptibility profile of riboflavin against *Candida* spp. Isolates: A preliminary study

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Candidiasis remains a significant cause of high mortality and morbidity in immunocompromised populations, with *C. albicans* being the most frequent *Candida* species isolated from infected patients. Inefficacy of the currently available antifungal drugs, both inherent and acquired drug resistance developed in *Candida* species and increasing numbers of immunocompromised patients are some factors predisposing to the increase in the mortality of patients infected with *Candida* infections. This warrants the search for newly potential antifungal compounds that are capable of resolving *Candida*-associated infections. Riboflavin is an essential vitamin for humans that contains antimicrobial activity. However, its therapeutic effect in tackling *Candida* infections remains elusive. Therefore, this preliminary study aimed to determine the antifungal susceptibility of riboflavin against a number of ATCC control *Candida* species, including of *Candida albicans* 90028, *Candida krusei* 6258, *Candida parapsilosis* 22019 and *Candida tropicalis* 750. The ATCC *Candida* spp. strains were identified using CHROMagar. Antifungal susceptibility testing and minimal inhibitory concentration (MIC) of riboflavin and fluconazole (reference drug) for *Candida* isolates were determined using broth microdilution method. Our preliminary findings showed that upon treatment with riboflavin at 24 h post-incubation, the MIC values for *C. krusei* and *C. tropicalis* were 0.125 µg/ml while *C. albicans* and *C. parapsilosis* were 32 and 1 µg/ml, respectively. At 48 h post-incubation, the MIC values for *C. albicans* and *C. tropicalis* were 0.125 µg/ml whilst the MIC values for *C. krusei* and *C. parapsilosis* were 16 µg/ml and 0.5 µg/ml, respectively. On the other side, the MIC values of the ATCC control *Candida* species were determined to be 0.125 µg/ml after treatment with fluconazole at 24 and 48 h post incubation. Our findings showed that riboflavin possesses comparable antifungal efficacy with fluconazole, in particular against *C. tropicalis* and *C. krusei* at 24 h post incubation and *C. albicans* and *C. tropicalis* at 48 h post incubation. Further investigation on the antifungal capacity of riboflavin should be extended to clinical *Candida* spp. Isolates.

Keywords: *Candida albicans*; non-*albicans Candida* species; riboflavin; antifungal susceptibility and broth microdilution methods

Synthetic mRNA-based genetic reprogramming of insulin-producing cells differentiated from mesenchymal stem cell for pancreatic beta cell regeneration

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Diabetes Mellitus (DM) is the most common chronic metabolic disease affecting 422 million people worldwide and cause 1.6 million deaths yearly. DM characterized by uncontrolled blood glucose level due to disruption in insulin homeostasis pathway which lead to serious complications such as cardiovascular disease and neuropathy. Autoimmune destruction of beta cell and insulin resistance is the main pathology hallmark of diabetes. For diabetic patient, intensive insulin regiment commonly prescribed are not sustainable in the long run due to possible adverse effect and economic burden. Alternative therapeutic option is important as it tackle the core pathology of DM. Using cell reprogramming, we can correct underlying insulin issue and prolong or stop diabetic from occurring. This study focuses on using synthetic mRNA to differentiate pluripotent stem cells into insulin producing cells via temporal expression of transcription factors that activate the trans-differentiation program. The main aim of this study is to reprogram human mesenchymal stem cells (huMSC) into functional beta cells through modulation of transcription factors PDX1 (ID: 3651), NGN3 (ID: 50674), MafA (ID: 389692), PAX4 (ID: 5078) and GLIS3 which have been found to be involved in the natural process of cells differentiation into pancreatic beta cells. The study is divided into three phases, (1) production of synthetic mRNA, (2) *in vitro* study and (3) *in vivo* cell transplantation. huMSCs will be separately transfected with synthetic mRNA expressing PDX1-NGN3-MafA (PNM), PDX1-NGN3-MafA-PAX4 (PNMP), PDX1-NGN3-MafA-Glis3 (PNMG) and PDX1-NGN3-MafA + PAX4-Glis3 (PNM + PG). Successful transfection will be determined via gene expression analysis, FACS, cell viability, apoptosis assay, immunohistochemistry staining and glucose-stimulated insulin assay.

Keywords: *Diabetes mellitus; synthetic mRNA; cell reprogramming; transcription factor and insulin-producing cell*

E-cigarette aerosol exposure leads to coronary endothelial cell dysfunction and restricted angiogenesis

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Cardiovascular disease (CVD) is a leading cause of mortality world-wide, with cigarette smoking being a major preventable risk factor. Smoking cessation can be difficult due to the addictive nature of nicotine and the withdrawal symptoms following cessation. Electronic cigarettes (e-Cigs) were designed as a smoking cessation tool, however, it is increasingly used for recreation with the health effects not fully understood. To investigate the effects of e-Cigs tobacco flavor containing 0 mg/mL or 18 mg/mL nicotine (i) *in vitro* using human coronary artery endothelial cells (HCAEC) and (ii) *in vivo* using a mouse model of 'vaping'. *In vitro*: HCAEC were exposed to e-Cigs aerosol condensate (EAC) for 24hr to assess the effects on cell viability, oxidative stress and inflammation using an MTT assay, dichlorofluorescein assay or an ICAM-1 ELISA, respectively. To assess the indirect effects following metabolism through the lungs, a coculture-like model using conditioned-media of A549 lung epithelial cells was used to treat the HCAEC. *In vivo*: Female BALB/c mice (7 weeks of age) were exposed to e-Cigs aerosol for half an hour twice daily or ambient air for 12 weeks. Left ventricles (LV) were collected for analysis. The *in vitro* results show a decrease in cell viability in HCAEC when exposed to EAC with or without nicotine and either directly or after exposure to conditioned lung cell media ($p < 0.005$, vs control). Reactive oxygen species levels and ICAM-1 expression were increased in HCAEC when exposed to EAC with or without nicotine directly ($p < 0.0005$, vs control). The *in vivo* results show ICAM-1 mRNA expression increased in the hearts of mice exposed to nicotine-containing e-Cig aerosol ($p < 0.05$, vs control) and angiogenic markers (FKBPL, CD31) were dysregulated. Mice hearts exposed to nicotine-free e-Cigs aerosol had no significant changes. e-Cig vapor exposure can have a negative impact on endothelial cell function and cardiovascular health. Caution should be taken before using e-Cigs. Further work is now needed to elucidate the full impact of e-Cigs in CVD.

Keywords: Smoking; e-cigarette; endothelial cell; atherosclerosis and FKBPL

Immunohistochemical detection of parvovirus B19 in head and neck carcinomas

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most frequent malignancy worldwide. Although some subsets of HNSCCs have been attributed to viral etiologies, their potential association with parvovirus B19 (B19V) has not yet been investigated extensively. Herein, we have included formalin-fixed paraffin-embedded (FFPE) tissue specimens from 103 HNSCC patients along with 40 oral mucocele FFPE specimens as benign controls to investigate the possible role of B19V in HNSCC and its association with the overexpression of nuclear factor kappa B (NF- κ B) as a cellular transcription factor involved in inflammatory pathways, and p16INK4a as a tumor suppressor protein whose dysregulation has been previously reported in HNSCCs. Immunohistochemistry assay was carried out on these tissue specimens using anti-parvovirus B19, anti-human p16INK4a and anti-human NF- κ B (p65) monoclonal antibodies. Data were analyzed using GraphPad Prism 8. Two-sided Fisher's exact test was applied for statistical analysis. The immunoreactivity against B19V was found in 24 (23.3%) HNSCC cases and none of mucocele specimens which revealed a significant difference ($p=0.0003$). B19V infection was found to be significantly associated with nuclear NF- κ B overexpression ($p<0.0001$), but not cytoplasmic NF- κ B overexpression ($p=0.599$) in HNSCC patients. Furthermore, there existed no significant association between B19V infection and p16INK4a overexpression in these patients ($p=0.610$). These results for the first time indicate a possible role of B19V in the pathogenesis of HNSCC. Furthermore, our findings suggest that B19V infection is associated with increased NF- κ B activation in HNSCC tissues.

Keywords: *Head and neck squamous cell carcinoma; parvovirus B19; nuclear factor kappa B; p16INK4a and immunohistochemistry*

Development of nanoemulsion formulation containing *Centella Asiatica* (L.) Urb crude extract as a promising parenteral delivery for epilepsy treatment

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Centella asiatica L. Urb (pegaga) is a medicinal plant and commonly served as a salad by Asians that believes the leaves have good memory enhancing and antiepileptic properties. In epilepsy treatment, maximum therapeutic effect on the targeted site could not be achieved as crossing the blood-brain-barrier (BBB) has been a major challenge even in the parenteral route. Thus, a nanoemulsion formulation containing *C. asiatica* crude extract needs to be developed to penetrate the BBB for better efficacy. Nanoemulsion formulation was prepared using a low-energy emulsification technique with desired particle size, polydispersity index (PDI) and zeta potential. The formulation remained physically stable at room temperature within 90 days. The morphological characteristic of the nanoemulsion was in spherical shape which correlated well with the particle size analyzer. Cytotoxicity analysis showed that the nanoemulsion containing *C. asiatica* crude extract is nontoxic against Vero and 3T3 cells lines (IC₅₀ > 500 µg/ml). The percentage release of the nanoemulsion formulation at pH 8.4 indicated an intermediate release. Thus, the formulation was found to be a promising drug delivery in parenteral application against BBB.

Keywords: Blood-brain barrier; *C.asiatica*; drug delivery; nanoemulsion and particle size

An optical nanobiosensor for the sensitive detection of MicroRNA-155 in breast cancer

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In 2020, breast cancer accounts for 2.3 million cases and 680,000 mortalities. Early detection has been known to improve the prognosis and overall survival-rate of breast cancer. In breast cancer patients, the expression of microRNAs (miRNA)-155 is commonly upregulated as compared to healthy individuals. Hence, extensive research has been focused on miRNA-155 as diagnostic and prognostic biomarkers for breast cancer. Herein, we present a nanobiosensor that integrates fluorogenic silver nanoclusters (AgNCs) into a hybridization chain reaction (HCR)-amplified system to detect miRNA-155. To construct this nanobiosensor, DNA hairpin probes were first mixed with miRNA-155 to initiate HCR. Silver salt was subsequently added and reduced to form fluorescent AgNCs. The performance of HCR was validated through gel electrophoresis. Meanwhile, the fluorescence emission from AgNCs was analyzed qualitatively and quantitatively with UV-transilluminator and spectrofluorometer, respectively. The HCR- AgNCs nanobiosensor exhibited dual-emissive fluorescence response, and a strong fluorescence enhancement in the presence of miRNA-155. In addition, the nanobiosensor was highly sensitive with a wide linear range between 100 fM and 1 μ M, and a limit-of-detection as low as 1.13 fM. Besides, the nanobiosensor also displayed high selectivity towards miRNA-155, with capabilities of discriminating single-base mismatch. In real sample analysis, the nanobiosensor showed exceptional reproducibility and stability when tested with diluted human serum samples. In lieu of current breast cancer and miRNA detectors, the HCR-AgNCs nanobiosensor displayed relatively comparable performance at a miniscule fraction of cost, effort and time required. Likewise, the direct and highly-responsive HCR-AgNCs nanobiosensor potentially offers a non-invasive and safe approach towards the clinical detection miRNA- 155 and point-of-care early diagnosis of breast cancer.

Keywords: *Biosensor; hybridization chain reaction and silver nanoclusters*

Synergism effect of asymmetrical curcumin derivative and paclitaxel combination in triple-negative breast cancer (TNBC) cell lines

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Triple-negative breast cancer (TNBC) is one of the most aggressive breast cancer subtype. Due to the non-existence of therapeutic markers, chemotherapy is predominantly used to treat TNBC. Paclitaxel (PTX) is a widely used TNBC chemotherapeutic drug, however, PTX-resistance can be acquired through the activation of TLR4/MyD88 signalling in TNBC. It has been reported that a combination treatment of PTX with curcumin could overcome the resistance. Recently, a series of curcumin analogs have been synthesised by our group and have shown promising anti-inflammatory properties possibly by targeting TLR4 signalling. Based on this curcumin analogs structure, a new series of curcumin analogues were synthesised and evaluated for their growth inhibitory effects in TNBC^{TLR4+} (MDA-MB-231) and TNBC^{TLR4-} (HCC-1806) cell lines. Docking simulation was applied to explain the binding interactions of the compounds with MD2/TLR 4 active binding site. *In silico* pharmacokinetic and toxicity properties of compounds were determined. A selected lead compound was tested in combination with PTX against TNBC cells growth. The level of MyD88 expression (TLR4 co-adaptor protein) was measured using ELISA assay. Results were analysed using Compusyn software. Lead compound **Dp7** was significantly downregulated PTX-induced overexpression of MyD88 in MDA-MB-231^{TLR4+}. When **Dp7** treated in combination with PTX, it synergistically improved the growth inhibition in both TLR4⁺ and TLR4⁻ TNBC cells. **Dp7** potentially improved PTX chemoresistance *via* downregulation of PTX-induced MyD88 overexpression in TNBC^{TLR4+}. It could also target other important cellular proteins in TNBC^{TLR4-} thus synergise PTX growth inhibitory effects.

Keywords: Curcumin derivatives; TNBC and Paclitaxel

Cytotoxicity, apoptosis and cell cycle study in human leukemia cells (Jurkat E6.1) exposed to triphenyltin(IV) dithiocarbamate with different ligands

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Following the discovery of cisplatin, many attempts have been made to study the anticancer effects of other metal complexes. Organotin(IV) dithiocarbamate is a type of compound that is getting more attention as an anticancer agent due to their uniqueness and properties of the individual structures of the organotin and the dithiocarbamate moieties. The ligands attached to the parent structure can also control the reactivity of the metal. Our focus study is on leukemia, which is a type of blood cancer causing abnormal and uncontrolled growth of blood cells with mortality rate of 3.1% of all cancer cases worldwide. In this study, a series of newly synthesized organotin compounds known as triphenyltin(IV) diisopropyl (Compound 1), diallyl (Compound 2), and diethyl dithiocarbamate (Compound 3) have been assessed for their cytotoxic effects toward Jurkat E6.1 cells. WST -1 assay was employed to determine the cytotoxic effect for each of the compounds at different concentrations. Mode of cell death induced by these compounds was assessed using Annexin V-FITC/PI staining. The morphological characteristics of the treated cells were observed using inverted microscope. Cell cycle analysis was done using RNase/PI staining. All triphenyltin(IV) dithiocarbamate compounds exhibited cytotoxic effects toward Jurkat E6.1 with $IC_{50} < 1.0 \mu M$. The compounds were found to induce cell death via apoptosis. Morphological observation conducted showed the characteristic of apoptosis. Jurkat E6.1 treated with Compound 1 arrest the cell cycle in G0/G1 phase. Different ligand on triphenyltin(IV) dithiocarbamate compounds caused different cytotoxic effects on Jurkat E6.1 cells. Compound 1 showed the most potent effects with the lowest IC_{50} value and highest percentage of apoptotic population. Compound 1 was also able to arrest the cell cycle at early phase. Therefore, further studies are needed to be done to determine the mechanisms of action and potential of this compound to be developed as anti-leukemic agents.

Keywords: *Anti-leukemia activity; cytotoxicity; apoptosis; cell cycle and organotin(IV) dithiocarbamate compounds*

Evaluation of phenolic composition of *Hibiscus cannabinus* L. (Kenaf) leaves extract and its role in the green synthesis of silver nanoparticles (AgNPs)

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Kenaf leaves have been researched extensively for its therapeutic properties due to the presence of various functional, bioactive compounds. In this study, silver nanoparticles (AgNPs) were synthesised using ethanolic kenaf leaves extract (KLE) whereby the compounds present in the KLE were also assessed to identify the phytochemicals responsible for the synthesis. The phenolic composition of KLE was determined by the total phenolic content (TPC) and total flavonoid content (TFC) whereas the identification of compounds and functional groups were determined using ultra high performance liquid chromatography (UPLC) and Fourier transformed infrared spectroscopy (FT-IR) respectively. The synthesised AgNPs were characterized by using UV-visible spectrophotometer and dynamic light scattering (DLS). It could be deduced that the hydroxyl and carbonyl groups present in the phenolic and flavonoid compounds act as the reducing agents during the synthesis of AgNPs. The UV-vis spectrum showed that the AgNPs peaked at wavelengths of 400-440nm. The particle size of the green synthesised AgNPs ranged from 21nm to 52nm with favourable negative zeta potential values (lower than -30mV) which indicated that they were highly stable. This study had revealed that the ethanolic extract of KLE contained vital phenolic and flavonoid compounds and serves as potential natural substitute for the synthesis of AgNPs. Subsequently, the characterization of AgNPs can be further confirmed by FT-IR, X-ray diffraction (XRD) and Transmission electron microscopy (TEM). Further investigation also involves the anti-acne efficacy test of AgNPs on *Propionibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228. It is expected that KLE-synthesised AgNPs will have inhibitory effect on the three tested microorganisms at low concentrations which highlights the role of KLE-synthesised AgNPs in cosmeceutical.

Keywords: *Kenaf; phytochemical; bioactive compounds; silver nanoparticles and nanotechnology*

Microencapsulation of *Lysiphyllum strychnifolium* extract using pectin as a carrier matrix for nutraceutical product: Development and characterization

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Lysiphyllum strychnifolium (LS, Fabaceae) is one of the folklore medicines in Thailand. The previous studies have demonstrated several pharmacological activities and high polyphenolic substances possessed by this plant. However, the suitable dosage form of LS for nutraceutical uses has not been discovered. Thus, this study aimed to develop LS microcapsules using spray drying technique. Pectin was used as a carrier to protect the active compounds of the extract. The effects of inlet temperatures (80, 100, and 120°C) and carrier concentrations (1, 5, and 10% w/v) on the encapsulation yield (EY), encapsulation efficiency (EE), total phenolic content (TPC), and main markers of LS microcapsules were studied. Finally, the characterization was investigated by Fourier transform infrared (FTIR), x-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). The obtained results indicated that S2 formulation, pectin to extract ratio 10:1 (w/w) at 100°C, was chosen as the best condition because of the positive tendency to exert higher EE as pectin level was increased. On the contrary, the level of TPC and markers was reduced due to the addition of pectin. The FTIR, XRD, and DSC results suggested that the well-encapsulated microcapsules were obtained for S2 formulation and SEM represented the semi-spherical structure of its microstructures. The development of LS microcapsules with the proximity to gain the advantageous powder analysis and characteristic has been achieved. Therefore, this approach could be used for the subsequent manufacturing of LS extract.

Keywords: *Lysiphyllum strychnifolium*; pectin; microencapsulation; spray-drying and characterization

The biogenic synthesis of reduced graphene oxide using *Hibiscus sabdariffa L.* as natural precursor

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Graphene have been identified by many industries sectors as a key material that will drive future product developments such as electronics, supercapacitors, drug delivery. However, to get a high quality graphene remains a challenge. Thus, as solution graphene derivatives is being explored to produce a quality graphene. Commonly, reduced graphene oxide (RGO) is fabricated using Improved Hummers Method which involves oxidation and exfoliation of pristine graphite. It contains numerous functional groups. For future functionalization we reduce the oxygen group using green synthesis method. Green synthesis is the cost-effective method due to its rapidity, renewable nature, and sustainability. This study emphasis on the graphene oxide (GO) reduction using a simple one-pot technique that does not require the use of toxic reducing agents. This article reports the green synthesis of RGO using *Hibiscus sabdariffa L.* calyxes extract as the natural reducing agent. This article analyse RGO using X-ray diffraction (XRD), UV-Visible spectroscopy (UV-Vis), and Raman spectroscopy. XRD result showed that the GO peak at 11 ° diminished, and a new hump appear at 22 ° indicating that the GO is fully reduced when it is refluxed for 6 hours, at 100 °C with 1:3 ratio of GO:PE. The UV-Vis data indicated absorption peak of GO (237 nm) and RGO (265 nm) at distinct locations. This finding shed new light on the enormous potential of *Hibiscus sabdariffa L.* calyxes extract for green GO reduction. As a result, this environmentally friendly method can help reduce dependence on chemical materials and can potentially be used for biomedical field such as biosensor, drug delivery and bioimaging application.

Keywords: Graphene oxide; green synthesis; reduced graphene oxide; *Hibiscus sabdariffa L.* and calyxes extracts

Effect of Thai traditional herbal formula, Ummalukkawatee and Prasamawaeng, on cytochrome P450, 3A4 activity

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Ummalukkawatee and Prasamawaeng are the traditional anti-cough formulae that were listed in the Thai Herbal Medicinal Products A.D. 2019. There was some research showed that some component herbs of these formulae involved in herb-drug interactions, especially via cytochrome P450 (CYP); CYP3A4. Hence, this research aimed to determine the effect of both formulae and their components on the main CYP enzyme activity, CYP3A4. In this research, both anti-cough formulae were tested in recombinant human CYP3A4 using fluorometric technique. The concentrations of CYP3A4 that were treated with plant extracts were observed and calculated as the percentage of relative inhibition. Ketoconazole was used as the inhibited positive control. The results showed that Ummalukkawatee showed inhibitory effects 61.99 % at 50 µg/ml, 70.89 % at 100 µg/ml, and 88.39 % at 150 µg/ml. Prasamawaeng had inhibitory effects 67.14 % at 50 µg/ml, 82.27 % at 100 µg/ml, and 88.24 % at 150 µg/ml, respectively. Both formulae are intended to be the strong inhibitors when compared with the controls at 30 µM and exhibited dose-dependent effects. Although these results are only from *in vitro* observation which needed to be confirmed by *in vivo* and clinical studies, it can be useful in prediction of herb-drug interactions to minimize risks of interactions.

Keywords: Herb-drug interaction; Ummalukkawatee; Prasamawaeng; CYP3A4 and fluorometric assay

Biogenic reduced graphene oxide from local herbs for potential biomedical applications

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Due to their extraordinary properties, carbon-based nanomaterials are gaining attraction in biomedicine. Green synthesis is the most cost-effective method for fabricating carbon-based nanomaterials due to its rapidity, renewable nature, and sustainability. The purpose of this study is to synthesize graphene oxide (GO) and reduce it to reduced graphene oxide (rGO) using a simple one-pot technique. This article reports the green synthesis of rGO using *Clinacanthus nutans* (*C. nutans*) leaf extracts as the natural reducing agent. The X-ray spectrum demonstrated that GO and rGO were successfully produced based on the illustrated 2θ angles at peak 10.09° and 22.12° with d-spacing of 0.88 nm and 0.40 nm for GO and rGO, respectively. The FTIR analysis demonstrated that the amount of oxygen group in rGO was reduced, while the UV-Vis spectrum demonstrated the absorption peak of GO (225 nm) and rGO (270 nm) at distinct locations. The analysis clearly demonstrates the successful removal of oxygen-containing functional groups from GO and the formation of rGO. Taken together, the findings in this study shed new light on the enormous potential of *C. nutans* leaf extract for green GO reduction. As a result, this environmentally friendly method can help to reduce reliance on chemical materials.

Keywords: Green synthesis; reduced graphene oxide; graphene oxide; *Clinacanthus nutans* and Leaf extract

Luminescent nanoparticles from cocoa pod husks as potential bioimaging tools of cancerous cells

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This study conducted was to investigate the properties of the nanomaterials known as luminescent nanoparticles (LNPs). These LNPs was synthesized with an organic precursor known as cocoa pod husk (CPH). Malaysia. has a large agricultural potential that can support economic activity. Rubber, palm oil, and cocoa are major agricultural export in Malaysia. Polyphenols are naturally occurring antioxidants found in foods like fruits and vegetables. They have been linked to numerous health benefits. Luminescent nanoparticles (LNPs) have attracted a great attention of researchers in the current era due to their unique properties. The LNPs used in this process could be obtained by a green approach such as the one-step hydrothermal method. The top-down synthesis method of LNPs has side effects such as toxicity which are still a major concern while using as pharmaceutical excipients or drug delivery transporter. Owing to that, herein we proposed green LNPs from agricultural waste for bio-applications. Green technology opens new windows as there are readily available, renewable, economic, and environmental-friendly. The synthesis, structural and optical properties, as well as photoluminescence mechanisms of prepared nanoparticles are reviewed. These LNPs also underwent characterization to show its presence by a facile method of reducing CPH into nanomaterials. The characterizations include UV-Vis, PL, XRD and FTIR. The results confirm the presence of these LNPs. Furthermore, the LNPs exhibited fluorescence properties when exposed to UV light. To determine the toxicity, the LNPs are tested unto brine shrimps. It proved that these LNPs are nontoxic.

Keywords: *Nanotechnology; cocoa pod husk; green synthesis; bioimaging and cytotoxicity test*

Extraction and characterization of phytochemicals from *Scolymus hispanicus* L.

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Scolymus hispanicus L. or Golden thistle belongs to the family Asteraceae and is an edible medicinal plant found in several countries worldwide. It is one of the traditional medicinal plants that has been in use for decades to treat various diseases by the local residents as first aid treatment in the villages of Turkey. Recently, it has been revealed that *S. hispanicus* can be harvested throughout the year. In this study, the field experiment was conducted at the research and experimental unit of Aegean Agricultural Research Institute, Izmir, Turkey, from November 2016 to July 2017. Various phytochemicals and total antioxidant activities of aerial parts, root barks, and root internal tissues of *S. hispanicus* were measured. Extraction of phytochemicals was performed by using 90% aqueous ethanol. Several phytochemical groups and antioxidant activities were analyzed qualitatively and quantitatively by colorimetric methods during the year. The results indicated the presence of major phytochemical groups such as phenols, alkaloids, flavonoids, triterpenoids, proteins, and tannins. These phytochemicals possess various beneficial health effects i.e anticancer, antiviral, cardioprotective, anti-inflammatory, anti-arthritic and antispasmodic effects. Quantitative analysis showed that some of the phytochemicals were dominant in the aerial parts while others were dominant in the root parts. Moreover, the phytochemicals were also affected by the harvesting time to a different extent. Total phenol, flavonoid, and tannin contents were higher in the aerial parts, whereas total triterpenoid contents were higher in the root parts. Similarly, total antioxidant activities were higher in the aerial parts than in the root parts. The results also showed that the concentration of phytochemicals and total antioxidant activities were affected by seasonal conditions, suggesting that cultivation conditions and harvesting time are important parameters for the synthesis of phytochemicals in *S. hispanicus* L.

Keywords: *Scolymus hispanicus* L; golden thistle; phytochemicals and antioxidant activity

***In silico* molecular docking of new xanthone derivatives as inhibitors of the Keap1-Nrf2 interaction**

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Xanthenes are heterocyclic organic compounds possessing a dibenzo- γ -pyrone structure and are versatile scaffolds to design novel compounds with diverse biological activities, depending on the type and position of substituents. Recently, natural xanthenes were reported to generate antioxidant and anti-inflammatory effects by activating the Nrf2/ARE signaling pathway, the most important cellular defense against oxidative stress. The pathway is regulated by the transcription factor Nrf2, which is negatively regulated by Keap1. Hence, we aimed to design a series of 30 new derivatives from 3-hydroxyxanthone to disrupt the Keap1-Nrf2 interaction and activate the Nrf2/ARE pathway and evaluate their binding energies using molecular docking. The physicochemical properties were evaluated and filtered for pan-assay interference compounds (PAINS) using SwissADME. Molecular docking was performed using AutoDock Vina with the crystal structure of Keap1 bound Nrf2 peptide (PDB ID: 2FLU). The xanthone derivatives were constructed using Chem3D, energy minimized by MM2 force fields, and docked into the Kelch domain of the protein. *Tert*-butylhydroquinone (tBHQ), an established Nrf2 activator was docked as a reference. All derivatives met the druglikeness criteria of Lipinski Rule of Five and were cleared for PAINS. Remarkably, all derivatives demonstrated good binding energies between -9.9 to -10.9 kcal/mol which was comparable to tBHQ (-9.6 kcal/mol). The xanthone with a 5-bromo-1,3-benzodioxole substituent (**1**) showed the best binding energy. Visualization using PyMOL and LigPlot revealed that **1** formed hydrogen bonds with Val-463, Ile-416 and Leu-559 via the xanthone's γ -pyrone oxygens and ether linker oxygen, while the benzene rings of the xanthone and benzodioxole stabilized the compound within the binding site through hydrophobic interactions with surrounding non-polar amino acids. All designed xanthenes are potential Nrf2 activators as ligands with $\Delta G_{\text{binding}}$ of less than -8.0 kcal/mol were suggested to be good Nrf2 activators. Further studies to synthesize and evaluate the biological activity of these xanthone derivatives are recommended.

Keywords: Drug design; Nrf2/ARE signaling; antioxidant and anti-inflammatory

Characterization and antioxidant activities of supercritical CO₂ extracted palm-pressed fiber oil and virgin coconut oil

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Oil palm and coconut are the main commodities in Southeast Asia. Oil palm fruits are processed and 20% of the oil is extracted, leaving behind pressed mesocarp fiber (PMF) that contain 5% to 6% remaining oil with phytonutrients. Virgin coconut oil (VCO) is extensively used to treat various skin disorders. However, PMF is normally used to generate electricity in palm oil mills. Various extraction techniques were used to recover palm-pressed fiber oil (PPFO) and VCO but phytonutrient degradation was observed, and the methods involve several processing stages. Therefore, this study aimed to extract PPFO and VCO using supercritical CO₂ and assess their physicochemical, compositional and antioxidant properties. PPFO was extracted from fresh PMF using supercritical CO₂ at 300 bar and 80°C while VCO from dried grated coconut kernel at 345 bar and 45°C. The extracted PPFO was found to contain high FFA (4.92%) and low DOBI value (1.59). The co-extracted phenolic-rich water with PPFO contributed to higher moisture content in PPFO (0.72%) than VCO (0.04%). No peroxide was detected in both oils. High iodine value detected in PPFO shows higher degree of unsaturation while its high unsaponifiable matter content correlates with its richer phytonutrients than VCO. The presence of carotenoids in PPFO (1,497 – 1,779 ppm) is responsible for its orange color and the vitamin E detected in PPFO is 50 times higher than VCO. Conversely, high amount of phytosterols and saponification value were detected in VCO. Lastly, the antioxidant activities of the methanol extract of these oils were evaluated using DPPH and ABTS radical scavenging assays. PPFO showed significantly stronger antioxidant activities than VCO with IC₅₀ value of 0.62 g/mL and 1.04 g/mL, respectively. In summary, SFE extracted PPFO and VCO contain complex natural compositions and possess antioxidant activity which leads to potential development of health supplement and anti-aging products.

Keywords: Oil Palm; fatty acids; coenzyme Q10; phytonutrients and phytosterols

Tualang honey and its silver nanoparticles reduced neuroinflammation and neurodegeneration in the hippocampus of kainic acid-induced rat

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The effect of Tualang honey (TH) was studied on kainic acid-induced neuroinflammation and neurodegeneration, however, no study has explored on its silver nanoparticles (THSN). Therefore, this study aimed to determine the effects of TH and THSN on TNF- α level and hippocampal injury on KA- induced rats. Sprague-Dawley rats were randomized into eight groups: (i) control, (ii) THSN (10 mg/kg), (iii) THSN (50 mg/kg), (iv) KA only, (v) KA+TH, (vi) KA + THSN (10 mg/kg), (vii) KA + THSN (50 mg/kg), and (viii) KA + Topiramate (TPM). All rats were pretreated orally with either distilled water, THSN (10 or 50 mg/kg), TH (1.0 g/kg), or TPM, an antiepileptic drug (40 mg/kg) five times at 12 hours intervals. Saline or KA (15 mg/kg) were injected subcutaneously 30 min after last oral treatment and all rats were sacrificed 24 hours post KA induction. Hippocampus was harvested for histological examination using fluoro jade C (FJC) staining. TNF- α level was measured using commercially available ELISA kits. The number of FJC- positive cells in hippocampal CA2 and CA3 region is significantly ($p < 0.05$) reduced in KA only group and is improved on pretreatment with TH as well as THSN. Meanwhile, the elevation of TNF- α level was significantly ($p < 0.05$) reduced in KA + THSN 10 mg group when compared with KA only group. In conclusion, both pretreatments of TH and THSN improve neurodegeneration in the rats' hippocampus. Remarkably, only pretreatment of THSN (10 mg/kg) reduces TNF- α level after KA-induced.

Keywords: Tualang honey; silver nanoparticles; hippocampus; inflammation and neurodegeneration

Killing effect of coconut oil on *Staphylococcus aureus*: an implication of *Staphylococcus epidermidis* induced fermentation

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Staphylococcus epidermidis, a commensal bacterium of human skin, possesses lipolytic activity to digest skin surface lipids into the smallest unit, fatty acids (FAs). Most of FAs hold antimicrobial properties which were important functions for protecting skin from invading microorganisms. In this study, we were interested in virgin coconut oil (VCO) that was the source of several medium-chain fatty acids (MCFAs) such as lauric acid and myristic acid. Those MCFAs products demonstrated remarkable antibacterial activity. Our results showed that crude supernatant from the culture medium of *S. epidermidis* with VCO fermentation exhibited the growth inhibition effect on *Staphylococcus aureus*, a bacterium that cause a wide range of skin diseases. A co-culture of *S. epidermidis* and *S. aureus* in a rich medium with 2.5% (v/v) VCO significantly reduced the growth of *S. aureus*, comparing to those without VCO (p-value <0.05). Moreover, time-kill assays indicated that the supernatant from culture medium of *S. epidermidis* with VCO fermentation showing an efficient antimicrobial activity against *S. aureus* after 18 hours of incubation. Our results can be concluded that the culture of *S. epidermidis* with VCO plausibly induced fermentation of naturally lipid source aiming the production of MCFAs with antibacterial activity, particularly suppression of skin pathogen *S. aureus* growth. The skin commensal bacterium, *S. epidermidis* might help to produce MCFAs from skin product containing VCO and made more benefit for protection of skin infection.

Keywords: Coconut oil; medium-chain fatty acids; skin commensal bacteria; *Staphylococcus aureus* and *Staphylococcus epidermidis*

Controllable synthesis of silver nanoparticles using brown seaweed *Sargassum polycystum* and its mosquito larvicidal potential

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A simple green synthesis method was developed to produce silver nanoparticles (AgNPs) involving the use of seaweed extract, an efficient reducing and stabilising agent. The effect of varying synthesis parameters on the size and production of AgNPs was studied using brown seaweed, *Sargassum polycystum*. pH and temperature were found to have the most impact on the size and production of AgNPs. At pH 11, the reduction of Ag⁺ ions to AgNPs occurred more rapidly, completing in 3 hours. Synthesis carried out at pH 11 produced AgNPs with much smaller diameter (7.11 ± 1.00 nm) in comparison to pH 3 (20.83 ± 1.03 nm). Higher synthesis temperatures also increased the reaction rate and produced AgNPs of smaller size (0.72 ± 0.02 nm) with lower polydispersity (PDI). Besides that, the concentration of seaweed extract and silver nitrate (AgNO₃) solution was studied to yield higher production of AgNPs with smaller size with 0.4 g/L of extract with 2 mM AgNO₃ found to yield the most favourable results. XRD and EDX characterisation of the synthesised nanoparticles verified the identity of nanoparticles to be silver metal. HR-TEM images showed that the synthesised AgNPs have a spherical shape and size range of 6-10 nm. The larvicidal potential of AgNPs was studied against Instar III *Aedes aegypti* larvae. The LC₅₀ against *Ae. aegypti* larvae were found to be 103.10 ppm at 24 hours and 46.67 ppm at 48 hours. Furthermore, the AgNPs did not exhibit any noticeable toxicity against non-target species *Artemia salina* compared to *Ae. aegypti* larvae. This study has demonstrated that controlled synthesis of AgNPs is possible by tailoring the synthesis parameters to obtain high production of AgNPs in desirable size range and that the AgNPs has the potential to be used to control *Aedes* mosquito larvae population.

Keywords: Silver nanoparticles; brown seaweed and *Sargassum polycystum*

***Cordyceps militaris* as potential therapeutic target against amyloid beta-mediated neuronal toxicity in Alzheimer's disease**

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Alzheimer's disease (AD) is recognized as a prevalent neurodegenerative disease, inflicting tremendous health and economic burden worldwide. Main pathological hallmarks of AD are associated with extensive amyloid plaques comprised of amyloid beta (A β) deposition and neurofibrillary tangles. Though cholinesterase inhibitors (ChEIs) are introduced as standard treatment against AD, their use are often accompanied with medicinal complications. Hence, attention has been shifted towards researching novel potent inhibitors capable of circumventing side effects besides ensuring drug efficacy. Despite exhibiting vast medicinal effects, reports on the neuroprotective properties of *Cordyceps militaris* (CM) are rather limiting. Hence, this study aims to elucidate the neuroprotective effects of CM against A β 42-induced neurotoxicity using SH-SY5Y neuroblastoma cell model. First, SH-SY5Y cells were pre-treated with various concentration of CM extracts or donepezil followed by A β 42 exposure. After 24-hour incubation, the effects of singular treatment of CM extracts or donepezil on cell viability were measured using MTT assay. Next, Thioflavin T (ThT) assay was conducted to evaluate the inhibitory activity of CM extracts or donepezil on A β 42 fibrillar aggregation. Furthermore, molecular docking analysis was performed to explore the interactions of CM ligands (adenosine and cordycepin) with selected AD-related target proteins (Akt and GSK3 β). Our results show that singular treatment of CM extract and donepezil enhanced the survival and growth of A β 42-treated cells. At 7.8125 μ g/mL, CM extract significantly increased A β 42-treated cell proliferation ($p < 0.01$) as compared to donepezil-treated cell proliferation at 1 μ M ($p < 0.05$). Adenosine exhibited the strongest binding energy towards GSK3 β (-8.8 kcal/mol), followed by Akt (-6.9 kcal/mol); whereas cordycepin binds most favorably to Akt (-7.1 kcal/mol) followed by GSK3 β (-6.5 kcal/mol). These results indicate that CM extract reduce A β 42 *in vitro*, and the bindings inhibit Akt and GSK3 β proteins from engaging in A β 42-mediated neurotoxicity. We believe that CM extract confer neuroprotection against A β 42-induced neuronal damage.

Keywords: Alzheimer's disease; amyloid beta; natural product; *Cordyceps militaris* and neuroprotective effects

Polyphenol-rich extract of *Hibiscus Sabdariffa* Linn. Calyx (HPE) improves cardiac function and limits hyperglycemia-induced oxidative stress and apoptosis progression in diabetic heart

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Diabetes mellitus increases the risk of cardiac functional and structural changes mediated by oxidative stress and apoptosis, leading to diabetic cardiomyopathy development. This study aimed to determine the cardioprotective effects of polyphenol-rich extract of roselle calyx (HPE) in diabetic cardiomyopathy rat model. Type 1 diabetes mellitus (DM) was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg). The non-diabetic rats (NDM) acted as control group (n=6). All rats were left untreated for four weeks. At the end of the four weeks, the diabetic rats were randomly divided into three groups: diabetic group (DM), diabetic group treated with HPE (DM+HPE) (100mg/kg) and diabetic group treated with metformin (DM+MET) (150mg/kg). Metformin group served as a positive control. Treatment for HPE and metformin were given daily for another four consecutive weeks. The results showed that HPE treatment was able to improve cardiac function, whereby the left ventricular developed pressure (LVDP), cardiac contraction rate (+dP/dt), and coronary flow were increased significantly ($P < 0.05$) while cardiac relaxation time (tau) was significantly decreased ($P < 0.05$) in DM+HPE compared to DM. However, the left cardiac relaxation rate (-dP/dt) was not significantly increased in DM+HPE group. HPE supplementation also attenuated cardiac oxidative damage as indicated by low malondialdehyde and advanced oxidation protein product as well as increased in glutathione level, catalase and superoxide dismutase activities. These findings correlate with reduction in cardiac apoptosis, exhibited by the increment of BCL-2 expression and reduction of Bax and Bax/BCL-2 ratio significantly ($P < 0.05$). Histological analysis showed marked decrease in cardiomyocyte hypertrophy and fibrosis in DM+HPE compared with the DM group. In conclusion, HPE showed a potential in improving diabetic cardiomyopathy condition via alleviating oxidative damage and apoptosis.

Keywords: *Diabetic cardiomyopathy; cardiac dysfunction; structural changes; antioxidant and roselle*

Pharmacokinetic interactions of triphala and midazolam *in vitro* and *in vivo*

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Triphala is well-known as an ayurvedic medicinal herbal formulation consisting of three fruits such as *Emblica officinalis*, *Terminalia bellerica*, and *Terminalia chebula*. The pharmacokinetic interactions with cytochromes P450 (CYP) activity of gallic and ellagic acids, the major phytochemicals in Triphala extracts, have been reported. Thus, the potential of herb-drug interactions of Triphala is concerned. The objectives of this study were to determine the *in vitro* and *in vivo* inhibitory effects of Triphala extract on CYP3A4 activity on pharmacokinetic evaluation. The *in vitro* inhibitory effect and enzyme kinetic types of inhibition of Triphala were investigated on drug metabolizing enzymes of CYP3A4 using testosterone as a probe substrate in human liver microsomes. Furthermore, the effect of Triphala on P-glycoprotein (P-gp) transporter was determined using Caco-2 cells. The interaction of Triphala on CYP3A4 activity was confirmed using midazolam, as CYP3A4 probe, in rats. The pharmacokinetics of midazolam were determined after oral and intravenous administrations of midazolam alone and combination with oral administration of Triphala extract at dose of 100 and 500 mg/kg. We found that Triphala inhibited CYP3A4 activity with an IC₅₀ value of 23.64 ± 9.18 µg/mL and exhibited a non-competitive inhibition with K_i value of 21.98 µg/mL. However, Triphala did not change P-gp activity in Caco-2 cells. Oral co-administration with Triphala (500 mg/kg) in rats significantly increased AUC_{0-2.5hr} and C_{max} of midazolam and decreased V_{dss/F} and CL_{tot/F} compared with control group (p<0.05). Moreover, bioavailability (F) of midazolam was enhanced by about 12%. In contrast, co-oral administration of Triphala did not change pharmacokinetics of midazolam when it was intravenously administered. These results demonstrate that Triphala extract showed pharmacokinetic interaction with midazolam probably due to inhibitory effect on CYP3A4. Further clinical studies are needed to examine interaction in human.

Keywords: Pharmacokinetic interaction; Triphala extract; midazolam; cytochrome P450 and p-glycoprotein

Synthesis and anti-inflammatory activities of new amine derivatives from 1,3,6-trihydroxyxanthone

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In responses to inflammatory process, inflammatory mediators and cytokines are released to combat pathogens and repair damaged tissue. However, excessive production of these signals can prolong the inflammation which leads to the development of chronic inflammations. Therefore, the discovery of new lead compounds for anti-inflammatory drugs via suppressing pro-inflammatory cytokines production is crucial. This project aimed to synthesize new xanthone derivatives that possess anti-inflammatory effects from 1,3,6-trihydroxyxanthone. A total of nine new xanthone derivatives with alkyl, benzyl, halogenated benzyl and methoxy benzyl groups substituted through amine were synthesized, purified and structurally characterized by spectroscopy methods including NMR, MS and FTIR. All the xanthenes were evaluated for their anti-inflammatory activities through NO production inhibition assay in LPS-induced RAW 264.7 cells. The non-cytotoxic xanthenes demonstrated good NO inhibitory effect with the IC₅₀ values ranging from 48.50 – 66.72 μ M, which are 2-3 times stronger than the standard drug, diclofenac sodium. The xanthone with dibromobenzyl substituent attached through the amine group showed the strongest NO inhibitory effect and was further evaluated for its suppression effect on the pro-inflammatory cytokines production of IL-1 β , TNF- α , and IL-6 at a concentration of 100 μ M. This xanthone exhibited stronger inhibition effect towards IL-1 β (81%) and TNF- α (75%), postulating the brominated aromatic ring tend to form π -interactions within the active site of IL-1 β and TNF- α . In contrast, IL-6 is likely to form hydrogen bond only with the hydroxyl group from xanthone, which is also expected to be present in IL-1 β and TNF- α . The results suggest that the presence of withdrawing group in the aromatic ring such as bromine atom could contribute to the anti-inflammatory activities of the xanthone. Further studies on the protein expression of LPS-induced MAPK phosphorylation and nuclear translocation of NF- κ B (p65) are recommended to elucidate the anti-inflammatory signaling pathway of the xanthone.

Keywords: *Chromatography; cytokines; nitric oxide; structure elucidation.*

Iranian medicinal plants role in COVID-19 prevention

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SARS-CoV-2 is the third pandemic of the 21st century, causing an alarming number of deaths in 209 countries worldwide. Vaccines, serums, monoclonal antibodies, immunomodulators, and antiviral drugs are the most promising options to prevent the disease. Moreover, different herbal medicines with promising results have been used alone or combined with conventional drugs to treat infected patients. In this review, the keywords Herbal Medicine, Phytotherapy, Traditional Medicine, Complementary Medicine, Alternative Medicine, Integrative Medicine, Prevention, COVID-19 Prevention and, SARS-CoV- 2 were used in combination to search for relevant articles indexed in Scopus, ScienceDirect, Google Scholar, and PubMed databases by May 2021. The retrieved articles were screened and the full texts were reviewed. According to the studies, plants such as Thyme, Green tea, Echinacea, Aloe vera, Black seed, Eucalyptus, Chicory, Cloves, Licorice, Garlic, and Saffron are effective in preventing COVID-19. These plants prevent COVID-19 effectively through strengthening the immune system, anti-inflammatory effects, and inhibiting virus attachment to the host cell. Plants categorized as Host cell receptor-attaching Inhibitors, inhibit viral protein transport in MDCK cells and attaching to ACE by 70-100%. Immune system boosters and modifiers are able to strengthen the immune system by increasing the proliferation of lymphocytes and white blood cells, enhancing phagocytosis, modulating the expression of cytokines, enhancing humoral and cellular immunity. Antioxidants are also involved in destroying CD4 T cells by apoptosis. Antioxidants along with factors interfering with the harmful effects of cytokines and lipid mediators, may play a role in treating viral diseases. As mentioned, many medicinal plants available in the Iranian medicinal plants market have *in vitro*, *in vivo* and clinical trials evidence for potential using in COVID-19 prevention. Since these plants are available and popular, additional clinical studies under physicians and pharmacists' supervision can elevate public health.

Keywords: *Phytotherapy; prevention; COVID-19; herbal medicine and alternative medicine*

Diagnostic imaging capabilities of neutron-activated Samarium-153 polystyrene microspheres as a theranostics agent after direct intra-tumoural injection on liver tumour bearing rats

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Neutron-activated Samarium-153 (¹⁵³Sm) is ideal for theranostics applications because it emits both beta ($E_{\beta_{\max}}=810$ keV) and gamma ($E_{\gamma}=103$ keV) radiations with a desirable physical half-life of 46.3 h and it can be produced by low-cost neutron activation technique. Samarium oxide loaded polystyrene (¹⁵³Sm2O3-PS) microspheres has been proposed for intra-tumoural theranostics treatment of solid tumours. This study aimed to evaluate the diagnostic imaging capabilities of the microspheres on tumour-bearing rats. Three male Sprague-Dawley rats (150 – 200 g) were implanted with N1-S1 hepatoma cell line orthotopically and once the liver tumour size larger than 5 mm, the rats received an intra-tumoural injection of 37 MBq ¹⁵³Sm2O3-PS microspheres into the centre of the liver tumour. The rats were subjected to static gamma imaging at 24 h, 48 h, 72 h and 168 h post-injection using a compact gamma camera to assess the biodistribution pattern. Clinical single photon emission computed tomography/computed tomography (SPECT/CT) system was then used to scan the rats at 120 h post-injection. Static gamma images clearly showed the distribution of ¹⁵³Sm radioactivity in the tumour at 24 h post-injection with no significant leakage of the microspheres from tumour up to 168 h (nearly after 4 half-lives). The SPECT/CT images similarly displayed a high uptake of ¹⁵³Sm radioactivity in the liver tumour at 120 h post-injection for all the rats. Additionally, the injection site of the ¹⁵³Sm2O3-PS microspheres was visible on the CT images. This has added to the benefit of ¹⁵³Sm as a CT contrast agent. Neutron-activated ¹⁵³Sm2O3-PS microspheres demonstrated excellent diagnostic imaging capabilities after intra-tumoural injection on tumour-bearing rats. Biodistribution and injection site of the ¹⁵³Sm2O3-PS microspheres can be clearly visualised on SPECT and CT images, respectively. Further studies on the therapeutic efficacy of the microspheres are needed to evaluate its feasibility as a theranostics agent.

Keywords: *Diagnostic imaging; intra-tumoural; neutron-activated; Samarium-153 and theranostics*

Minocycline modulates astrocytes activation in Lipopolysaccharide-induced Alzheimer's disease rat model

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Lipopolysaccharide (LPS) was used to induce Alzheimer's disease. The effects of minocycline on astrocytes-activation induced LPS was not elucidated. The aim of this study is to elucidate the effects of LPS and minocycline on astrocytes in comparison to clinically approved drug memantine. A total of twenty-five male SD rats were divided into: (i) control, (ii) LPS, (iii) LPS-treated with minocycline 25 mg/kg, (iv) LPS-treated with minocycline 50 mg/kg, and (v) LPS-treated with memantine 10 mg/kg. Minocycline and minocycline treatments were administered intraperitoneally once daily for 2 weeks and LPS was injected once on day 5. Immunohistochemistry and Western blot for glial fibrillary acidic protein (GFAP) astrocyte marker were performed to measure its expression and level in hippocampus and cortex. This study showed that LPS significantly activated astrocytes ($p > 0.05$). Minocycline treatment dependent on dose modulated astrocytes activation ($p > 0.05$) comparable to memantine effect. Dependent on dose, minocycline reduced astrocytes activation in LPS rat model of AD in comparison to memantine. Thus, minocycline has a potential preventive-therapeutic effects in Alzheimer's disease.

Keywords: *Astrocytes; lipopolysaccharide and minocycline*

Uncovering the immunogenicity of Neonatal Nav1.5 by utilising serum samples from 4T1 orthotopic mice and breast cancer patients

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Neonatal Nav1.5 (nNav1.5) potentiates breast cancer (BCa) metastasis. However, its immunogenicity remains unexplored. The study's objective was to confirm the presence of circulating anti-nNav1.5 antibodies (anti-nNav1.5-Ab) in 4T1 orthotopic mice model and BCa patients. In the pre-clinical phase, 37 female BALB/c mice, were divided into two groups: a) control ($n=20$) b) 4T1 orthotopic mice ($n=17$). 4T1 cell injection was administered subcutaneously at the 3rd mammary fat pad of the 4T1 orthotopic mice, whereas PBS was introduced at a similar site of the control mice. After tumour development, both mice groups were sacrificed, followed by the collection of serum, 4T1 tumours and target organs. In-house indirect ELISA was conducted to detect anti-nNav1.5-Ab in the serum. Histopathology of the organs was carried out to validate BCa metastasis within the model. As for the clinical study, healthy females ($n=64$, mean age= 39.98 ± 1.79) and BCa patients ($n=64$, mean age= 47.81 ± 1.43) were recruited. Approximately 3 ml of blood was withdrawn, and the serum was separated. Similarly, an in-house indirect anti-nNav1.5- Ab ELISA assay, specifically for human antibodies, was designed. Both pre-clinical and clinical studies portrayed the positive presence of anti-nNav1.5-Ab. There was a significant difference in the absorbances of anti-nNav1.5-Ab between the control and 4T1 orthotopic mice ($P<0.0001$ ****). There were signs of metastasis on the heart, lungs, spleen, liver and kidneys of the 4T1 mice. Based on the ROC analysis, all the 4T1 mice exhibited absorbances of anti-nNav1.5-Ab above the cut-off value (>2.759). Similarly, the clinical study also reported a significant difference in the absorbances of anti-nNav1.5-Ab between healthy participants and BCa patients ($P<0.0001$ ****). The ROC analysis for the clinical study presented a cut-off value of >0.280 . Unlike the pre-clinical study, 9.38% of control samples had a reading greater than the cut-off value. In conclusion, nNav1.5 is an immunogenic protein.

Keywords: Neonatal; Nav1.5; breast cancer; metastasis and immunogenicity

Economic, clinical and humanistic outcomes (ECHOs) of type 2 diabetes: A systematic review

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The diabetes figures are projected to increase with the number of individuals with the condition rising from 19.8 million in 2013 to 41.5 million in 2035, representing a 110% absolute increase. Economic, clinical, and humanistic outcomes (ECHOs) research is essential in the improvement of clinical practice, outcome research, and disease management. Therefore, we conducted a systematic review of existing literatures that measured the cost, clinical and quality of life resulting from specific interventions among type 2 diabetes patients and explore its use in the management of type 2 diabetes. The search was done through two electronic databases PubMed and Scopus. Studies published between 2009 and 2019, and that reported the three outcomes by comparing two or more interventions were selected. The search resulted in the inclusion of 3 randomized controlled studies. The three studies fully represented the differences in the outcomes between the interventions; therefore, showing which intervention was more beneficial for the patients. The selected studies were conducted in the high middle-income countries, two were conducted in the USA and one in Singapore. The length of study was at least 6 months. Regarding the assessment of the outcomes, clinical characteristics differed between the selected studies, however, HbA1c analysis was the common considered characteristic. For the humanistic outcome, different questionnaires are currently available to measure health-related quality of life of patients. Only one study used a generic instrument, the remaining studies used a specific instrument for diabetes. The cost was estimated from the provider and insurer perspective. ECHOs model provide sufficient knowledge on the outcomes resulting from any intervention, therefore, this model is important in the improvement of diabetes management. However, it is time consuming and expensive to conduct.

Keywords: *Type 2 diabetes mellitus; clinical outcome; humanistic outcome; economic outcome and cost of illness*

Electron Paramagnetic Resonance (EPR) as a biophysics method: Future of biodosimetry

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According to the dangers of nuclear plant accidents such as Chernobyl and Fukushima and hazards of terrorist sabotage, exist a trustworthy, accurate and ubiquitous biodosimetry system is more necessary than ever. EPR or electron paramagnetic resonance is a biophysics dosimetry that is accomplished by changing the magnetic behaviour of materials due to radiation can measure the absorbed dose in different biological and non-biological materials. In this review, we tried to have a comprehensive survey about EPR to reveal strengths and weaknesses of it. This study is a systematic review based on articles, books and studies available in scientific databases such as PubMed, Web of Science, Scopus, Springer and Google Scholar from 2000 to 2021. The criteria for entering (reviewing) the types of studies in this article were the time of publication and the appropriate quality of articles, thematic relevance and keywords. Generally, EPR has beneficial applications in biodosimetry because of being retrospective (about 10 years), get fast results (about 5 Minute), widespread measurable absorbed dose range, good linear relationship in the response-dose curves via tooth, fingernail, toenail, chitin and so on. Although, this method can be used for natural materials such as chalk and cane sugar. EPR, on the other hand, requires expensive equipment and includes a number of limitations (weaknesses), such as the fact that it requires a higher resolution at a minimum measurable dose threshold (>0.5 Gy). The effectiveness of the EPR can be screened on an outpatient basis in the event of a nuclear disaster or terrorist attack, so that people who have actually been irradiated can be identified from those who have not been exposed to radiation, and precautions must be taken. The future development and upgrading of such a technique in biodosimetry will be a major step in promoting health.

Keywords: *biodosimetry; electron paramagnetic resonance; nuclear accidents and radiation exposure*

Gene Expression and biodosimetry perspective

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Biodosimetry is an approach for dose estimation after exposure to ionizing radiation by means of changes in biological endpoints or biomarkers in order to ensure efficient use of medical resources following a radiological incident. The most frequently used method of biological dosimetry at present is the dicentric chromosome assay (DCA), which is poorly suitable, because this method is time consuming, laborious and requires experienced personnel. In the present study, we reviewed the applicability of a gene expression signature for the estimation of exposure dose as well as the time elapsed since irradiation. This study is a systematic review based on articles, books and studies available in scientific databases such as PubMed, Web of Science, Scopus, Springer and Google Scholar from 2000 to 2021. The criteria for reviewing the types of studies in this article were the time of publication of articles, thematic relevance and keywords. Gene expression analysis is a time-efficient biodosimetry approach, hence it is an appropriate method for triaging patient in radiation accidents. Different genes that response to radiation can be used in this method. In previous studies different genes have been introduced as biomarkers including: GADD45A, ACTA2, AEN, ASCC3, BAX, DDB2, BBC3, CD70, FDX. These genes are involved in various cellular processes including: apoptosis, DNA repair, cell proliferation, cell cycle regulation, metabolism and cell cycle arrest. One of the disadvantages of this approach is highly dynamic and transient nature of signal. It means that gene expression alterations can be reduced within time so knowledge of the time between exposure and measurement is essential for correct dose estimation. According to what has been said, there are convincing reasons to state that analysis of gene expression is important for future use as one of the most reliable, well established and practical biodosimetry methods.

Keywords: *biodosimetry; dose estimation; gene expression and radiation exposure*

Different domains of dengue research in the Philippines: A systematic review and meta-analysis of questionnaire-based studies

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Dengue is the world's fastest-spreading mosquito-borne viral disease, affecting people all over the world, including Southeast Asia. This study includes a comprehensive summary of questionnaire-related dengue studies conducted in the Philippines, as well as an assessment of the surveys' questionnaire reliability and validity. The methodology, research design, search strategy, and selection criteria were all developed using a review protocol created by a panel of qualified academic reviewers. Between March and June 2020, a comprehensive literature search was undertaken in PubMed, EMBASE, MEDLINE, and ScienceDirect, as well as other significant electronic biomedical databases. The preferred item reporting approach was a systematic review and meta-analysis (PRISMA). There were 34 peer-reviewed dengue-related KAP papers found, 15 of which matched the inclusion criteria and were published between 2000 and April 2020. Knowledge (68.89), attitude (49.86), and preventative behaviour each had a poor mean score based on the meta-analysis (64.69). Most respondents were well-versed in the major clinical signs of dengue. Worryingly, 95% of respondents had negative attitudes on dengue prevention, believing that it was impossible and that it was not their responsibility to implement preventive measures. Surprisingly, television or radio was cited as the primary source of dengue information (range 50–95%). Finally, only five articles (33.3%) piloted or pretested their questionnaires before surveying, with three of them reporting Cronbach's alpha coefficient (range 0.70 to 0.90). According to the findings, we need the active participation of local communities, complete engagement of healthcare workers, promotion of awareness programmes, and access to safe complementary and alternative medicines to battle the growing public health danger of dengue in the Philippines. Importantly, the psychometric properties of any survey questionnaire should be thoroughly examined before being used in a survey.

Keywords: *Dengue; Philippines; knowledge; prevention and systematic review*

Exogenous metabolite-polymyxin B combination against *Klebsiella pneumoniae* based on metabolite feeding with *in-silico* genome-scale metabolic modeling and *in-vitro* static time-kill studies

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Klebsiella pneumoniae is an opportunistic pathogen that often causes antimicrobial-resistant healthcare-associated infections. To combat multidrug-resistant *K. pneumoniae* with limited newer antibiotics available, polymyxins have resurged as the last-line therapy. Combination of polymyxin B with antibiotic adjuvant, exogenous metabolite is a promising approach to rescue activity of this last-resort antibiotic. Exogenous metabolite feeding demonstrate ability to alter metabolic response through regulation of metabolic flux. To this end, four genome-scale metabolic models (GSMMs) were constructed to elucidate the metabolic adaptation in *K. pneumoniae* ATCC 10031, 700603, 700721 and BAA-2146 isolates upon addition of seven metabolites. Metabolic simulation revealed that addition of 3-phosphoglycerate (3pg), D-ribose 5-phosphate (r5p), uridine 5'-diphospho-*N*-acetylglucosamine and *Sn*-glycerol 3-phosphate stimulated flux of central carbon metabolism. In addition, metabolic flux of purine and pyrimidine metabolism were also upregulated upon addition of 3pg and r5p. This resulted in increased ATP utilization, increased metabolic burden to the bacteria and may disrupt the cell hemostasis. Furthermore, static time-kill studies showed additive and near additive antibacterial activity against *K. pneumoniae* through feeding of metabolite 3pg and r5p combination with polymyxin B, respectively. Exogenous metabolite feeding induced metabolic modulation offers an attractive approach to improve antibiotic efficacy. This study involves integration of *in-vitro* antimicrobial pharmacodynamics experiment and *in-silico* network modelling to discover a novel exogenous metabolite-antibiotic combination.

Keywords: Metabolite feeding; Genome-scale metabolic modelling; antibiotic adjuvant; polymyxin and *Klebsiella pneumoniae*

Health perceptions influence self-care behaviour in hypertension prevention

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Self-care behaviour is vital in preventing hypertension. Preventive behaviour can be influenced by several theoretical constructs such as perceived severity, motivation, barriers, and self-efficacy according to the Health Belief Model (HBM). Therefore, this study aimed to examine predictors that influence self-care behaviour through an expanded application of HBM, and to identify common motivators and barriers faced in adopting these strategies. A web-based survey was administered to Malaysians residing in Selangor or Kuala Lumpur without formal diagnosis of hypertension. The survey evaluated hypertension knowledge, HBM constructs and frequency of self-care behaviour. From 238 valid responses, statistical analyses showed that perceived motivation and barriers significantly predicted self-care behaviour ($R^2 = 0.485$, $p < .001$). Further analysis of self-care behaviour frequency revealed that an alarming majority (79.5%) displayed poor habits of reducing intake of salt and foods high in saturated or trans fats, followed by having regular blood pressure checks (58%). Collectively, the lack of time to prepare healthy foods (48.1%) and lack of healthy food choices in school or workplaces (32.6%) were the top two barriers in preventing good eating habits. Those with poor blood pressure checking habits mostly displayed an apathetic attitude, as some had never thought about it (71%), or had assumptions of their health status (54.5%). In summary, perceived motivation and perceived barriers are the main determinants of self-care behaviour. Development interventions or education programmes that selectively target motivations and barriers related to the prevention of hypertension, specifically in reducing salt and calorie intake, and improving blood pressure measurements in the community may prove most efficacious to reduce the burden of hypertension.

Keywords: *Health belief model; high blood pressure and preventive strategies*

Tocotrienol rich fraction promotes 2-cell embryo retrieval in ovalbumin-induced asthma model

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Embryo quality and quantity are associated with successful preimplantation embryonic development. They may be affected by allergen-induced respiratory exacerbation via inflammatory-oxidative stress (OS) mechanism. The role of tocotrienol rich fraction (TRF) as a dietary antioxidant, on the quality and quantity of embryos in such exacerbation is poorly understood. Here we reported the effect of TRF on the quality and quantity of 2-cell embryos in ovalbumin (OVA)-induced asthma mice. Female BALB/c (5 w/o, 20–25 g) were randomly divided into control (PBS), asthmatic (OVA) and intervention (TRF+OVA) groups. Over a 24-day treatment duration, animals were induced with allergic asthma through a series of sensitization, superovulation and challenge followed by methacholine (MCh) test. TRF were concomitantly supplemented in the intervention group. Following treatment, animals were euthanized. Two-cell embryos were retrieved and cultured *in-vitro*. Serum 8-hydroxydeoxyguanosine (8-OHdG), total antioxidant capacity (TAOC), interleukin (IL)-4 and immunoglobulin (Ig)-E were measured using ELISA. 150 µg/mL OVA reduced the number of retrieved 2-cell embryos compared to control ($p < 0.0001$), accompanied by increased serum 8-OHdG ($p < 0.0001$), IL-4 ($p < 0.0001$), Ig-E ($p < 0.0001$) but decreased TAOC ($p < 0.0001$) suggestive of inflammatory-OS. However, TRF supplementation raised the number of retrieved 2-cell embryos in the intervention group ($p < 0.0001$), accompanied by decreased serum 8-OHdG ($p < 0.0001$), IL-4 ($p < 0.0001$), Ig-E ($p < 0.0001$) but increased TAOC suggestive of normalised inflammatory-OS. Our results indicated that 60 mg/kg bw TRF improved the number of retrieved 2-cell embryos in allergen-induced maternal asthma condition, possibly through alleviating inflammatory-OS. Further investigation into identifying the properties of TRF is paramount to clarify its capability in ensuring successful cleavage stage division and development in asthma model.

Keywords: Embryo; antioxidant; oxidative stress; inflammation and asthma

Knowledge, use, and attitudes of complementary and alternative medicine among dental students in Iran: A questionnaire based study

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In recent years, complementary and alternative medicine (CAM) has become one of the most widely used fields of medicine among patients, which necessitated awareness of health care staff in regard to CAM. However, to date, little is known about the knowledge of Iranian dental students toward CAM. Therefore, the aims of this study were to examine the knowledge, attitude towards, and usage of CAM among dental students, to identify the source of acquired information about CAM, and to explore recommended traditional Iranian medicine treatment methods. This national cross-sectional study was carried out to collect data from dental students enrolled in Complementary and Persian medicine university courses from April to August 2020. An online self-administered questionnaire was utilized to attain the aims of the study. Data from 119 participants were analyzed using descriptive and analytic statistics. Most participants had little knowledge about different methods of traditional Iranian medicine. The lowest level of knowledge was related to combination herbal medicines (with a frequency of 43% no information response). Among the students who completed the questionnaire, only 2 Students (1.7%) used cupping reported classic training courses as a source of getting information. The majority of subjects (74.8%) declared to have used maintaining health based on temperamentology, stated that they got familiarized with these methods by media and the Internet. The most frequent reason reported for traditional Iranian medicines use was the adjuvant treatment method. The most and the least recommended traditional Iranian medicine methods were nutritional recommendations as adjuvant treatment method (59.7% suggestions) and leech therapy (64.7% no recommendations). Overall, this study showed poor knowledge and usage of CAM among Iranian dental students. More broadly, in the future, further research is needed to investigate the capacity of CAM for integration into the dental university curriculum.

Keywords: *Complementary and alternative medicine; dental students; knowledge and use*

The effects of hypoxia inducible factor prolyl-hydroxylase inhibitors on human lung cancer progression

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Hypoxia is defined as low oxygen levels in tissues that can occur due to low atmospheric oxygen levels or in diseases such as cancer. The transcription factor hypoxia-inducible factor, HIF, dictates the cellular transcriptional response to hypoxia, activating genes that are responsible for adaptations to low oxygen levels such as erythropoietin and vascular endothelial growth factor. At normal oxygen levels, HIF is regulated by HIF prolyl-hydroxylases (PHD), leading to the degradation of HIF and prevention of an unnecessary hypoxic response during normoxia. PHD inhibitors have been developed by the pharmaceutical industry to activate the HIF pathway to drive erythropoiesis. These inhibitors have been clinically-trialed in anaemic patients, and some have gained approval for medical use in selected countries. A clinical trial has begun testing one inhibitor, Roxadustat, on chemotherapy-induced anaemia in non- myeloid cancer patients. Although there is a lack of research on the effects of clinical inhibitors in cancer, some studies have shown that the more selective clinical PHD inhibitors stop cancer progression, while pan-inhibitors seem to promote cancer. In this study, we study the effects of a specific inhibitor (IOX2), two clinical inhibitors (Roxadustat and Daprodustat) and a pan-inhibitor (DMOG) on lung cancer cell progression. Human lung cancer cell line A549 was treated with the inhibitors and assayed for cell proliferation and migration as a measure of cancer progression. Preliminary results show that Roxadustat reduces lung cancer cell proliferation, while Daprodustat, IOX2, and DMOG increase proliferation. Roxadustat increases cell migration at concentrations of 100 and 200 μ M. While this unveils promising aspects for the use of Roxadustat as an anti-cancer drug, the same drug may induce metastasis. This study will highlight the importance of identifying the differing properties between specific and non-specific PHD inhibitors that would result in contrasting results on cancer progression.

Keywords: Hypoxia; HIF1- α ; lung cancer and PHD inhibit

The use of dietary supplement among the elderly in Yangon, Myanmar

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The use of dietary supplement (DS) among elderly has increased worldwide. Little previous research in this area has been found in Yangon, Myanmar. This survey research aimed to explore the prevalence of DS use and factors associated with DS use among the elderly in Yangon, Myanmar. Two hundred elderly were systematic sampling from four wards in South Okkalapa Township, Yangon. Face-to-face interviews with a structured questionnaire were conducted in February, 2020. Descriptive statistics, chi square test and a binary logistic regression were used for data analysis. Findings showed that most of elderly (71%) used DS in the last three months. The top three frequently used were multivitamins and minerals, vitamin B complex, and a combination of ginseng and multivitamins/minerals (59.9%, 17.6%, 15.5% respectively). The majority used only one DS, once daily, and between one and five years. Nearly half of them took DS to promote their health. They received DS from purchasing at pharmacies (45.1%), obtaining from family/relatives and friends (33.2%), and hospitals (18.5%). Nearly 75% reported that they consulted with their healthcare providers when taking DS. There were no any associations among independent variables (demographic factors, health behaviors, as well as health status) and DS use of elderly. The prevalence of DS use among the elderly in Yangon, Myanmar was high. Thus, some interventions by healthcare organizations might be created to raise awareness of appropriate use of DS, especially for the elderly who are at high risk group.

Keywords: *Dietary supplement; elderly; prevalence and Myanmar*

Chemo-radioembolization Therapy: A novel biodegradable formulation of microspheres loaded with radioactive samarium-153 and doxorubicin

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Liver cancer is the 6th most common cancer and the 4th leading death of cancer worldwide. Its high mortality rate is primarily due to late diagnosis when treatment options are limited. Chemoembolization and radioembolization are both encouraging treatments for advanced stage liver tumors. The combination of chemotherapy, radiation therapy and embolization aim to attain the collaborative anti-tumor effects. However, the procedures are currently administered at separate occasions. This study aims to develop a novel hybrid formulation that contains both radioactive agent, Samarium-153 (Sm-153) and chemotherapy drug, Doxorubicin (Dox) for intraarterial chemo-radioembolization of liver tumors. Biodegradable polyhydroxybutyrate-co-3-hydroxyvalerate (PHBV) microsphere loaded with non-radioactive ¹⁵²Sm₂O₃ (12.80±0.01%) and Dox (66.00±0.12%) was synthesized via solvent evaporation method. The formulation was then sent for neutron activation using a research reactor at the Malaysia.n Nuclear Agency. The physicochemical properties (i.e., size, morphology, density, thermal stability, Sm-153 and Dox concentration) were analyzed before and after neutron activation. Radioactivity and radiochemical purity were measured using a hyper-pure Germanium radiation detector. The radiolabeling efficiency and in-vitro Dox release study was conducted in phosphate buffered saline at 37°C for 430 hours. The hybrid Sm-Dox-PHBV microspheres showed smooth and spherical structure with a mean diameter of 33±1.05 µm. The specific radioactivity of Sm-153 was 4.20±0.16 GBq/g after 6 hours neutron activation in a neutron flux of 10¹² cm⁻²s⁻¹, and the concentration of Dox was 1.89±0.36 mg/g. The microspheres have shown thermal stability up to 150°C. The in-vitro release study showed a cumulative release of Dox of 41.5 ± 0.15% at 430 hours. A hybrid microsphere formulation loaded with both radioactive Sm-153 and chemotherapy drug, Dox was successfully developed in this study. The Sm-Dox-PHBV microsphere demonstrated desirable physicochemical properties as a chemo-radioembolic agent for liver cancer treatment. Further studies are needed to study the cytotoxicity and anti-cancer effects of the formulation.

Keywords: chemo-radioembolization; liver cancer; Samarium-153; Doxorubicin and hybrid microspheres

Hospital pharmacists' perspectives on antimicrobial resistance management in Thailand

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Since 2017, antimicrobial resistance (AMR) management has been one of the key performance indicators (KPIs) of tertiary public hospitals in Thailand. Pharmacists are an integral part of a multidisciplinary team combating AMR in hospitals. There has been no previous research on Thai pharmacists' perspectives on AMR management. The purpose of this study is to investigate hospital pharmacists' opinions, attitudes, and job satisfaction on AMR management as well as the relationships between variables. A cross-sectional survey was conducted. Self-administered questionnaires were posted to both public (n=1,005) and private (n=293) hospitals across Thailand. Between April and June of 2021, data was collected. Descriptive statistics, Mann Whitney U test, and Kruskal Wallis test were used for analysis. A total of 249 pharmacists who were actively involved in AMR management in their hospitals completed questionnaires. The response rate was 19.18%. The respondents' average age of was 37.20±8.2 years. Most of pharmacists (77.5%) were female. The average number of years spent as a hospital pharmacist was 12.3±8.2. Approximately 63% of them believed it was worthwhile to work in AMR management and they liked their current job. Secondary hospital pharmacists had more favorable attitudes than tertiary hospital pharmacists. In terms of job satisfaction, pharmacists in secondary hospitals reported higher level of satisfaction with their salary/compensation than pharmacists in the tertiary hospitals (p=0.000). Regarding gender, work competency and career path were found to be statistically significant (p < .05). Males expressed greater satisfaction with work competency than females, whereas females expressed greater satisfaction with their career path. It can be stated that the hospital pharmacists were optimistic about AMR management. While the majority of them expressed satisfaction with their jobs, improving some factors such as ability and career path is necessary to retain health care professionals in hospitals.

Keywords: *Antimicrobial resistance; attitude; hospital pharmacist; job satisfaction and opinion*

Pain control and adverse effects of morphine among cancer pain patients in National Cancer Institute, Malaysia

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Cancer pain remains inadequately treated despite management and treatment guidelines. In Malaysia, only 24% of cancer pain patients received regular analgesia in 2008 and there is limited recent clinical data available. The aim of this study was to determine the pain control and adverse effects of morphine among cancer pain patients. A cross-sectional study was carried out in the Oncology and Palliative wards of National Cancer Institute from August 2020 to July 2021. A total of 38 patients were recruited. Pain control was determined using Brief Pain Inventory questionnaire. Incidence and severity of morphine adverse effects were quantified using a validated questionnaire with a Likert scale of 0 (not at all) to 4 (overwhelming). Cancer pain patients who received morphine achieved a mean pain relief of $81.6 \pm 22\%$ and 76.3% reported with significant pain relief with morphine. Enjoyment of life had the highest mean for pain interference score of 6.03 ± 3.94 . 92.1% of patients presented with at least one adverse effect, with the most common being constipation (39.5%) and drowsiness (26.3%). There were significant associations between ethnicity and the incidence of urinary retention ($p=0.004$), the severity of vomiting ($p=0.044$); hallucination ($p=0.035$); and headache ($p=0.004$) adverse effects and pain control of patients ($p<0.05$). Gender was significantly associated with the incidence of dry mouth ($p=0.03$) and severity of insomnia ($p=0.04$). This study highlighted that 23.7% of patients had insignificant pain relief and 92.1% were reported with adverse effects with morphine. The pain control and adverse effects were significantly influenced by ethnicity and gender. Therefore, the optimization cancer pain treatment regimen using precision medicine is crucial to enhance the quality of life among cancer pain patients.

Keywords: Cancer; morphine; pain control and adverse Effects

Impact of motivational interview on medication adherence among renal failure patients on hemodialysis: A pilot study

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Poor medication adherence is the major issue causing frequent hospitalization and mortality among hemodialysis (HD) patients. Motivational interviewing (MI) is an efficient method to cause behavioral changes in a patient with chronic disease. However, its impact in improving medication adherence untested among HD patients. This study aimed to evaluate the impact of motivational interviewing to improve medication adherence among HD patients. This pre-post analytical cohort study was conducted by assessing the patients' medication adherence using 11-item General Medication Adherence Scale (GMAS). This study was conducted in a tertiary hospital in Kuala Lumpur for 3 months. Patients who had mental disorders, surgical interventions, malignancies, cognitive impairment, and pregnant women were excluded from the study. The recruited patients received two sessions of motivational interviewing, where the motive is to identify and break the barrier to medication adherence using a motivational approach. The patients' adherence was measured before and after interventions and analyzed using the paired t- test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 25, Chicago, IL, USA) ($p < 0.05$ was considered significant). A total of 34 patients were included in the study. The mean age of the patients was 61 ± 4.83 (years). Most (58.8%) of patients included in the study were females. The mean adherence score of the patients, before the MI was 25.76 ± 3.91 and improved to 27.94 ± 2.55 after the intervention. Paired *t*-test showed a significant increase in medication adherence score, $p < 0.001$. Majority (85.3%) of the patients have adequate adherence after the motivational interview sessions. Pharmacist intervention through psychosocial method using MI skills can influence the positive behavior change among HD patients to adhere medication. The study emphasized that MI technique can result in better medication adherence which leads to better management of their disease.

Keywords: *Pharmacist; medication; adherence; motivational and hemodialysis*

The economic burden of malaria in Burundian children: An evidence for disease management in resource limited settings

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In Burundi, malaria is the leading cause of morbidity and mortality especially in children aged under five years. This results in high clinical burden of the disease; however, its economic burden remains unknown. The aim of this study was to estimate the economic burden associated with malaria in Burundi and explore the factors that can affect the costs. This study was a prospective incidence-based cost-of-illness study analysed from the societal perspective. The study included children aged under five years with malaria infection, who visited and received treatment at any of the two study health facilities in the period of November to December 2019. Data collection was done by reviewing medical and financial records and by interviewing caregivers of the patients. Micro-costing approach was used to estimate the economic costs of malaria per episode. The cost was presented in international dollars (Int\$) for the year 2019. Stepwise multiple linear regression method was applied to examine the factors affecting the costs and to generate a cost model. Eighty-five children with the average age of 29 months were included in the study. Most of them (70.5%) were treated as in-patient. The out-patient visits costed Int\$23.53, while the in-patient hospitalization costed Int\$218.28. The types of medical services, health facilities, antimalarials used, and duration of fever before seeking appropriate medical care were found to affect the costs. The model indicates that up to Int\$18.76 can be saved per malaria episode if treated early (in less than 2 days). This can save up to Int\$24,257,748 per year at national level. This study demonstrated that malaria is associated with a considerable economic burden in Burundi. It will support decision makers in deciding an appropriate clinical management for malaria prevention like the community case management program.

Keywords: *Malaria; Burundi; economic and cost of illness*

Thermal oxidation of the Ti-4Al-6V alloy in different temperature for surface roughness improvement

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The Ti-6Al-4V alloy represents the gold standard metallic biomaterial implant due to its excellent biocompatibility and mechanical properties. Various techniques have been developed to modify the surface roughness to improve the surface characteristics of titanium alloy, and therefore, enhance osseointegration. The thermal oxidation method is simple and effective for enhancing surface roughness. Thus, this study aimed to produce thermally oxidised Ti-6Al-4V at different temperatures and evaluate the surface roughness. The Ti-6Al-4V plate was oxidized in normal air for 1 hour at temperatures of 500°C and 600°C to produce a thin surface oxide layer. The surface topography of thermally oxidized Ti-6Al-4V samples was evaluated using an atomic force microscope (AFM). The 2D and 3D images were obtained to evaluate the surface topography of the untreated and thermally oxidized samples. The surface roughness, Ra of the smooth surface was 0.0055µm (SD = 0.0420 µm) and thermally oxidised sample at 500°C was 0.0206 µm (0.008 µm), and 600°C was 0.0298 µm (0.0199 µm). The smooth surface roughness of the was significantly different at $p < 0.05$ from the thermally oxidised surfaces. The surface roughness of thermally oxidised at 600°C shows the highest surface roughness.

Keywords: *Thermally oxidation; Ti-6Al-4V and surface roughness*

Response of community pharmacists to the presentation of a minor ailment

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Community pharmacists are the most easily accessible healthcare providers to the general public. They can play a major role in counselling clients on the appropriate use of medicines to optimise its therapeutic outcomes. Therefore, it is essential that community pharmacists respond appropriately even to management of minor ailments. The objective of this study was to assess the response of community pharmacists to the presentation of minor ailments such as a cough. This study utilized a simulated client method. A research assistant acted as the simulated client and visited 100 community pharmacies in the Klang Valley which were randomly selected, from September to October 2018. The simulated client consulted the pharmacists on cough treatment for his father. Pharmacists' responses were recorded in a data collection form developed based on pharmacy mnemonics for the response to symptoms and OBRA'90. Most community pharmacists (97%) asked about symptoms, but only 38.0%, 25% and 13% asked about who the medicine was for, medical condition and any medication of the patient. Among these pharmacists, 68.3% were deemed to have recommended appropriate treatment to the patient but only 9% realised that it could be a drug related problem. More than 80% of the community pharmacists provided information about the dose and frequency of the medicine but none mentioned potential side effects and storage information. The present study showed that community pharmacists did not gather adequate patient's medical information before recommending a treatment nor provided comprehensive medication counselling for the management of a cough. Such practice may lead to inappropriate or ineffective use of medication besides posing potential safety concerns to the patient and a waste of financial resources.

Keywords: *community pharmacist; minor ailment; self-medication; simulated client and counseling*

Simultaneous detection of curcumin and piperine using Reversed-Phase High Performance Liquid Chromatography (HPLC)

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Curcumin and piperine has been recognized to exert synergistic effects against a variety of diseases. Thus, both compounds have been widely used in the development as potential treatment options, either as individual compounds or in combination. However, only limited studies were focused on simultaneous detection of both curcumin and piperine. The present study aimed to develop a simple, sensitive, and precise high performance liquid chromatography (HPLC) method for simultaneous detection of both curcumin and piperine. HPLC method was developed and optimized with different mobile phase-buffer ratios, at different temperature for better separation of analyte peaks. Successful simultaneous separation of both curcumin and piperine was achieved using C18 column reverse phase column with mobile phase combination of acetonitrile, methanol and 5% acetic acid at ratio of 28:34:36 v/v and flow rate of 0.9 ml/min by using 17-beta-estradiol as internal standard (IS). Further, the temperature was set at 45°C. Intraday and interday precision tests as well as accuracy tests at different percentage (80%, 100% and 120%) were performed to validate the optimized method. The simultaneous detection of both the analytes was monitored at wavelength of 275 nm. The retention time of curcumin, piperine and IS were recorded at 4.92 min, 5.91 min and 12.63 min, respectively. The limit of detection (LOD) for curcumin and piperine was at 0.35 µg/ml and 0.15 µg/ml respectively. The limit of quantification (LOQ) of curcumin and piperine was at 1.05 µg/ml and 0.146 µg/ml respectively. Moreover, the percentage of relative standard deviation (RSD) for precision and accuracy of the method was found to be less than 2%. More than 98% of recovery for both curcumin and piperine was detected. In conclusion, an optimized HPLC method was successfully developed and validated for accuracy, precision, robustness, and recovery for the simultaneous detection and estimation of curcumin and piperine.

Keywords: Curcumin; piperine; HPLC and simultaneous detection

Effect of meal timing and frequency on lipid profile in adults: An overview of systematic reviews

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Dyslipidaemia is a modifiable risk factor of cardiovascular diseases, which is the leading cause of death due to non-communicable diseases globally. There is increasing evidence to show that lipid metabolism, which is closely related to the circadian rhythm, is regulated by eating patterns. The fast-paced lifestyle has disrupted our conventional meal pattern, potentially impacting lipid metabolism. Hence, this overview of reviews intends to summarise the effect of meal timing and frequency on lipid profile by critically evaluating all available systematic reviews. Five databases were searched from inception to 19 July 2021 to identify systematic reviews that investigated the effects of interventions related to meal timing and frequency on lipid profile. Two reviewers independently screened, extracted data, and assessed quality of the systematic reviews. Fifteen reviews were included, with 12 reviews on intermittent fasting and one on breakfast skipping, night-time eating and meal frequency, respectively. Current available evidence suggests that breakfast skipping has potential detrimental impact on lipid profile while night-time eating could increase postprandial triglyceride more than daytime eating. Increasing meal frequency while maintaining the total calorie intake is reported to reduce total cholesterol and low-density lipoprotein-cholesterol. Intermittent fasting, particularly alternate day fasting and periodic fasting (i.e., 1 to 2 fast days per week), has positive impact on lipid profile compared to the usual diet. To conclude, the evidence suggests that increased meal frequency, breakfast eating and certain types of intermittent fasting lead to positive outcomes on lipid profile. Future high-quality studies with a bigger sample size are warranted to provide strong evidence of this positive association.

Keywords: *lipid; meal timing; meal frequency and umbrella review*

Effects of sugars on adsorption kinetics of gelatin extracted from black soldier flies (*Hermetia illucens*) larvae at air-water interface

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Gelatin is one of the most versatile food biopolymers widely used in the food industries whereas bovine and porcine are the primary sources for gelatin production. However, some countries banned gelatin extracted from certain sources due to religious constraints or human transmitting disease. Therefore, the alternative source of gelatin extraction is warranted. Recently, gelatin extracted from edible insects was reported to be an alternative source which is free from the drawbacks. Black soldier fly (*Hermetia illucens*) larvae (BSFL) have been found as a potential alternative source of gelatin because of its high protein content, abundant in sources, cost-saving, and eco-friendly. In this study, BSFL gelatin was extracted under 0.05 M acetic acid pretreatment condition. SDS-PAGE analysis of the extracted BSFL gelatin showed a major protein fraction at 75 kDa. Minor protein fraction was observed from 75 kDa to 17 kDa. All gelatin-based food products in the market are generally added with sugars such as glucose or sucrose to improve the functional properties of gelatin. However, overconsumption of sugars leads to overweight and obesity that could increase the risk of developing type 2 diabetes and cardiovascular disease. Hence, non- or low-calorie sweeteners (NLCS) are used in this study to replace the 'energy-dense' free sugars. Majority of NLCS are chemically synthesized and only required in small quantities to improve the sweet flavour in food products without providing enormous energy intake. The sugars chosen in this study were xylitol and acesulfame-K. Therefore, the study herein is to investigate the adsorption kinetics mechanisms of extracted gelatin from BSFL at Air-Water interface in addition of sugars.

Keywords: *Acesulfame-K; black soldier fly; edible insect; gelatin and xylitol*

Physicochemical and functional properties of oil palm leaves protein hydrolysate extracted under different pH conditions

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Oil palm is an important industry in Malaysia., contributing approximately 37.7% of the agriculture sector to the gross domestic product (GDP) in 2019. However, its plantation and processing in mills produced large quantities of oil palm biomass (OPB) such as fronds, leaves, trunks, empty fruit bunches, palm oil mill effluent and decanter cake that causes environmental pollutions. OPB has been found to contain nutritional components including proteins with a range of 3 – 13%. Many studies reported the extraction methods of protein hydrolysate from various sources of animals and plants but limited from OPB, leading to our interest to study on the protein hydrolysate from one of the OPB, oil palm leaves. This study aimed to extract protein hydrolysate from oil palm leaves for 2 h with different pH at 2, 7 and 8.5 and evaluate their physicochemical properties including protein content, degree of hydrolysis (DH) and zeta potential, in addition to correlated functional properties including solubility and emulsifying properties. All the protein hydrolysates showed high DH in a range of 73-79% and the protein hydrolysate extracted at pH 7 had the highest protein content of 11.4%, followed by pH 8.5 that showed the highest absolute zeta potential and resulted in the highest solubility and emulsifying properties. The results postulated that pH 8.5 is optimum for endogenous enzyme to cleave the peptides with good functional properties, thus it reveals a wide range of application in food system to improve in the texture, volume and stability of food. Further studies on the bioactivities of these protein hydrolysates are highly recommended to discover other potential usages of these protein hydrolysates.

Keywords: *Oil palm biomass; physicochemical properties and functional properties*

Structural and rheological properties of sonicated κ -carrageenan gel

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Sonication is gaining popularity in food processing for its low cost, low energy consumption, and low impact on the environment. It is primarily used for the modification of chemical composition and techno-functional properties. This study aims to characterise the structural and rheological profile of sonicated κ -carrageenan gel, which could potentially be utilised to engineer a melt-in-the-mouth texture in reduced-fat products. κ -carrageenan (2%) was hydrated in water at 60°C before undergoing sonication. The sonicated gel was then freeze-dried at -45°C, 0.05 mbar for 48h before proceeding with Fourier-transform infrared spectroscopy (FTIR) analysis and field emission scanning electron microscopy (FESEM). FTIR results showed that sonication did not alter the chemical composition of κ -carrageenan, thus retaining the native status of the material. However, when viewed under FESEM, the sonicated sample had a porous and fibrous structure as compared to the flake-like film structure in the non-sonicated samples. This change in microstructure has affected the rheological properties of the κ -carrageenan. Amplitude of sonication reduced the yield stress, τ_0 , of the κ -carrageenan gel, but a peak was observed at an amplitude of 40% ($\tau_0 = 255\text{Pa}$). As the duration of the sonication increases from 15s to 60s, a peak of yield stress was observed at 15s ($\tau_0 = 132\text{ Pa}$) and then dropped below 108 Pa ($\tau_{0\text{control}}$). In terms of flow behaviour, the increase of sonication amplitude rendered the gel more shear-thinning (Herschel-Bulkley fluid). However, as the duration increased at the amplitude of 100%, the gel started to behave closer to Bingham plastic fluid. This change in microstructure and rheological properties including reduced yield stress and improved shear thinning properties while retaining the chemical structure of the κ -carrageenan provided a window to create a clean label food stabiliser that could enhance the perception of in-mouth meltability of reduced-fat product.

Keywords: *freeze-drying; κ -carrageenan; microstructure; rheological properties and sonication*

The association between sweet taste perception and fasting blood sugar status among FELDA settlers in Kuantan

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Sweet food, tantalizing human's taste buds has always sugar coated the increased risk of diabetes mellitus. In Malaysia., where sugary food is easily accessible, it is no surprise that the prevalence of diabetes is skyrocketing especially in rural areas. Meanwhile, sweet food consumption is closely related to how an individual perceive sweet taste and their preference for sweetness level. This study aims to find the association between sweet taste perception and fasting blood sugar status among FELDA settlers in Kuantan. A cross-sectional study involving 76 adults aged ranging from 20 to 80 years old was conducted in FELDA Bukit Sagu 1, 2, and 3. Participants were advised to fast overnight on the previous day to determine fasting blood sugar. 10 different concentrations of sucrose solutions ranging from 1.63 gm/L to 821.52gm/L were freshly prepared. Participants need to drink 10 ml of each solutions using full-mouth technique. The first solution that they able to detect glucose presence was their taste threshold (TT) and the solution with the level of sweetness that they prefer in their daily drinks was their taste preference (TP). The mean FBG obtained was slightly higher at 7.6 mmol/L (S.D 0.6). However, majority of them had normal FBG level at 69.7%. Most of the hyperglycaemic participants had medium TT and TP at 87%, while only 1 of them had high TP. Interestingly, no one from the hyperglycaemic group had high TT. The association between TT and TP with FBG was statistically insignificant as P value was more than 0.05 at (P= 0.334 and P=0.750 respectively). No association found between sweet taste perception and fasting blood sugar among FELDA settlers in this study. Nevertheless, it is noteworthy to consider the high mean of FBG for future studies while incorporating other aspects of blood sugar status such as HbA1c level.

Keywords: Sweet taste perception; blood sugar; fasting blood sugar and adults

Toxicity study and antioxidant activity of *Plukenetia volubilis* L. (sacha inchi) shell extract

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Sacha inchi (*Plukenetia volubilis* L.) is one of the edible plants popularly consumed as functional foods in Thailand. The processing of its nut yields the shell as underutilized agricultural waste. Recently, the shell has been commercially available as tea which claimed to reduce the risk of chronic diseases. Some consumers made the tea from the shell by infusion, while some used decoction method. At present, there is no data on the safety of sacha inchi shell. Moreover, its antioxidant activity has not been completely reported. The aim of this study was to evaluate toxicity and antioxidant activity of sacha inchi shell extract. The extracts of sacha inchi shell obtained from infusion and decoction methods were subjected to toxicity study by using *Caenorhabditis elegans* as a model. The extract obtained from infusion method is safer than that from decoction method. Then infusion method was selected as the extraction method used to prepare the extracts from outer, inner, and whole shell. The study on their antioxidant activity showed that the extract from the outer shell of sacha inchi contained the highest total phenolic and total flavonoid contents, followed by whole shell, and inner shell extracts, respectively. The same trend was observed for their DPPH scavenging activity tested in both microplate and TLC assays and FRAP value. This study suggested the safety of sacha inchi shell extract obtained from infusion method. The extract of the outer shell of sacha inchi showed the highest antioxidant potential in which phenolics and flavonoids might be the active compounds. Thus, it is interesting to perform further study on other biological activities in order to support the use of sacha inchi shell as functional foods.

Keywords: *Plukenetia volubilis* L.; sacha inchi; *Caenorhabditis elegans*; toxicity and antioxidant activity

Development and validation of new questionnaire on knowledge, attitude and practice towards food poisoning among secondary school in Tangkak, Johor

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Food poisoning among school students are at an alarming state which can be avoided by educating them with knowledge, attitude and practise of food poisoning prevention. There is, however, no validated questionnaire on preventing food poisoning among secondary school students. A validated questionnaire on food poisoning and prevention was produced as part of the current study. In Tangkak, a cross-sectional pilot survey of 50 consumers was undertaken. Consumers were recruited through convenience sampling based on inclusion and exclusion criteria. The questionnaire included four sections: knowledge, attitude, practise, perception and health seeking behaviour. The difficulty and discrimination index was used to analyse knowledge questions, while construct validity and reliability analysis were used to examine attitude, practise, and perception. A difficulty index of less than 0.3 is regarded difficult, 0.30-0.70 is deemed acceptable, and more than 0.70 is considered easy. Items with a discrimination index of less than 0.2 are deemed poor, those with a score of 0.2-0.24 are considered good, and those with a score of more than 0.35 are considered exceptional. Cronbach's alpha, inter-item correlation, and the value of Cronbach's alpha were used to determine construct validity. Cronbach's alpha, inter-item correlation (0.30), item-total correlation (0.30), and Cronbach's alpha if item eliminated were all used to determine construct validity. According to the discrimination index, five items were poor, 35 were excellent and four items needed to be revised. None of the items from attitude, practise and health seeking behaviour sections were removed. Meanwhile three items from perception sections were deleted based on construct validity. The reliability analysis of attitude, practice, perception and health seeking behaviour sections were 0.941, 0.855, 0.770 and 0.791 respectively which were at acceptable level of reliability. In conclusion, this questionnaire is a valid and reliable tool for assessing students' knowledge, attitudes and practice about food poisoning prevention.

Keywords: *Questionnaire development; reliability; food poisoning and school children*

Citation:

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