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Taylor's University Graduate Research Symposium 2024: Beyond the Framework, Leaping Between Disciplines

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ABSTRACT

The theme "Beyond the Framework: Leaping between Disciplines" addresses the growing importance of cross-disciplinary research, particularly in Life Sciences, Medicine, and Biomedicine. This Special Issue explores how research that transcends traditional disciplinary boundaries can lead to breakthroughs in complex health challenges. The convergence of these fields fosters innovation by integrating diverse methodologies, perspectives, and technologies. This Special Issue highlights the value of interdisciplinary approaches in advancing understanding of diseases, developing novel treatments, and improving patient outcomes. Our contributors delve into a wide range of topics, including regenerative medicine, personalized healthcare, and biomaterials, showcasing collaborative research that has led to significant advancements. By breaking down silos, researchers from these domains can create more holistic solutions to pressing health concerns such as cancer, neurodegenerative disorders, and metabolic diseases. This issue aims to inspire researchers and clinicians to pursue collaborative projects that transcend traditional boundaries, fostering innovation and promoting sustainable healthcare solutions.

Keywords: *Interdisciplinary research; innovative research and collaborative science*

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ABSTRACTS

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Taylor's University- Xi'an Jiaotong Liverpool Unversity-Mae Fah Luang University Graduate Research Symposium 2024

The apoptotic effects of Trichostatin A and Zebularine in combination with TRAIL (TZT) on E-cadherin re-expressed human breast adenocarcinoma cells

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Abstract

Despite advancements in current treatments, breast cancer remains a leading cause of death among women. Particularly, the triple negative breast cancer (TNBC) poses a significant challenge due to its aggressive nature and lack of hormonal receptor expression, which reduces the effectiveness of chemotherapy. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) has shown promise in selectively killing cancer cells while sparing healthy cells, but its effectiveness is limited by the development of resistance. Therefore, this study aims to explore a potential therapeutic approach to overcome TRAIL resistance in TNBC cells that have re-expressed E-cadherin (E-MDA-MB-231). After treatment with Trichostatin A (TSA), Zebularine (Zeb), TSA and Zeb (TZ), TRAIL, and TZ followed by TRAIL (TZT), the cells were subjected to MTT assay, Reverse Transcription-Polymerase Chain Reaction (RT-PCR), and enzyme-linked immunosorbent assay (ELISA). First, the MTT cell viability assay revealed a significant reduction in the viability of E-MDA-MB-231 after TZT treatment compared to MDA-MB-231 (TNBC) and MCF-7 (Luminal A). Notably, no significant reduction in the cell viability was observed in MCF10A (normal breast epithelial cells). Following RT-PCR analysis, *Bax/Bcl-2* ratio was the highest in E-MDA-MB-231 treated with TZ, followed by Zeb and TRAIL. On the other hand, Bax/Bcl-2 ratio was the highest in MDA-MB-231 treated with Zeb, followed by TRAIL. Although there was no difference in the Bax/Bcl-2 ratio after TZT treatment in both cell lines, ELISA demonstrated a significant increase in caspase-3 expression in E-MDA-MB-231 after TZT treatment. These findings suggest that TSA and Zeb, along with E-cadherin re-expression, may enhance the sensitivity of cancer cells to TRAIL-induced apoptosis, potentially at the protein level. However, further molecular analysis is needed to validate this therapeutic approach and improve future clinical outcomes.

Keywords: *Breast cancer; TRAIL resistance; E-cadherin; Trichostatin A and Zebularine*

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New mono-carbonyl curcumin analogues as potential anti-inflammatory lead compounds

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Abstract

Chronic inflammation may cause severe cell and tissue damage and may lead to the development of chronic inflammatory disease. Novel compounds with significant anti-inflammatory therapeutic potential is of utmost importance. Natural product, such as curcumin exhibits remarkable anti-inflammatory activities but is hindered by its low stability and pharmacokinetic properties due to the existence of β-diketone linker. Thus, this study aimed to design and synthesize mono-carbonyl based curcumin analogues by replacing the linker with *N*-methyl-4-piperidone and 1-benzyl-4-piperidone for their structure-activity relationship study on anti-inflammatory activities. The curcumin analogues were synthesized through aldol condensation and their structures were confirmed through MS, NMR and FTIR spectroscopy. Evaluation of their inhibitory effects against prostaglandin E_2 (PGE_2) production in LPS-induced RAW264.7 cells showed that the curcumin analogues possessed moderate to good inhibition with IC50 values ranging from 8 to 24 μM compared to dexamethasone (IC_{50} = 14 μM). Furthermore, the potent analogues inhibited interleukin-1β cytokine release with IC_{50} values ranged from 6 to 21 μM. Structure-activity relationship analysis revealed that the 1-benzyl-4-piperidone linker enhanced the anti-inflammatory activity, with fluorine substituted benzene rings providing beneficial effects. Further mechanistic study demonstrated that the potent analogues reduced COX-2 protein expression and suppressed total NF-κB/p65 phosphorylation in LPS-induced RAW 264.7 cells, suggesting that their anti-inflammatory effects are associated with the modulation of NF-κB signalling pathway. The study outcomes highlight the potential of curcumin analogues as lead compounds for the development of anti-inflammatory therapeutics. Thus, further study using animal model is highly recommended to validate drug metabolism, safety and efficacy of the potent analogues.

Keywords: *COX-2; NF-κB signalling pathway; Piperidone linker; PGE_2 and Structure-activity relationship*

Taylor's University- Xi'an Jiaotong Liverpool Unversity-Mae Fah Luang University Graduate Research Symposium 2024

Radio Embolization Samarium-153 Therapy (REST): From synthesis to dosimetry

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Abstract

Radio Embolization Samarium-153 Therapy (REST) is a novel theranostic treatment for liver cancer. Samarium-153-loaded polystyrene (^{153}Sm-PS) microspheres are injected into tumors to deliver 808-keV beta particles for therapy and 103-keV gamma rays for imaging. This treatment can be produced locally using research reactors, available in over 50 countries. REST seeks to address limitations of current yttrium-90 radioembolization, such as challenges in imaging, high costs, and limited accessibility. Previous in vivo studies on REST microspheres demonstrated tumor shrinkage within 28 days post-treatment, with no observed acute or chronic toxicity. This study aims to synthesize and characterize ^{153}Sm-PS microspheres and develop a dosimetric framework using voxel-based dosimetry. The ^{153}Sm-PS microspheres were synthesized via solvent evaporation and neutron-activated at the TRIGA PUSPATI Research Reactor, Malaysian Nuclear Agency. In a pilot study, 17 MBq of ^{153}Sm-PS microspheres were injected into a liver tumor-bearing Sprague-Dawley rat and imaged 24 hours later using pre-clinical single photon emission computed tomography (SPECT). The SPECT images were converted to cumulated activity and convolved with a ^{153}Sm dose voxel kernel (DVK) using Fast Fourier Transform in MATLAB to generate an absorbed dose map. Electron microscopy showed that the microspheres had smooth, spherical morphology with diameters ranging from 39.9 to 56.7 μm. Thermogravimetric analysis revealed that ^{152}Sm constituted 22% of the formulation, resulting in a specific activity of 4.47 GBq/g. DVK calculations provided a self-dose factor of 10.9 Gy•kg/GBq for ^{153}Sm. SPECT images confirmed localization of the microspheres at the injection sites, and the dose map indicated mean and maximum doses of 45 Gy and 194 Gy, respectively. Future studies will include additional animal groups and dose points to establish subject-specific dosimetry for REST

Keywords: *Radioembolization; Samarium-153; Voxel-based dosimetry; SPEC and neutron activation*

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Targeting Candida infections: The role of gold nanoparticles on Candida biofilms

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Abstract

Candida infections, especially in immunocompromised individuals, pose a serious global health challenge due to increasing resistance to antifungal treatments like fluconazole. This resistance complicates treatment, particularly for biofilm-forming species, leading to higher mortality rates. Gold nanoparticles (AuNPs) have shown potential to combat this issue by disrupting Candida cell membranes, inhibiting biofilm formation, and enhancing drug delivery, reducing the risk of resistance development. Additionally, AuNPs can penetrate biofilm matrices and function as drug carriers, encapsulating antifungal agents and delivering them to the biofilm core, where conventional treatments struggle. This study evaluated the antifungal efficacy of AuNPs against seven Candida species using broth microdilution following CLSI guidelines. Serial dilutions of fluconazole and AuNPs were prepared in 96-well plates, with readings taken every 24 hours over 72 hours to determine MIC50 and MIC80 values. Results showed significant antifungal activity. The lowest MIC50 values for planktonic cells were found in C. albicans (3.9 µg/mL), C. parapsilosis (5.63 µg/mL), and C. kefyr (5.26 µg/mL). For biofilm-forming species, AuNPs also demonstrated efficacy, with C. glabrata (2.15 µg/mL), C. rugosa (0.81 µg/mL), and C. kefyr (0.81 µg/mL) showing the lowest MIC50 values after 72 hours. MIC80 values followed similar trends, highlighting AuNPs' ability to inhibit biofilm growth effectively. These findings underscore the potential of AuNPs not only to inhibit Candida growth but also to overcome the challenges posed by biofilms and antifungal resistance. Their ability to penetrate biofilms and function as carriers for other antifungal drugs suggests a dual mechanism of action that could significantly improve treatment outcomes. AuNPs, therefore, represent a promising alternative to conventional antifungal therapies, warranting further research into their mechanisms of action at the cellular and genomic levels, as well as their potential clinical applications.

Keywords: *gold nanoparticles; antifungal properties; candida infection; candida biofilms and fungal infection*

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Prebiotics promote pro-inflammatory cytokine secretion in THP-1 macrophages *in vitro*

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Abstract

Prebiotics are indigestible compounds selectively utilised by beneficial intestinal microbes to grow and generate metabolites, conferring health benefits to the host. Additionally, most prebiotics are carbohydrate-based. Hence, they can modulate host immune responses by interacting directly with pattern recognition receptors expressed by immune cells such as macrophages. Macrophages display remarkable plasticity and can be polarised into pro-inflammatory or anti-inflammatory phenotypes upon receiving specific signals. Most research on prebiotic immunomodulation employed murine cell models but not human cells. Our study aims to determine if prebiotics can directly modulate cytokine production by human macrophages with the THP-1 cell line as a macrophage model. An MTT assay was first carried out to assess the effect of three prebiotics on cell viability: inulin, fructooligosaccharides and lactulose. The cells were subsequently conditioned with non-toxic concentrations of prebiotics for 24 hours and activated with lipopolysaccharide. Cell culture supernatants were harvested to determine pro-inflammatory IL-1β, IL-6, TNF-α and MCP-1, and anti-inflammatory IL-1Ra and IL-10 using Luminex cytokine assay. Data was analysed using one-way ANOVA, followed by Dunnett's multiple comparisons tests. Our results show that prebiotic concentrations up to 32 mg/mL were non-toxic to THP-1 cells. Inulin and FOS significantly increased MCP-1 and TNF-α production, while FOS also significantly reduced IL-10 secretion. Lactulose had a greater effect on cytokine production than inulin and FOS, significantly increasing MCP-1, IL-6 and TNF-α levels while decreasing IL-1Ra and IL-10 levels. Our data indicates these prebiotics have direct immunomodulatory properties on human macrophages, promoting pro-inflammatory and suppressing anti-inflammatory responses. Ongoing work involves quantifying macrophage activation receptor expression and phagocytosis of bacterial cells.

Keywords: *Prebiotics, fructans; lactulose; macrophage immunomodulation and cytokine secretion*

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Dual payload delivery using CD44-specific PEGylated liposome: Enhancing efficacy of doxorubicin hydrochloride and miR-145 mimics in breast cancer *in vitro*

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Abstract

Doxorubicin hydrochloride (Dox-HCl) is recognized as one of the widely utilized anti-cancer drugs and serves as the first-line option for breast cancer treatment. However, the clinical applications of Dox-HCl are restricted, particularly in the context of triple-negative breast cancer (TNBC), attributed to its associated toxicity and aggressive treatment profiles. Thus, this study aims to synthesize and characterize CD44-specific PEGylated liposomes co-loaded with Dox-HCl and miR-145 mimics. This initiative aims to evaluate their in vitro anti-proliferative and anti-metastasis activity against MDA-MB-231 cell line, representative of triple-negative breast cancer. The optimized formulation, developed using the composite central design approach, exhibited the highest desirability function (D = 0.814) and demonstrated excellent stability over a 60-day period at 4°C. Moreover, it showed an enhanced drug release profile under acidic conditions and facilitated optimal cellular internalization within 4-hours incubation period. By further conjugating this optimized formulation with CD44-specific A6-peptide, the resultant liposomal formulation (A6-PEG-lipo-Dox-miR145) exhibited increased cytotoxicity (IC_{50} = 1.67 µM) against MDA-MB-231 cells, surpassing the non-conjugated formulation and the free Dox-HCl and miR-145 regimen. Subsequent analysis through western blotting indicated that the crucial anti-proliferative role of A6-PEG-lipo-Dox-miR145 is closely associated with the modulation of PI3K/AKT and downstream NF-κB signaling pathways. Additionally, further investigations using scratch motility, as well as transwell invasion and migration assays provided evidence of the enhanced anti-migration and anti-invasion properties of A6-PEG-lipo-Dox-miR145. Notably, western blot analysis highlighted the ability of A6-PEG-lipo-Dox-miR145 in enhancing the suppression of epithelial-mesenchymal transition (EMT) by modulating the N-cadherin and E-cadherin expression. Our findings suggest that CD-44-specific PEGylated liposomes can be effectively tailored for the concurrent delivery of anticancer drugs and therapeutic miRNAs into tumor cells, representing a promising alternative approach for targeted drug development in cancer treatment.

Keywords: *Breast cancer treatment; liposomal formulation; anti-cancer drugs; nanoformulation and miRNAs*

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Formulation and optimization of hyaluronic acid – oleic acid ester based nanoemulsion

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Abstract

Ultraviolet radiation is a major contributor to skin damage and aging due to reactive oxygen species (ROS)-induced oxidative stress. Innate antioxidant and repair mechanisms are insufficient to hinder the damage, requiring supplementation from external sources. Hyaluronic acid - oleic acid (HA-OA) is an ester derived from hyaluronic acid (HA) and oleic (OA) acid, which possess antioxidant activity stronger than HA and OA alone. In this study, HA-OA was formulated into an oil-in-water nanoemulsion as a carrier to penetrate the skin. The nanoemulsion was synthesized using ultrasonication and high shear homogenizer and D-optimal mixture design was used to obtain the optimized formulation for HA-OA nanoemulsion. The effects of Tween 80, almond oil, xanthan gum, HA-OA and deionized water on the nanoemulsion droplet size were determined. Additionally, the rheological response and in vitro cytotoxicity of HA-OA nanoemulsion on human dermal fibroblast (HDF) cell line were also investigated. Results showed that the optimized formulation of HA-OA nanoemulsion with the desirable criteria was 3.38% of Tween 80, 3.70% of almond oil, 0.60% of xanthan gum, 4.89% of HA-OA and 86.74% of deionized water. This optimum formulation had a predicted droplet size of 183.80 nm, which showed good agreement with the actual value (178.57 nm), having a residual standard error <3%. Rheological studies on the nanoemulsion revealed that it has a shear thinning and pseudoplastic behavior with elastic properties. Moreover, in vitro cytotoxicity of HA-OA nanoemulsion on HDF cell line showed no cytotoxic effect, with cell viability more than 90% at concentrations ranging from 0.2 to 1.0 mg/mL. To conclude, the data revealed that HA-OA nanoemulsion is non toxic with favorable properties, which suggests its potential to become a topical carrier for the antioxidant agent, HA-OA.

Keywords: *Nanoemulsion; D-optimal mixture experimental design; hyaluronic acid; oleic acid and antioxidant*

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Oral nutrition supplementation to treat protein energy wasting in hemodialysis patients by measuring response using global untargeted and targeted metabolomics

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Abstract

End stage kidney disease (ESKD) patients undergoing hemodialysis (HD) are vulnerable to protein energy wasting (PEW). PEW is an abnormal metabolic condition associated with malnutrition, muscle wasting and increased mortality in HD patients. Oral nutrition supplementation (ONS) and dietary counselling are recommended to optimise energy and protein intakes in PEW patients. But the metabolic impact of ONS treatment is unknown and metabolomics may elucidate this role. We applied global untargeted and targeted metabolomics in tandem with multivariate data analysis (MVDA) to study the change in metabolism occurring in HD patients with PEW either treated with ONS (n=29) or only receiving standard dietary counselling (n=27) over six months. Their plasma samples were subjected to nuclear magnetic resonance (NMR) spectroscopy at 600 MHz. MVDA, through a non-supervised principal component analysis (PCA) and a supervised orthogonal projection latent square-discriminant analysis (OPLS-DA), were used to provide insights on separation of high-dimensional spectral measurement from NMR, whilst NMR spectra corresponding to these differences were subsequently identified and quantified. PCA and OPLS-DA did not discriminate plasma metabolites between the two groups at baseline but detected 5 significant metabolites at 3-month and 6-month as indicators of differences between PEW treatment. N-methyldantoin (p=0.046), 3-aminoisobutyrate (p=0.011) and 3-hydroxybutyrate (p=0.015) significantly reduced whilst myo-inositol (p=0.046) and 2-oxoglutarate (p= 0.031) significantly increased in the ONS treated patient group compared to the patient control group. Pathway analysis showed that these metabolites were associated with tricarboxylic cycle, butanoate metabolism, inositol phosphate metabolism, synthesis and degradation of ketone bodies, and alanine, aspartate and glutamate metabolism. Our results demonstrated that metabolomics is a useful platform for identifying the changes in metabolism attributed to nutritional treatment of HD patients with PEW.

Keywords: *metabolomics; supplementation; hemodialysis and nutrition*

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The role of Nrf2 on cellular senescence and autophagy-lysosomal pathways in macrophage foam cells

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Abstract

Atherosclerosis, a major cause of cardiovascular diseases, is characterized by the accumulation of lipid-laden macrophages known as foam cells. The balance between senescence and autophagy is critical for foam cell survival and it also influences plaque stability in atherosclerosis. While Nrf2 is known to regulate oxidative stress and autophagy, its role in managing the balance between senescence and autophagy in foam cells remains unclear. This study investigated the effect of Nrf2 on foam cell senescence and autophagy in THP-1 macrophage foam cells model using siRNA-mediated Nrf2 silencing. The findings revealed that Nrf2 silencing reduced oxLDL-induced foam cell formation by decreasing lipid accumulation and enhancing cholesterol efflux. Nrf2 silencing also inhibited senescence, as indicated by lower β-galactosidase activity and reduced expression of senescence-associated secretory phenotype (SASP) markers, such as MMP-9 and TNF-α. It also promoted autophagy as evidenced by increased autophagosome formation and LC3B expression, alongside with reduction of p62 expression. To further examine the role of autophagy, hydroxychloroquine (HCQ) and 3-Methyladenine (3-MA) were used to inhibit different early and late stages of autophagy, respectively. Inhibition of autophagy with HCQ significantly decreased cell viability, indicating that foam cells became more reliant on late-stage autophagy for survival after Nrf2 silencing. These results reveal a previously unrecognized role of Nrf2, where its inhibition paradoxically reduces lipid accumulation and senescence, while autophagy plays a crucial regulatory role in foam cell survival. Altogether, this study provides valuable insights into how Nrf2 regulates foam cell formation, cellular senescence, and autophagy.

Keywords: *Atherosclerosis; foam cells; Nrf2; senescence and autophagy pathway*

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The future of neurodegenerative therapy: phytochemicals at the forefront

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Abstract

Neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease, pose significant global health challenges due to their progressive nature, consistent decline in higher cognition, mobility and memory, and lack of curative treatments. Several clinical trials of pharmaceutical drugs in the past decade have revealed mild symptomatic relief with a wide range of toxicities, suggesting these diseases have highly complex and multifaceted etiology. Nootropic herbal plants are gaining attention as complementary treatments and valuable sources for drug development in neurodegenerative diseases. Several studies highlight their potential in treating neurodegenerative diseases through their key phytochemicals, also known as bioactive compounds. This presentation explores the neuropharmacological properties of various phytochemicals, including flavonoids, polyphenols, alkaloids, phytosterols and terpenoids, in modulating pathological mechanisms implicated in neuroprotective activities. The mechanisms such as antioxidant activity, anti-inflammatory effects, anti-amyloidogenic, modulation of neurotransmitters, cognitive-enhancing effects, and inhibition of protein aggregation and cholinesterase will be discussed. Furthermore, recent preclinical and clinical studies highlight the efficacy of specific phytochemicals, such as curcumin (from *Curcuma longa*), Huperzine A (from *Huperzia serrata*), asiaticoside (from *Centella asiatica*), bilobalide (from *Ginkgo biloba*), ginsenosides (from *Panax ginseng*) and Withanolide A (from *Withania somnifera*), in attenuating neurodegenerative processes. These phytochemicals are reported to influence neuronal metabolism in the central nervous system (CNS) synergistically, leading to improved cognitive function, particularly in cases of neuronal damage or degeneration. Hence, they are increasingly used in managing chronic, subacute, and acute disorders related to consciousness, memory, and learning. By integrating molecular insights and therapeutic applications, this presentation aims to underscore the potential of phytochemicals as adjunctive or alternative therapies in the management of neurodegenerative diseases, offering hope for enhanced cognitive health and neuroprotection.

Keywords: *Alzheimer's disease; phytochemicals; nootropic herbs and neurodegenerative diseases*

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Oil Palm Mesocarp Fibre (OPMF) derived nanocellulose: Surface functional groups and their characterisations

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Abstract

Oil Palm Mesocarp Fibre (OPMF), a biomass residue obtained after the extraction of palm oil from the oil palm fruits, can be a viable and sustainable source to produce nanocellulose (NC) as the raw fiber is rich in lignocellulose. In this study, we have transformed OPMF into NCs with different surface functional groups and degrees of substitution using acid hydrolysis (H_2SO_4 or HCl) and TEMPO oxidation methods. Various instrumentation and techniques such as dynamic light scattering (DLS), Fourier-transformed infrared (FT-IR) spectroscopy, Thermogravimetric Analysis (TGA), conductometric titration, X-ray diffraction (XRD), and transmission electron microscopy (TEM) were employed to characterise the physicochemical properties of the produced NCs. XRD analysis showed that the synthesised NCs have a higher degree of crystallinity than cellulose. FT-IR analysis confirmed the modification of surface functional groups of H_2SO_4-hydrolysed NCs with sulfated groups (-SO_3H) detected at around 1100 cm^{-1}; while TEMPO-oxidised NCs display carboxyl groups (-COOH) at around 1420 cm^{-1}. The results from the conductometric titrations reveal that increasing concentrations of H2SO4 and NaOCl used in the respective synthesis methods has increased the content of sulfur and carboxylate correspondingly. DLS analysis shows that the different size ranges of the NCs are produced with the smallest sizes produced by TEMPO-oxidation (33.5 - 57.1 nm) compared to acid hydrolysis with H_2SO_4 (78.0 - 145.9 nm) and HCl (66.4 - 123.4 nm). Moreover, TGA analysis reveals that TEMPO-oxidised and HCl-hydrolysed NCs demonstrate higher thermal stability compared to H_2SO_4-hydrolysed NCs. This study has demonstrated that NCs with different surface functional groups and physicochemical properties can be prepared from OPMF biomass residue which serve as high-value commodities for various potential biomedical and pharmaceutical applications.

Keywords: *Oil Palm Mesocarp Fibre; nanocellulose; TEMPO oxidation and acid hydrolysis*

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Synthetic mRNA mediated differentiation of human stem cell into insulin producing cells

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Abstract

Diabetes Mellitus (DM) is a global epidemic, projected to affect 629 million individuals by 2049, and poses significant healthcare challenges. Current treatment does not address the underlying issue of autoimmune β-cells destruction and insulin resistance. This study explored a novel therapeutic approach of using synthetic mRNA to induce the differentiation of human mesenchymal stem cells (huMSCs) into insulin-producing pancreatic β-cells. This is achieved by modulating transcription factors PDX1, NGN3, MafA, PAX4, and GLIS3, pivotal in natural β-cell development and maturation. This study involved three stages: (1) synthesis of synthetic mRNA, (2) in vitro investigations, and (3) in vivo transplantation. In vitro, huMSCs were cultured and transfected with synthetic mRNAs encoding different combination of the five transcription factors. Results showed that PDX1, NGN3, MafA, PAX4 and GLIS3 (PNMPG) enhanced cell viability and promoted the differentiation of huMSCs into pancreatic β-cell when compared to PDX1, NGN3 and MafA (PNM). RT-PCR demonstrates amplified mRNA expression of the transcription factor in both differentiated cells. In vivo, differentiated cells were injected into streptozotocin-induced diabetic BALB/c. Results demonstrated that PNM and PNMPG-differentiated cells improved blood glucose control; however, results were transient (~2 weeks). Oral glucose tolerance tests indicated improved glucose handling in PNMPG compared to PNM ($P<0.001$)-treated mice. The transient nature of the glucose-lowering effect and potential immune responses warrant further investigation.

Keywords: *Diabetes Mellitus; synthetic mRNA; human mesenchymal stem cells; pancreatic β-cells and transcription factors*

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NanoMUD: Profiling of pseudouridine and N1-methylpseudouridine using Oxford Nanopore Direct RNA sequencing

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Abstract

Nanopore direct RNA sequencing provided a promising solution for unraveling the landscapes of modifications on single RNA molecules. Here, we proposed NanoMUD, a computational framework for predicting the RNA pseudouridine modification (Ψ) and its methylated analog N1-methylpseudouridine (m1Ψ), which have critical application in mRNA vaccination, at single-base and single-molecule resolution from direct RNA sequencing data. Electric signal features were fed into a bidirectional LSTM neural network to achieve improved accuracy and predictive capabilities. Motif-specific models (NNUNN, N = A, C, U or G) were trained based on features extracted from designed dataset and achieved superior performance on molecule-level modification prediction (Ψ models: min AUC = 0.86, max AUC = 0.99; m1Ψ models: min AUC = 0.87, max AUC = 0.99). We then aggregated read-level predictions for site stoichiometry estimation. Given the observed sequence-dependent bias in model performance, we trained regression models based on the distribution of modification probabilities for sites with known stoichiometry. The distribution-based site stoichiometry estimation method allows unbiased comparison between different contexts. To demonstrate the feasibility of our work, three case studies on both in vitro and in vivo transcribed RNAs were presented. NanoMUD will make a powerful tool to facilitate the research on modified therapeutic IVT RNAs and provides useful insight to the landscape and stoichiometry of pseudouridine and N1-pseudouridine on in vivo transcribed RNA species.

Keywords: *Nanopore direct RNA sequencing; mRNA vaccines; pseudouridine; N1-methylpseudouridine; deep learning and epi-transcriptome*

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Rational design of peptides targeting EGFR Using EGF-EGFR interaction models

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Abstract

The epidermal growth factor receptor (EGFR) is a crucial prognostic biomarker, overexpressed in various malignant solid tumors. This has made EGFR a key target in cancer chemotherapy. Despite advances in EGFR-targeting therapies, drug resistance remains a major clinical challenge. In this study, we employed computational approaches to design small peptides that specifically target EGFR, with potential applications in cancer-targeted therapies such as colorectal, glioblastoma, breast, non-small cell lung, and pancreatic cancer. The peptide design strategy involved rational modeling of the epidermal growth factor (EGF)-EGFR interaction, using EGF-EGFR complex (PDB ID: 1IVO) to identify the key binding. This information was used to predict the amino acid residues with optimal fit for the EGFR binding site, generating a virtual peptide library. From an initial library of 180 peptides, 53 candidates were shortlisted based on stability, predicted binding potential, and hydrophilicity. Further binding affinity analysis had resulted in 17 peptides with promising interaction profiles against EGFR. Three peptides, CDP44, CDP85, and CDP179, were selected for detailed study due to their favorable binding energies, determined using the Alphafold3 and MDockPeP2 servers. Molecular dynamic simulations conducted with GROMACS using the CHARMM36 force field were performed to analyze peptide-EGFR complexes in a solvated environment over 10 ns. The results confirmed that all three peptides formed stable complexes with EGFR, with no significant conformational changes observed. These results demonstrate that small peptides can be effectively designed to target EGFR with high binding affinity, laying a strong foundation for the development of new cancer therapeutics. These peptides show potential for overcoming drug resistance in EGFR-targeted cancer treatments which could lead to more effective and durable therapies in the future.

Keywords: *Peptide computational; EGFR targeting; cancer targeted therapy and peptide therapeutics*

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Investigating transition metal ion interactions with histagged virus like particles to enhance metal affinity precipitation for protein purification

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Abstract

The polyhistidine-tag (His-tag) is a widely used protein tag that consists of six-twelve histidine residues at the N- or C- terminus of a protein. It is a useful tool for protein purification due to its ability to be easily fused to proteins via recombinant DNA technology with minimal impact on their folding and function. Immobilized Metal Ion Affinity Chromatography (IMAC) is a common technique used to purify his-tagged recombinant proteins, relying on the specific interaction between the his-tag and metal ions immobilized on a matrix. While IMAC has been shown to efficiently purify his-tagged proteins with high yield and purity, it has some drawbacks, including the requirement for matrix columns and various reagents, which can complicate the process and increase costs. A novel approach, nickel affinity precipitation, simplifies this process by introducing free nickel ions into culture feedstock, forming nickel-his-tag coordinate bonds that result in protein precipitation. In this project, other transition metal ions, including zinc, copper, cobalt, iron, and calcium were explored as alternatives to nickel to purify his-tagged turnip yellow mosaic virus coat (TYMVc) protein, which form virus-like particles (VLPs). SDS-PAGE and dynamic light scattering analyses revealed that trace amounts of zinc, copper, cobalt and iron were able to precipitate VLPs, achieving purities of approximately 90% at optimal ion concentrations (50 - 150 μM) with recovery yield above 50%. This method presents a promising, less toxic alternative to nickel-based approaches for VLP protein purification, offering greater flexibility for industrial adaptation in large-scale purification of VLP-based vaccines.

Keywords: *Polyhistidine-tagged protein; Turnip yellow mosaic virus (TYMV); Turnip yellow mosaic virus coat protein (TYMVc); Metal affinity precipitation and VLP purification*

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Network pharmacology of bioactive compounds in *Brassica oleracea* as potential neuroprotective agent in preventing Alzheimer's disease

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Abstract

Alzheimer's disease is characterised by chronic neuroinflammation in the brain, which results in neuronal dysfunction, abnormal protein folding and deterioration of cognitive abilities. The current work focuses on employing network pharmacology to identify the therapeutic prospect in kale (*Brassica oleracea*) on Alzheimer's disease through bioinformatics approach. Several studies revealed that kale exerted a neuroprotective effect in Alzheimer's model, however employment of network pharmacology has yet to be performed. The bioactive compounds were extracted from Pubchem and their target gene predictions were conducted through Swiss Target Prediction and SuperPred databases. Alzheimer's related genes were extracted from the OMIM, DisGeNet, and GeneCards databases. Both Alzheimer's and kale's target genes were intersected and used for constructing protein-protein interaction and compound target network by STRING >0.7. In the enrichment analysis, it was revealed that kale may prevent the progression of Alzheimer's disease by regulating neuroinflammation, toll-like receptor and neurotrophin signalling pathway. APP, MAPT, AKTI, NFKB1, APEX1, CTSD, NFE2L2, and NR1I2 were identified as core target genes, whereas quercetin, kaempherol, isohamnetin-II and narigenin-I were considered as core target compounds. Subsequent molecular docking revealed that these core target compounds exhibited a promising binding affinity towards NFE2L2, NR1I2, APEX1 and CTSD. In short, the current research unravels the gene expression behind the anti-neuroinflammation effect of kale, which serves as foundation for future studies on these compounds and core target genes to boost the research in neurodegenerative disease.

Keywords: *Alzheimer's disease; network pharmacology; kale; anti-inflammatory and core targets*

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Metadynamics-directed modelling of bioavailable and high stability quercetin analogues targeting calcineurin

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Abstract

Calcineurin inhibitors including popular drugs like cyclosporine and tacrolimus are a class of immunosuppressants conventionally prescribed for the treatment of autoimmune diseases and prevention of post-organ transplant rejection. However, calcineurin inhibitors are present with significant adverse effects including but not limited to nephrotoxicity and neurotoxicity. Recent research revealed the potential utility of quercetin as a calcineurin inhibitor with minimal adverse effect. Nevertheless, its poor bioavailability has led to its confined clinical utility, urging efforts to improve its pharmacologic properties. Prior studies demonstrated bioavailability improvement with methylation of free hydroxyl groups in quercetin, prompting the adoption of an *O*-alkylation approach in this study. Enhanced sampling methods, like binding pose metadynamics (BPMD), have markedly improved structure-based computational drug design through efficacy in accurately predicting ligand binding poses and their stabilities within protein binding sites. In this study, BPMD was applied to optimize the modeling of prominent quercetin analogues targeting calcineurin. Ninety-three analogues, satisfying Lipinski's rule of five were designed through alkylation of the free hydroxyl groups in quercetin. ADMETlab 2.0 was utilized to assess the pharmacokinetic properties of the analogues. Molecular docking was performed with AutoDock Vina, followed by molecular dynamics simulations using GROMACS with AMBER ff14SB force field. Finally, the analogues were subjected to BPMD simulations via PLUMED, implementing the OpenBPMD protocol. In silico ADMET profiling using ADMETlab 2.0 predicted fifty-one analogues to have higher bioavailability and permeability than quercetin. BPMD results predicted thirty-nine analogues to be more stable than quercetin when bound to calcineurin ($CompScore_{(analogues)}$ = 0.1432 to 5.9761 vs. $CompScore_{(quercetin)}$ = 5.986). This study found that structural modification of quercetin via O-alkylation can enhance its pharmacological properties and stability when bound to calcineurin, potentially treating autoimmune diseases with few side effects. Binding pose metadynamics represents a promising approach for enhancing the efficiency of lead optimization in drug discovery.

Keywords: *Quercetin; calcineurin; molecular docking; molecular dynamics and metadynamics*

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De novo whole genome assembly of two polar *Pseudogymnoascus* species and their secondary metabolite biosynthetic gene clusters

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Abstract

Previous studies have shown *Pseudogymnoascus* spp. to produce bioactive secondary metabolites (SM) with various pharmaceutical potentials. However, their related biosynthetic gene clusters (BGC) are not well studied, let alone strains from the Antarctic and Arctic regions. For this study, the whole genomes of the Antarctic *Pseudogymnoascus griseus* (strain AKSP4) and Arctic *Pseudogymnoascus australis* (strain HNDR4) were sequenced on Illumina NovaSeq6000 250PE platform at 150× sequencing coverage. With de novo assembly method, the first whole genome assemblies were produced for polar *P. griseus* with the genome size of 35.53Mb based on 999 contigs, whereas P. australis genome size was 33.26Mb assembled from 646 contigs. Additionally, a total of 58 and 52 BGC regions were predicted for *P. griseus* and *P. australis*, respectively. In both strains, seven predicted regions were identified to have 100% similarity to entries in Minimum Information about a Biosynthetic Gene Cluster (MiBiG). Four of those gene clusters have the potential to produce compounds similar to choline, YWA1, cichorine and UNII-YC2Q1O94PT. These metabolites hold potential applications in biotechnological industry such as pigments or discovery of bioherbicides. The results produced will be valuable for future studies involving polar fungi by providing a high-quality genome to be used as a reference. Finally, this study is also the first to report the genes involved in SM production where they can assist in narrowing down the search for genes potentially producing interesting SMs.

Keywords: *Leotiomycetes; antiSMASH; genome mining; Pseudogymnoascus australis and Pseudogymnoascus griseus*

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Enhanced removal of nickel and copper ions using biochar from oil palm empty fruit bunch fiber

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Abstract

This study investigates the adsorption of nickel (Ni (II)) and copper (Cu (II)) ions from aqueous solutions using biochar (BC) derived from oil palm empty fruit bunch fiber (OPEFB). The biochar was produced via pyrolysis of factory empty fruit bunch (FEB) and pre-treated empty fruit bunch (PEB) under high temperatures and inert conditions. Various characterization techniques such as FESEM, BET, EDX, FTIR, iodine number measurement, and methylene blue adsorption were employed to evaluate the BC. The BC exhibited a porous surface, with cylindrical tube-like structures identified through FESEM. BET analysis revealed an increase in surface area from 4.43 m^2/g to 424.98 m^2/g when pyrolysis temperature was raised from 500°C to 700°C. EDX data indicated that 0.30% and 13.05% of Ni (II) and Cu (II) ions, respectively, were adsorbed on the BC surface. FTIR analysis showed the loss of functional groups such as O-H, C-H, and C-O at higher pyrolysis temperatures. The iodine number and methylene blue adsorption results indicated that FEB 500, with higher surface area and adsorption capacity, outperformed PEB 700. The optimum removal efficiencies of Ni (II) and Cu (II) ions, at 98% and 91.91%, respectively, were observed with FEB 500. Adsorption data fit better with the Langmuir model for Ni (II) (R^2 = 0.9247) and the Freundlich model for Cu (II) (R^2 = 0.9961). FEB 500 was more effective than PEB 700 due to its higher potassium content, aiding in cation exchange during heavy metal adsorption.

Keywords: *Biochar; adsorption; nickel; copper and oil palm*

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Effect of whey protein isolate (WPI) on the physicochemical and digestive properties of potato starch

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Abstract

Starch is commonly used to enhance texture and water retention in food products, especially frozen or chilled items. However, its high amylopectin content can lead to rapid spikes in blood sugar levels. The incorporation of protein to form starch-protein complexes offers a way to slow down starch digestion, creating healthier food options with improved metabolic effects. This study aims to explore the effects of incorporating whey protein isolate (WPI) on the physical and digestive characteristics of potato starch (PS). Different blends of PS and WPI were formulated with differing WPI ratios. The WPI-PS complexes were analyzed using X-ray diffraction (XRD), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), rheological testing, and in vitro enzymatic digestion assays. XRD results indicated that the addition of WPI did not disrupt the natural crystalline structure of PS, rather its non-crystalline regions, suggesting that WPI compacts the starch matrix. SEM images showed morphological changes in PS as WPI content increased, with rougher surfaces and larger granules observed, indicating protein-starch interactions. DSC data suggested the presence of WPI altered the thermal behavior of PS, potentially affecting its gelatinization properties. Rheological tests revealed that incorporating WPI influenced the viscoelastic properties of PS, enhancing its solid-like behavior and stability. In vitro digestion tests demonstrated that increasing WPI levels led to higher amounts of slowly digestible starch (SDS) and resistant starch (RS), while reducing rapidly digestible starch (RDS). These changes suggest a modified nutritional profile for PS, with potential benefits for regulating blood sugar levels and promoting gut health through the formation of new starch-protein complexes. Overall, this study highlights the potential of PS-WPI complexes in creating customized food products with modified textures and health-promoting properties, which lay the groundwork for further exploration of starch-protein interactions and their applications in food innovation and nutrition.

Keywords: *Starch-protein complexes; whey protein isolate; potato starch; digestion and food modification*

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A roadmap to investigating gastric emptying and metabolic responses of solid meals with glycemic loads: A protocol to integrating MRI evaluation with postprandial feeding

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Abstract

Postprandial gastric emptying is crucial to understanding prandial effects from glycemic and lipemic of meals. We describe the development of a study protocol to measure gastric emptying responses to a rice-based solid meal in healthy human volunteers using the MRI technique. The study design required serial testing of crossover postprandial dietary permutations with healthy subjects consuming test meals. The protocol development required MRI imaging, with biospecimen collection being performed at baseline and repeated prandial timings after single test meal ingestion. Perceived fullness and satiety measures were collected at each time point using Visual Analogue Scale. Total gastric content volume pre- and post-meal was assessed using T2-weighted axial and coronal images which were analysed using Horos software. Three pilot test runs were required to optimize the desired protocol. Test Run 1 used one subject to establish the MRI protocol parameters for imaging settings and staged timing between biospecimen collection and scans. Test Run 2 optimized the imaging sequence by reducing slice thickness and staged timings enabling efficient coordination for two subjects. Test Run 3 allowed optimization of the MRI schedule for the inclusion of three subjects per trial day. The total gastric volumes obtained from axial and coronal MRI views from Test Run 2 demonstrated close agreement across all timepoints (ICC = 0.987, $p < 0.001$), with low coefficients of variance (0.2-7.8%). The ICC for inter-rater reliability was 0.996 (95% CI: 0.988 – 0.999, $p < 0.001$), indicating excellent agreement in TGV measurements between the two raters. This research successfully developed and optimized MRI protocol for measuring gastric emptying in response to rice-based solid meals, integrating sequential scanning with concurrent biospecimen collection. This protocol will greatly enhance future studies on gastric emptying, particularly for solid meals requiring precise and reliable measurements.

Keywords: *magnetic resonance imaging; gastric volumes and solid meals*

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Microbial transglutaminase-induced gelation of black soldier fly larvae (*Hermetia illucens*) protein: Aggregation and rheological characterization

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Abstract

The utilization of edible insects like black soldier fly larvae (BSFL) protein in food applications requires a comprehensive understanding of their techno-functionalities under various processing conditions. This study investigates the potential of microbial transglutaminase (MTG) to induce gelation in BSFL protein, which is a crucial step for its application in structured food products. BSFL proteins were subjected to different MTG incubation times, ranging from 0h to 24h, to modulate the degree of protein crosslinking. Analyses including particle size, zeta potential, free amino group, surface hydrophobicity, sulfhydryl group (SH) quantifications, SDS-PAGE, rheological measurements, and water-holding capacity (WHC) were employed to elucidate the structural, physicochemical, and network development changes during crosslinking. SDS-PAGE results revealed that BSFL protein is susceptible to MTG crosslinking, with intensified large molecular weight bands appearing after just 30 minutes of treatment. The degree of crosslinking increased over time reaction as evidenced by a steady reduction in free amino groups, with a 21.6% crosslinking degree observed after 24h. The particle sizes first increased then decreased after 20h–24h of incubation, suggesting that extensive crosslinking led to the formation of compact structures. MTG crosslinking also altered protein conformation, as seen by a decrease in surface hydrophobicity. The reduction of total SH groups in MTG-treated samples denotes the contribution of disulfide bonds in the formation of BSFL protein gel network besides the MTG-catalyzed covalent isopeptide bonds. Compared to untreated samples, MTG treated BSFL protein formed a stable viscoelastic gel network with lower frequency dependency, higher critical strain, and improved WHC. Collectively, this study provides insights into the aggregation and gelation properties of MTG-modified BSFL protein, laying the groundwork for manipulating the texture of BSFL protein gels. This information is important for the development of BSFL protein-based colloidal materials and texturized insect-based food..

Keywords: *Black soldier fly larvae; alternative proteins; transglutaminase; enzymatic crosslinking and gelation*

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Do dietary patterns of T2D patients carry cardiometabolic risk? Insights from the RICH study

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Abstract

Little is known about dietary patterns (DPs) of Malaysian T2D patients and their cardiometabolic risk. Given multicultural and dietary diversities in Malaysia, eating habits impacting cardiometabolic health of this population need to be elucidated. To identify DPs and investigate their associations with cardiometabolic risk markers (BMI, waist circumference, blood pressure, TC, HDL-C, LDL-C, fasting blood glucose, HbA1c) in T2D patients. An à posteriori approach was adopted to analyze 3-day dietary records of 191 T2D patients screened for the Rice Intervention in Chronic Health (RICH) clinical trial. DPs were identified using principal component analysis (PCA). Associations of DP tertiles with sociodemographic and lifestyle characteristics were determined using One-way Analysis of Variance (ANOVA) and Pearson Chi-Square tests. One-way Analysis of Covariance (ANCOVA) tested associations of DPs with nutrient profiles and cardiometabolic risk markers. Three DPs were identified: "Non-Prudent," "Prudent," and "Eating Out." Malay patients mostly followed the "Non-Prudent" pattern, while Chinese and Indian female patients were more likely to adhere to the "Prudent" pattern (all $P<0.05$). The highest tertile of the "Non-Prudent" pattern profiled a higher carbohydrate and lower fat, sodium, and fiber content (all $P<0.05$). This pattern is significantly associated with increased HDL-C levels (T3=1.3±0.0 vs T1=1.2±0.0, P=0.022) and showed a clinically significant trend for increased HbA1c (T3=7.8±0.2 vs T1=7.3±0.2, P=0.075). The "Prudent" pattern is associated with lower carbohydrate (P=0.048) and higher fiber (P=0.033) content. This pattern linked to lower fasting blood glucose (T3=7.3±0.3 vs T1=8.4±0.3, P=0.014) and HbA1c (T3=7.4±0.2 vs T1=7.8±0.2, P=0.042) levels. The "Eating Out" pattern is associated with higher fat and sodium content (both $P<0.05$) and increased HDL-C levels (T3=1.3±0.0 vs T1=1.2±0.0, P=0.022). The "Prudent" pattern inclusive of whole grains and vegetables, is associated with better glycemic control in these T2D patients.

Keywords: *Type 2 diabetes; dietary patterns and cardiometabolic health*

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Improving bamboo soda pulp by steam explosion and refining processes for packaging use

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Abstract

The growing demand for pulp and paper products, coupled with limited wood supplies, has driven the industry to seek alternative resources. Bamboo, the world's fastest-growing plant, is a promising alternative non-wood pulp resource for paper and packaging manufacturing. However, the bamboo pulping process is often chemically and energy-intensive. Thus, the purpose of this study was to evaluate a soda process using low alkali (NaOH) concentration and low pulping temperature. The experiment involved pre-soaking bamboo chips with 15-25% NaOH for 48 hours and cooking at 100±5°C for 1-9 hours. A pulping period of 4-6 hours enhanced the screened yield of the resulting pulp. Furthermore, increasing the NaOH concentration reduced the amount of uncooked bamboo (reject portion), but did not affect the lignin content of the resultant pulp. The optimal soda pulping condition, achieved with 15% NaOH and pulping time of 6 hours, resulted in a pulp yield of 70% and 47% delignification. The effects of steam explosion pre-treatment and pulp refining post-treatment were also studied. Steam explosion proved greatly beneficial for lowering the uncooked portion of bamboo down to 0.03%. At the optimum pressure of 18 bar, it was possible to improve fibrillation of bamboo chips by up to 61%. After pulping, the obtained bamboo pulps were wet-formed in the frame before being hot-pressed into pulp sheets using compression molding. It was revealed that using either a steam explosion or a refining process, treated bamboo soda pulps increased their tensile index by more than 8 times to 4.37-4.98 MPa compared to untreated bamboo pulp (0.39 MPa) due to enhanced fiber-fiber bonding from pulp fiber fibrillation. The tensile strength of the present pulp sheet was comparable to that of bamboo pulps prepared using previous alkali-based pulping processes, indicating that it is suitable for use in packaging and other paper-based products.

Keywords: *Bamboo; soda-pulping; steam-explosion; refining and tensile index*

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Place-based social innovation in urban living labs: A case study of an outdoor research and teaching site

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Abstract

Urban Living Labs (ULLs) are pivotal experimental spaces fostering sustainability transitions in urban areas. While extensive research in Europe and North America explores the link between social innovation in ULLs and urban sustainability, there is limited focus on their role in rapidly urbanizing regions like China. This study investigates how ULLs can drive innovation and sustainability in such contexts, focusing on the Outdoor Research and Teaching Space (ORTS) ULL at Xi'an Jiaotong-Liverpool University (XJTLU). We examined the iterative mechanisms through which diverse user preferences promote social innovation and multifunctionality within ORTS. Utilizing the Q-method, we classified user preferences based on interviews with local and international scholars and staff. Additional in-depth interviews and participatory action mapping helped categorize the types of social innovation within ORTS and summarize the operational processes. Our findings reveal that the diversity of user attitudes, approaches, and multicultural backgrounds enhances social innovation through shared preferences and coordinated space usage. This diversity drives the multifunctionality of the ULL, creating a cyclical effect where increased user engagement further enhances multifunctionality. Pre-existing shared values, such as environmental care and waste reduction, alongside specific place-based conditions—like ORTS's proximity to indoor research facilities and a canal for irrigation—encourage innovative behaviors that conserve resources and the environment. This study suggests that the ORTS ULL demonstrates how user diversity and shared goals can foster innovation and sustainability in urban experimental spaces. Future research should explore similar ULLs across different contexts and scales to validate whether this participatory, multifunctional model operates as a broader mechanism for innovation diffusion, offering sustainable solutions for urban spaces. Nonetheless, our findings highlight ORTS as a case where experimental spaces effectively support sustainability transitions in urban settings.

Keywords: *Urban living lab; place-based social innovation; multiple functionality; multicultural; urban sustainability and transformative changes.*

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A comprehensive study on the protein quality and toxicity analysis of black soldier fly larvae (*Hermetia illucens L.*) as an alternative food source

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Abstract

The rising of global awareness towards sustainable diets and their impact on food security has led to an increasing interest in alternative protein sources. Among these, black soldier fly larvae (BSFL) are one of the most promising options for their high protein content, low environmental footprint, and high fecundity. However, it is essential to ensure the quality of the protein to advance their application as a viable alternative for human consumption. High-quality protein is generally measured by its ability to provide all indispensable amino acids and the digestibility of protein for human growth and maintenance of body functions. Hence, the current study was designed to determine the protein quality of BSFL by evaluating their amino acid composition using the HPLC method and their digestibility through the in-vivo rat model, with a focus on determining the Digestible Indispensable Amino Acid Score (DIAAS). The amino acid profile of BSFL revealed the presence of all the indispensable amino acids essential for human health, classifying it as a complete protein source. However, in the in-vivo rat analysis, BSFL protein exhibited a significantly lower DIAAS value of 82.34% ± 3.76% ($p < 0.05$), compared to casein protein, which recorded a DIAAS of 114.04% ± 2.89%. An acute toxicity analysis was also conducted alongside the in vivo digestibility analysis following a 10-day oral intake period. This analysis focused primarily on kidney and liver function, as observed through biochemical and haematology tests. Results indicated a decrease in total serum protein and an increase in the albumin: globulin ratio in the BSFL group compared to the casein and protein-free groups, suggesting potential liver inflammation. However, no toxicological changes were observed in body weight, water and food consumption, or haematology markers in rats consuming BSFL protein. In conclusion, these findings suggest that while BSFL protein holds promise as a complete protein source, further toxicological evaluations over extended periods and with higher dosages are necessary to ensure their safety as food ingredients.

Keywords: *Black soldier fly larvae protein; edible insect; alternative protein source; protein quality and food safety*

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Mimicking the production of Philippine Buro using microbial isolates from its source of origin, Tilapia and shrimp

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Abstract

The popular traditional Filipino fermented food commonly referred to as "BURO," and in global terms known as Pickle is favored by the locals for its unique taste and was used in this study. Typically, it is made from a mixture of either brine or rice and several types of seafood. The study aimed to isolate Lactic Acid Bacteria (LAB) from commercial BURO specifically produced from *Oreochromis* sp., Tilapia and *Caridea* sp., Shrimp. A standard microbiological procedure for isolating LAB was implemented using MRS agar in a pour plate method. All sample products and microbiological assays were replicated three times. Likewise, Gram staining technique was employed to identify the Gram reactions of the microbial isolates. A total of three microbes were isolated from the Tilapia and Shrimp samples. Two isolates were Gram-positive rods from both samples, while one isolate appeared as a rod-shaped Gram-negative bacterium. These isolates were further used to mimic the production of BURO in the absence of tilapia and shrimp. This is to compare the physico-chemical change, texture, color, and odor after 10-day fermentation. Initial results showed that successful fermentation occurred by the decrease in pH and an increase in the moisture content. A slight change in the color and texture was noted. In addition, it produced a pungent smell after three days of observation, which further indicates fermentation. To date, microbial isolates were being processed for bioassays, organoleptic evaluation, metabolite extraction, anti-quorum sensing activities, and final identification.

Keywords: *BURO; fermentation; lactic acid bacteria; Tilapia and shrimp*

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Interactive effects of warming and benzophenone-3 on selected *Chlorella* strains from different climate regions

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Abstract

Benzophenone-3 (BP-3), a commonly used organic ultraviolet (UV) filter in sunscreen products, has raised concerns due to its prevalence in aquatic ecosystems and its potential for bioaccumulation. The accumulation of BP-3 in aquatic environments poses a significant threat to the survival of aquatic organisms and disrupts the delicate balance of aquatic ecosystems. In parallel, global warming, largely driven by human activities such as fossil fuel combustion and deforestation, presents critical environmental challenges. Rising global temperatures lead to a cascade of ecological disruptions—glacial melting, sea-level rise, and shifts in species distribution—all of which threaten ecosystem sustainability. Any adverse impacts on microalgae can have far-reaching consequences on higher trophic levels. In this study, we investigated the interactive effects of BP-3 and elevated temperatures by employing three *Chlorella* strains from different regions, spanning tropical (*Chlorella* UMACC 003), temperate (*Chlorella vulgaris* UMACC 248), and polar climates (*Chlorella* UMACC 234). The *Chlorella* strains were subjected to a combination of BP-3 at varying concentrations (0, 0.01, 0.1, 1.0, 10.0, 100 mg/L) and elevated temperatures (ambient, ambient +4 ℃, ambient +8 ℃) over a duration of 10 days. Our assessment included measurements of the specific growth rate (μ), photosynthetic pigments (chlorophyll-a, chlorophyll-b, carotenoids), and biochemical composition (carbohydrates, proteins, lipids) of the *Chlorella* strains, enabling a thorough understanding of the intricate multi-factorial impacts influencing the *Chlorella* strains from different climate regions. We conducted docking simulations to assess the binding affinity of BP-3 with metabolites of *Chlorella* strains. Notably, polar microalgae demonstrated increased sensitivity to BP-3 under warming conditions compared to tropical and temperate strains. BP-3 was found to exert inhibitory effects on the growth of the polar *Chlorella* UMACC 234 strain at all concentrations tested across all temperature conditions. This study observed a decline in specific growth rate, likely due to reduced levels of chlorophyll-a (chl-a), chlorophyll-b (chl-b), and carotenoids. Understanding the interaction between BP-3 and global warming on microalgae is essential for assessing potential long-term impacts on aquatic ecosystems.

Keywords: *Microalgae; emerging contaminants; benzophenone-3; temperature stress and biochemical compositions*

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Ultrasound-enhanced fermentation of soymilk with *Streptococcus salivarius* for improving hypoglycemic properties and biomass

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Abstract

This study investigates the impact of ultrasound-assisted fermentation of Soybean Isolate Protein (SPI) using *Streptococcus salivarius* ATCC9222, specifically examining hypoglycemic potential and bacterial biomass. Response Surface Methodology (RSM) was used to identify the optimal ultrasound parameters to maximize these effects. Through RSM optimization, the most effective ultrasound conditions were identified as 20 kHz frequency, 45% amplitude, 3.5-minutes treatment duration, and two 10 second pauses. Under these optimized conditions, the biomass of S. salivarius showed a significant increase ($P<0.05$). Hypoglycemic activity was evaluated using α-amylase and α-glucosidase inhibition assays, which revealed that ultrasound-assisted fermentation markedly improved bioactivity compared to the control. Specifically, anti-α-glucosidase activity increased to 51.77%, while anti-α-amylase activity reached 13.97%. Biomass also rose significantly to 3.14 × 10^8 CFU/cm^3. In contrast, the fermentation process without ultrasound achieved only a 41.9% inhibition rate for α-glucosidase, a 10.4% inhibition rate for α-amylase, and a biomass of 1.1 × 10^8 CFU/cm^3. This study demonstrates that ultrasound-assisted fermentation can significantly enhance the production of bioactives while boosting the growth of S. salivarius in SPI-based media. The observed improvements in both α-amylase and α-glucosidase inhibitory activity highlight the potential for developing this technique as a novel, efficient approach to producing functional food ingredients for diabetes management. This study underscores the broader impact of ultrasound technology in food biotechnology, opening pathways for more efficient production methods that improve the bioavailability and functional properties of peptides in fermented food products.

Keywords: *SPI; fermentation; Streptococcus salivarius; hypoglycemic properties and biomass*

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Antifungal activity of piperine-based nanoemulsion against Candida spp.

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Abstract

Candidemia leaves a trail of approximately 750,000 cases yearly, with a morbidity rate of up to 30%. While *Candida albicans* still ranks as the most predominantly isolated *Candida species*, *C. glabrata* comes in second, with a death rate of 40–50%. Although infections by *Candida* spp. are commonly treated with azoles, the side effects and rise in resistance against it has significantly limited its clinical usage. The current study aims to address the insolubility of piperine and provide an alternative treatment to *Candida* infection by formulating a stable piperine-loaded O/W nanoemulsion, comprised of Cremophor RH40, Transcutol HP and Capryol 90 as surfactant, co-surfactant, and oil, respectively. Characterization with zetasizer showed the droplet size, polydispersity (PDI) and zeta potential value of the nanoemulsion to be 24.37 nm, 0.453 and -21.10 mV, respectively, with no observable physical changes such as phase separation from thermostability tests. FTIR peaks confirmed the presence of piperine within the nanoemulsion and TEM imaging visualized the droplet shape and further confirmed the droplet size range of 20–24 nm. The MIC90 value of the piperine-loaded nanoemulsion determined with *in vitro* broth microdilution assay was approximately 20–50% lower than that of the pure piperine in DMSO, at a range of 0.8–2.0 mg/mL across all Candida spp. tested. Overall, the study showed that piperine can be formulated into a stable nanoemulsion, which significantly enhances its antifungal activity compared to piperine in DMSO..

Keywords: *Nanoemulsion; natural product; Candida spp and antifungal*

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The capacity building for improving the business growth of local enterprise (agricultural cluster) in Chiang Rai Province, Thailand

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Abstract

This project aims to develop entrepreneurial capabilities in agriculture for farmers and community-based enterprises, elevate the standards of herbal raw material production for community-based enterprises and farmers, develop a high-quality herbal raw material production business model, and enhance financial planning capabilities for community-based enterprises. It was conducted using the Area-based collaborative research for development approach, employing Participatory Action Research methods. These methods involve engaging in hands-on research, learning together as a group to understand oneself, the community, and the environment, to identify problems and find solutions through direct field surveys, observations, and interviews with stakeholders in the herbal value chain system. There were 117 participants including farmers, community-based enterprises, collectors, quality inspectors, processors, transporters, and entrepreneurs. The analysis and synthesis of data was summarized accordingly. The results found that the target groups of farmers and community enterprises can expand their business in producing high-quality herbal raw materials, both in terms of production capacity and financial management capabilities. Moreover, they can manage and expand their cultivation areas, allowing them to produce high-quality herbal raw materials with an average increase in curcuminoid content by 6.55% and an increase in production volume by 68.80%. Furthermore, the target groups in the project have knowledge and understanding in financial and marketing planning, production cost calculation, and future financial planning for cultivation. They also have increased their ability to engage in businesses and an average increase in business income by 35.54%. These efforts not only contribute to the development of the target groups' personnel capacity and the production of high-quality herbal raw materials but also improve the quality of life for the target group members in terms of economy, society, and health.

Keywords: *Curcuma longa L.; curcuminoid content; local enterprise and high-quality herbal raw material*

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Development of community enterprises in Chiang Rai province in herbal processing and raw material management: Enhancing product diversity and economic sustainability through carrageenan herbal jelly production

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Abstract

The development of community enterprises involved in herbal processing and raw material management is essential for fostering economic stability at the community level. This research focuses on enhancing production quality and diversifying herbal products by utilizing locally abundant herbs, specifically *Clitoria ternatea L., Hibiscus sabdariffa L.*, and *Chrysanthemum indicum L*. These herbs were processed to create a new product carrageenan herbal jelly designed for health-conscious consumers, including those with chronic conditions like diabetes. The research began by analyzing their phenolic content and antioxidant activity, leading to the development of a prototype herbal jelly product. The results revealed that the water extracts of the three herbs demonstrated strong antioxidant activity, correlating with the total phenolic content. The microbial analysis showed that the total plate count for bacteria, yeast, and mold in the prototype herbal jelly was less than 10 CFU/g, complying with the Ministry of Public Health's Announcement (No. 416) B.E. 2563 under the Food Act B.E. 2522, ensuring the product's safety for consumption. This innovation not only adds value to herbal products but also enhances their competitiveness in the herbal supplement market. By diversifying the herbal market, the product increases the income of community enterprises in a sustainable manner. This study integrates business management strategies, advanced herbal processing technologies, and the development of raw material quality standards, empowering community enterprises to add value efficiently. Additionally, the project promotes the conservation of local natural resources and creates new employment opportunities. Overall, this initiative plays a critical role in driving economic growth and improving the quality of life within the community.

Keywords: *Community enterprises; production quality; value-added products and carrageenan herbal jelly*

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Truncated pardaxin derivative targets MAPK8 to induce selective breast cancer apoptosis via the MAPK/JNK pathway with reduced toxicity to normal cells

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Abstract

Cancer remains a global health challenge, with conventional anticancer drugs often causing adverse effects like pulmonary issues and drug resistance. This highlights the need for alternative therapies. Anticancer peptides are promising candidates due to their higher selectivity against cancer cells and ease of modification. Among these, pardaxin has demonstrated anti-proliferative effects on different cancer types. Nevertheless, concurrent hemolysis effects were also reported. The study proposes to shorten and induce residue replacement to discover pardaxin derivatives retaining anticancer yet alleviating hemolytic effects via *in silico* analyses followed by *in vitro* validation. Pardaxin was truncated to 27 or 18 residues and involved single-residue replacement by AntiCP 2.0. Besides, the 27 residue peptide was replaced with multiple arginine residues. These designed peptides were further predicted for anticancer, hemolysis, toxicity, and cell-penetrating capabilities. Potent anticancer derivatives with non-toxic and low hemolytic probability were subsequently predicted for alpha-helices and stronger docking affinity towards the FAS death receptor. Potential peptides were synthesised for validation, including MTT assays for breast cancer and normal cells, hemolysis assays on human erythrocytes, and FITC-Annexin V binding and lactate dehydrogenase (LDH) assays for apoptosis and necrosis investigation. Potential targets for MAPK/JNK apoptosis were investigated through HDOCK docking screening and validated via molecular dynamics (MD) simulation and MM/GBSA free binding energy calculation. Among the synthesised derivatives, *in vitro* results validated D18.13 as the most potent peptide due to retaining its breast cancer-killing ability in MDA-MB-231 and MCF-7 (IC_{50}=123.3±3.3; 89.8±6.7 μM) by inducing apoptosis over necrosis. It also exhibited lowest toxicity on normal cells (IC50 > 200 μM) and alleviated hemolytic effects. In the MAPK/JNK pathway, docking analysis identified MAPK8 as the potential target of D18.13, further validated by MD simulation and MM/GBSA. In conclusion, *in silico* approaches facilitated anticancer peptide discovery, like D18.13, which could treat breast cancer effectively without side effects.

Keywords: *Bioactive peptide; pardaxin; shortened anticancer peptide; breast cancer and in silico*

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Preparation of protein hydrolysates from *Schizophyllum commune* and its biological activities

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Abstract

Schizophyllum commune, an established potential source of bioactive compounds, has been primarily focused on polysaccharides and other metabolites. However, the studies that focused on proteins and protein hydrolysates are limited. This research aimed to investigate the optimal conditions for producing protein hydrolysates from *S. commune* and assessed their biological activities due to limited knowledge about protein functionality. Proteins were hydrolyzed using Alcalase® under various conditions based on protein concentration. The optimal result was exhibited at 80 mU, 37°C for 60 minutes. The acquired hydrolysates were further fractionated using a molecular weight cutoff (MWCO) spin column, and their antioxidant, anticancer, and anti-inflammatory properties were examined. The 10-50 kDa fractions exhibited potent antioxidant activity, as indicated by the IC_{50} values of 0.78 ± 0.02 mg/mL for ABTS radical scavenging activity and 1.98 ± 0.07 mg/mL for DPPH radical scavenging activity. Additionally, the hydrolysates decreased nitric oxide production in RAW 264.7 macrophages (IC_{50} = 3.31 mg/mL) and exhibited cytotoxicity against SW480 human colon cancer cells (IC_{50} = 1.5 mg/mL). The CC_{50} results showed that the hydrolysates exhibited significantly lower cytotoxicity toward L929 cells compared to the non-hydrolyzed proteins. These results suggested that S*. commune* protein hydrolysates possessed promising biological activities. Future studies should focus on large-scale production and further elucidating the factors influencing their biological properties..

Keywords: *Schziophyllum commune; optimal conditions; protein hydrolysates and biological activities*

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Anterior foregut endoderm differentiation from bone marrow derived-human induced pluripotent stem cells

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Abstract

Anterior foregut domain is known to be one of the regions that will develop within the endoderm post-gastrulation during embryonic development. In humans, the anterior foregut domain is known to develop major cell types present within the esophagus, lung, salivary glands, thymus, parathyroid and thyroid. Being able to understand the developmental biology and signaling pathways of different organs originate from the anterior foregut domain will enable research in three-dimensional (3D) *in vitro* models. This study aimed to observe the morphological changes during the stepwise induction from human induced pluripotent stem cells (hiPSCs) to the anterior foregut endoderm. Anterior foregut endoderm (AFE), a group of cells that can be derived from the anterior foregut domain, was developed using human induced pluripotent stem cells (hiPSCs) obtained from a commercial cell line. Firstly, hiPSCs were maintained and cultured to obtain desired hiPSCs colonies suitable for induction. The desired hiPSCs colonies were subjected to stepwise differentiation to be induced into definitive endoderm by maintaining in a medium supplemented with signaling molecules that regulate Transforming growth factor beta (TGF-β) morphogens, which are the key regulating factors within the Nodal signaling pathway that give rise to the definitive endoderm. The successfully developed definitive endoderm was subsequently used to derive anterior foregut endoderm by means of controlling the necessary growth factors and signaling molecules that regulate the SMAD signaling pathway and Wnt signaling pathway. Morphological changes along the stepwise differentiation from hiPSCs to AFE were evident. Other than that, the anterior foregut endoderm differentiation was successful as AFE spheroids managed to self-aggregate and detach from the monolayer. Stepwise differentiation from hiPSCs to AFE was proven to be successful as anterior foregut endoderm spheroids were present on the last day of AFE induction.

Keywords: *Human induced pluripotent stem cells; definitive endoderm; anterior foregut endoderm; organogenesis and Signaling pathways*

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Comparative bioactivities of essential oils from kaffir lime peel wastes and bentong ginger rhizomes

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Abstract

Essential oils (EOs) from kaffir lime (*Citrus hystrix*) peel and Bentong ginger (*Zingiber officinale* var. Bentong) possess valuable aromatic and bioactive properties. There is a growing interest in their potential applications in various industries, especially as a natural alternative ingredient to improve the biological performance and market value of other existing products. This study aimed to compare the phytochemical profiles, antioxidant, and anti-inflammatory activities of EOs from fresh kaffir lime peel and Bentong ginger rhizome. The EOs were extracted using the ultrasonication-assisted hydrodistillation method. Determination of total phenolic (TPC) and total flavonoid (TFC) contents along with GC-MS analysis were conducted to elucidate the chemical compounds present in both samples. Antioxidative evaluation included DPPH radical scavenging, metal chelation, and ferric reducing antioxidant power (FRAP) assays, while bovine serum albumin (BSA) denaturation inhibition test was used to determine anti-inflammatory activities of the EOs extracted. Through GC-MS analysis, kaffir lime peel EO was found to contain β-Pinene (19.9%) and Citronellal (18.7%) as the major volatile compounds whilst Bentong ginger EO possessed α-Citral (22%), β-Citral (13%), and α-Curcumene (15.5%). Bentong ginger EO exhibited higher TPC and TFC, resulting in stronger antioxidant performances based on DPPH radical scavenging and FRAP assays at concentrations 10 to 50 mg/mL. Kaffir lime peel EO, on the other hand, demonstrated stronger ferrous ion-chelating activity besides exerting superior anti-inflammatory properties as reflected by higher inhibitory effect towards BSA denaturation. This interesting phenomenon could be attributed to the presence of β-Pinene in the kaffir lime peel EO. This volatile compound had displayed anti-inflammatory as well as metal chelating properties in previous studies. As such, kaffir lime peel, a food waste, may offer a more cost-effective and eco-friendlier raw material for EO production as compared to the high-priced Bentong ginger.

Keywords: *Kaffir lime peel; Bentong ginger; essential oils; antioxidant and anti-inflammatory*

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Effect of soybean intercropping with sugarcane on the dynamic distribution of systemic bacteria, nitrogen-fixing bacteria and nitrogen nutrition

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Abstract

Sugarcane is currently one of the most important sugar crops and also a potential bioenergy crop. Guangxi is the largest sugar production base in China. Sugarcane has a long growth cycle, high biomass, and a high demand for fertilizers, with the highest demand for nitrogen fertilizer. Long-term and large-scale application of nitrogen fertilizer can lead to soil nutrient imbalance, soil acidification and compaction, water and air pollution, and low nitrogen utilization efficiency of sugarcane. Sugarcane can combine nitrogen fixation with nitrogen-fixing bacteria to obtain part of nitrogen required for growth and development. Through intercropping with leguminous crops, an appropriate combined nitrogen fixation group can be formed, and less nitrogen fertilizer is applied to promote sugarcane combined nitrogen fixation, which greatly saves nitrogen fertilizer and production cost, and realizes sugarcane high yield, high sugar and efficient environmental protection production. Sugarcane intercropping and the utilization of sugarcane combined nitrogen fixation resources are important ways to save cost and increase efficiency. In this study, sugarcane and soybean intercropping field experiments will be conducted with three sugarcane varieties and one soybean varieties intercropping respectively under the condition of reduced nitrogen application. Community structure, species diversity and temporal and spatial changes of nitrogen-fixing bacteria in sugarcane-soybean intercropping system will then be further analyzed by 16S and nif*H* amplified sequencing based on the high-throughput third generation sequencing platform, and the migration and colonization of target rhizobia will be monitored by fluorescence in situ hybridization. Then, the relationship between the spatial and temporal distribution of nitrogen-fixing bacteria and the changes of community structure in the intercropping system and the changes of nitrogen-fixing activity and nitrogen accumulation of sugarcane plants will be studied, so as to provide theoretical basis for the production practice of sugarcane-bean intercropping and the construction of efficient sugarcane nitrogen-fixing system that will be of benefit to the sugarcane industry..

Keywords: *Intercropping; sugarcane; nitrogen-fixing bacteria; nitrogen nutrition and diversity*

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Effects of different pretreatments on hydrodistillation extraction of essential oils from *Aquilaria sinensis* (agarwood)

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Abstract

Agarwood is a highly regarded non-timber forestry product widely used in fragrances, incense, medicinal preparations, aromatherapy, and religious rituals. Agarwood essential oil (AEO), valued for its fragrance, is extracted from the oleoresinous heartwood of the Aquilaria tree. The tree secretes this dark, viscous resin as a natural defense mechanism in response to injury, primarily within its stem tissues. Currently, most available studies on AEO extraction focus on optimizing a single extraction method. However, combining different extraction methods has been shown to enhance both efficiency and yield. Among the various techniques, a combination of ultrasound and microwave extraction offers many advantages over traditional methods. Despite this, there is no existing report or application of continuous combined extraction methods in the AEO extraction process. This study explores the effects of different pre-treatments (soaking, ultrasound, microwave, and ultrasonic-microwave synergy) along with material size on the yield of *Aquilaria sinensis* (agarwood) essential oil. The study examines the impact of these pre-treatments on AEO yield, color, and microstructural changes. Ultrasonic pre-treatment, identified as the most environmentally friendly method, yielded 0.140%, surpassing soaking (0.109%), direct hydrodistillation (0.104%), microwave pre-treatment (0.111%), and the combination of microwave-ultrasound (0.137%). According to SEM analysis, ultrasonic pre-treatment disrupts the material's surface structure, enhancing AEO production, diversifying functional substances, and improving extraction efficiency. Additionally, using a 20-mesh screen increased the contact area of the raw material, improving the solubility of active components and stabilizing AEO output. This study lays the groundwork for further improving and utilizing AEO extraction processes. The results suggest that ultrasonic-assisted hydrodistillation (UHD) could significantly improve AEO extraction and utilization

Keywords: *Aquilaria sinensis; agarwood essential oil; extraction yield; hydrodistillation and ultrasound*

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Enhancing molded pulp for food packaging applications via cellulose derivatives surface coating

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Abstract

Molded pulp (MP) is a highly attractive biodegradable packaging compared with non-degradable single-use plastic packaging. However, its porous fiber structure and hydrophilic nature make it unsuitable for direct food packaging due to high oil/water absorption and significant deformation when exposed to hot foods or microwaved. Surface coating is thus necessary to improve the properties of molded pulp and expand its use in food packaging. Cellulose derivatives (CD) are attractive biopolymers as coating materials for conventional plastics (e.g., polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP)) due to their biocompatibility, film-forming capabilities, low toxicity as well as good thermal, water, and oil resistance. In this study, CD was applied onto a molded pulp surface using a solution dip-coating technique, with an optimal coating thickness of 50 µm. The uncoated MP and MP coated with CD (MP/CD) samples were tested for water absorption (Cobb test) and grease resistance (Kit test, Tappi T559). The results indicated that MP/CD sample exhibited as high as 98% reduction in water absorption and achieved a maximum grease resistance with Kit value of 12. In addition, the tensile strength of the MP/CD sample increased by 146%. MP and MP/CD samples were tested for hot water and oil resistance (GB18006-2008) at 98±5°C for 20 minutes. The MP/CD sample showed no penetration from hot water or hot oil. Furthermore, after 3.5 minutes of microwave testing at 600 watts, it was found that the MP/CD sample holding water within exhibited no shape deformation or water leak, as opposed to the MP sample. These findings demonstrated that bio-based CD coating could enable pulp or paper packaging to be used as a hot water/oil resistant, microwave-safe, biodegradable alternative to conventional food plastic packaging.

Keywords: *Cellulose derivatives; molded pulp; hot water resistance; hot oil resistance and microwavable*

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Tumour-homing peptides targeting breast cancer

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Abstract

The use of tumour-homing peptides (THPs), is an effective approach for diagnosing breast cancer that belongs not just one but several major breast tumor subtypes. THPs were designed based on their binding affinity with highly expressed receptors by breast cancer cells and synthesized for imaging detection purposes. Cytotoxicity assay was performed to determine the minimum non-toxic dose of the peptides. These peptides were also incubated in human blood samples to determine the hemolytic activity. The IFN-γ ELISPOT assay was employed to determine the possibility of inflammatory response upon peptide treatment on human peripheral blood mononuclear cells (PBMC). The binding efficacy of the THPs with breast cancer cells was confirmed by fluorescence imaging. Flow cytometry analysis was carried out to measure fluorescence intensity to predict binding between the FITC-labelled THPs and breast cancer cells. Finally, breast cancer tissue microarray slides were incubated with FITC-labelled THPs to determine ex vivo peptide binding capacity. Minimum non-toxic dose selected for succeeding tests was fixed at 12.5 µg/mL and 25 µg/mL as percentage of cell viability was in the range of 80-92 %. No inflammatory response (IFN-γ) and hemolytic activity were observed ensuring the safety of the THPs. Uptake of FITC-labelled peptides MBC-P1, MBC-P2 and MBC-P3 was observed in breast cancer cells and uptake of FITC-labelled MBC-P2 was observed in breast cancer tissue. There is no significant uptake of FITC-labelled THPs in normal breast cells (MCF-10) and normal breast tissue. Among the THPs, MBC-P2 has the highest percentage of fluorescence detection indicating higher rate of binding to breast cancer cells at both concentrations of 12.5 µg/mL [MDA-MB-231(88.05 ± 0.95 %), MDA-MB-453 (88 ± 5.7 %) and T47D (89.1 ± 2 %)] and 25 µg/mL [MDA-MB-231(95.5 ± 0 %), MDA-MB-453 (99 ± 0.6 %) and T47D (91.3 ± 1.1 %)]. The findings of this study shows bioinformatic analysis can be utilised to produce potential diagnostic THP which shows efficacy towards breast cancer cells that belong to several major breast tumour subtypes.

Keywords: *Tumour-homing peptide; diagnosis and breast cancer*

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Selection of skin-penetrating peptides (SKPs) for non-invasive transdermal delivery

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Abstract

Skin-penetrating peptides (SKPs) have emerged as a novel and promising approach for enhancing permeation in non-invasive transdermal delivery. This can be attributed to the affinity of SKPs for skin constituents. Their application could potentially help to address the challenges associated with current drug delivery methods, such as skin invasiveness, enzymatic degradation and first-pass metabolism. This study aims to select and identify novel SKPs for their potential use in enhancing topical delivery of therapeutic agents. SKPs were isolated through biopanning of phage display peptide libraries onto *ex vivo* excised living human skin models. Potential SKPs were identified, and their sequences were analysed *in silico* using PepCalc (https://pepcalc.com) to determine molecular weight and isoelectric point, as well as the GRAVY Calculator (http://www.gravy-calculator.de/) to calculate the grand average of hydropathy (GRAVY) index (hydrophobicity/hydrophilicity). These parameters indicate their physicochemical properties, which influence their interaction with the skin. Five SKPs were shortlisted for further skin-permeation studies on excised skin tissues. Peptides with the best penetration profiles can be used for further analysis and optimisation, such as fusing with a nanocarrier for drug delivery. The biopanning results demonstrate that these peptides can successfully facilitate the transport of the M13 phage across the skin, underscoring their potential as effective permeation enhancers by replacing the phage particles with therapeutic agents. This delivery method is also applicable to diagnostic approaches and cosmetics applications, offering enhanced penetration.

Keywords: *Biopanning; skin-penetrating peptides; transdermal drug delivery and permeation enhancers*

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In silico and *in vitro* identification of potent biotherapeutic anticancer peptides derived from bee venom and royalisin targeting metastatic breast cancer cells MDA-MB-231 and T47D

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Abstract

Cancer has emerged as a leading cause of death worldwide, with female breast cancer being the most frequently diagnosed and the second leading cause of mortality among women. Traditional cancer treatments, including chemotherapy and radiation, have been practiced for many years but are now less favored due to drug resistance, lack of tumor selectivity, and high costs. Peptide-based therapeutics is a promising and novel approach in targeting and disrupting tumour cells with various advantages such as low molecular weight and toxicity. In this study, our objective was to design potential biotherapeutic anticancer peptides (ACPs) derived from bee venom (specifically Melittin, Phospholipase A2, Tertiapin, Mast cell degranulating peptide, Apamin) and Royalisin from the honeybee *Apis mellifera*. Using advanced computational biology techniques, we fragmented a set of three overlapping peptides encompassing the entire sequence of different bee venoms. Each ACP consists of 15 amino acid residues, with one residue overlapping with the adjacent peptide. With amino acid substitution, we have successfully designed 19 ACPs with a high anticancer prediction score along with low toxicity and hemolytic activities. Through peptide-protein docking, M1_3 has the strongest inhibitory action targeting several anti-apoptotic proteins (Bcl-2, Bcl-xL and MCL1) in metastatic breast cancer cells. However, based on the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, it was discovered that while A_1 and R4I7I displayed strong anti-proliferative activity against MDA-MB-231 and T47D, they exhibit no hemolytic activity. Although these newly designed peptides have exhibited significant anti-proliferative activity against breast cancer cells, further investigations are required to determine the underlying mechanisms of these ACPs.

Keywords: *Apis mellifera; bioinformatics; metastasis and peptides*

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Anti-diabetic properties of piperidone curcumin analogues with α-amylase and α-glucosidase inhibitory activities

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Abstract

Diabetes mellitus, characterized by hyperglycemia due to impaired insulin secretion and activity, is a growing global health concern. With an escalating global incidence projected to reach alarming numbers by 2030 and 2045, Malaysia faces a particularly high prevalence within the Western Pacific region. Naturally occurring anti-diabetic compound curcumin can prevent diabetes complications due to antidiabetic properties as well as the attenuation of postprandial hyperglycemia. Thus, this study aimed to investigate the anti-diabetic properties of piperidone curcumin analogues through α-amylase (α-Amy) and α-glucosidase (α-Glu) inhibitory bioassay. The results showed that curcumin analogues with N-methyl 4-piperidone and 1-benzyl-4-piperidone linkers inhibited α-Glu and α-Amy enzymes with the former linker showing greater inhibition effects. In particular, the analogues with N-methyl 4-piperidone linkers bearing phenyl and fluorophenyl substituted rings are the most potent inhibitors of the α-Glu enzyme among twelve analogues, with 98% and 99% inhibition when compared to the positive control, acarbose (19%) at 1mM. Both analogues exhibited IC_{50} values at 0.35 mM and 0.69 mM in comparison to acarbose (3.12 mM). Furthermore, the same analogues demonstrated mild inhibitory action against the α-Amy enzyme with 34% and 43% inhibition if compared to acarbose (88%) at 1 mM. Given that the progression of severe gastrointestinal adverse effects is facilitated by strong inhibitors of pancreatic α-Amy, both potent curcumin analogues exhibited a lower α-Amy/α-Glu inhibitory ratio, which suggests that they have a high potential as lead compounds for an anti-diabetic drug. The curcumin analogues are presently under investigation for their potential to be used in anti-diabetic cell model assays, including glucose uptake, glycerol release, and cell viability (MTT).

Keywords: *Mono-carbonyl curcumin; hyperglycemia; glycohydrolase and metabolic disease*

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Identification and validation of programmed death-ligand 1 single nucleotide polymorphisms (rs4143815, rs822336 and rs2890658) in non-small cell lung cancer through DNA sequencing and bioinformatics

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Abstract

Programmed Death-Ligand 1 (PD-L1) is a critical immune checkpoint protein involved in modulating the immune response in cancer, particularly non-small cell lung cancer (NSCLC). Genetic variations within the PD-L1 gene, such as single nucleotide polymorphisms (SNPs), may play a role in cancer development and progression. Among these, SNPs rs4143815, rs822336 and rs2890658 have been observed in various cancer types. Despite their identification in various cancers, the role of these SNPs in NSCLC remains inadequately explored. This study aims to identify and validate the presence of these PD-L1 SNPs (rs4143815, rs822336, rs2890658) in NSCLC through DNA sequencing and bioinformatic analysis. Genomic DNA was extracted from snap-frozen NSCLC tumour tissues, and the target regions harbouring the SNPs were amplified via conventional polymerase chain reaction (PCR). SNP identification will be carried out using Sanger sequencing, followed by bioinformatic analysis to annotate the SNPs and determine their potential functional significance. The preliminary findings are expected to confirm the presence of these SNPs in NSCLC, with bioinformatic annotations providing deeper insights into their biological impact. Understanding these genetic variations is crucial, as they may serve as potential biomarkers for predicting patient responses to immunotherapy and shaping personalized treatment strategies. This research highlights the importance of integrating genetic analysis with clinical applications to enhance the efficacy of immunotherapy in NSCLC patients.

Keywords: *Programmed death-ligand 1; single nucleotide polymorphisms; non-small cell lung cancer and bioinformatics*

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Targeted DNA sequencing and bioinformatic characterization of Lymphocyte Activation Gene 3 polymorphisms in non-small cell lung carcinoma

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Abstract

Lung cancer is expected to remain a leading cause of mortality worldwide. Immunotherapy represents one of the most significant advancements in cancer treatment, as it can reverse tumor immune escape by inhibiting immune checkpoints. Lymphocyte Activation Gene-3 (LAG-3) is a critical inhibitory immune checkpoint that plays a significant role in novel cancer treatment strategies, particularly for non-small cell lung cancer (NSCLC). However, LAG-3 has not been extensively studied, and there is a lack of data regarding its role and associated single nucleotide polymorphisms (SNPs) in NSCLC. In this study, lung tumor samples will be collected from Hospital Kuala Lumpur and Hospital Serdang and snap-frozen in liquid nitrogen. DNA will be extracted for further analysis, and the regions of interest containing the SNPs will be amplified using conventional polymerase chain reaction (PCR). Sanger sequencing, employing a high-capacity genetic analyzer, will be utilized to detect specific polymorphisms (rs870849, rs1922452, and rs951818) in LAG-3. This analysis will confirm the presence or absence of these SNPs in NSCLC samples. Bioinformatic analysis will be conducted to identify and validate the LAG-3 polymorphisms, exploring their significance and associations with NSCLC. Retrieved databases will be utilized to evaluate their functional relevance and frequency. In conclusion, this study aims to enhance the understanding of the genetic landscape of LAG-3 in NSCLC, addressing a critical gap in current research by providing data on LAG-3 polymorphisms in cancer patients. This contribution will offer new insights into the genetic factors underlying NSCLC, potentially informing future research and clinical approaches.

Keywords: *Lymphocyte Activation Gene-3; non-small cell lung cancer; immunotherapy; single nucleotide polymorphisms and bioinformatics*

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Anti-leukemia potential of synthesized xanthone derivatives through cytotoxicity and apoptosis mechanisms

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Abstract

Xanthone derivatives are a group of natural compounds known for their diverse pharmaceutical properties, including antioxidant, anti-inflammatory, antibacterial, and anticancer activities. While numerous studies have explored the anticancer effects of xanthone derivatives, there is a notable gap in research specifically addressing their anti-leukemia effects and the detailed mechanisms underlying these actions. This study aimed to evaluate the cytotoxic effects of synthesized xanthone derivatives by using A20, HL-60, and Jurkat cells in the MTT assay and investigate the underlying mechanisms of cytotoxicity for the most potent derivatives. Nineteen xanthone derivatives exhibited 57%-100% cytotoxic effects towards all cell lines at 10 μM. In particular, two derivatives that demonstrated the strongest cytotoxic effects were further assessed with a range of concentrations and their IC_{50} values obtained ranged from 1.9 to 4.9 μM. Their mechanism of cytotoxicity was evaluated by using a 3D *in vitro* culture system in which A20 cancer cells were co-cultured with mesenchymal stem cells (MSCs), where the Caspase-3 expression levels were measured, together with Annexin V staining analysis. The results confirmed that these xanthone derivatives induced apoptosis in A20 tumor cells. In summary, xanthone derivatives are promising lead compounds for anti-leukemia drugs, attributed to their induced apoptosis in cancer cells. Thus, a study on the signaling mechanisms involved in the cytotoxic effects of xanthone derivatives is ongoing..

Keywords: *Xanthones derivatives; leukemia; MTT assay; 3-D culture and apoptosis.*

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The growth and yield of choy sum and butterhead lettuce grown using different hydroponic systems with microbe-supplemented nutrient solution

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Abstract

Hydroponics is a soil-less crop cultivation technique that includes the use of nutrient-rich solutions. In Malaysia, there is a surge of interest in urban farming, especially using hydroponic techniques. Moreover, recent innovations have included effective microbes as a facilitator in crop nutrient uptake in hydroponic systems. However, there is a lack of comparative study of these various systems in Malaysia, especially regarding crop growth and nutritional values of the produce. Hence, this study aims to determine the effect of different hydroponic systems, as in Kratky, Drip Fertigation, and Substrate-based system using standard AB nutrient with and without effective microbes on the physical properties of urban crops. Two such favoured vegetables - choy sum and butterhead lettuce which are known for various health benefits, and commonly grown commercially were chosen. The crops were grown in three different systems (Kratky, Drip Fertigation, Substrate-based), with two different nutrients (AB, AB with effective microbes), and with three replicates of fifteen samples. The results indicated that the application of effective microbes impacted crop growth in all systems. When tested with different nutrients within the same treatments, physical properties were overall improved in substrate-based treatment, while others weren't significantly affected. Additionally, treatments also affected crop growth based on crop-specific comparison as the growth of butterhead lettuce was significantly impaired when grown in drip fertigation system. In summary, the findings of this study suggest that application of effective microbes in hydroponics can potentially improve crop growth and yield depending on the system used.

Keywords: *Hydroponics; Kratky; drip fertigation; substrate-based and effective microbes*

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Targeted internal radiation therapy: Biodegradable radioactive rod for liver tumours

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Abstract

By 2045, the incidence and mortality rates of liver cancer are projected to increase by 63.1% and 66.6%, respectively worldwide. Liver cancer at intermediate and advanced stages has limited treatment options. Emerging studies suggested that internal radiation therapy provides a viable choice for liver cancer patients who are unsuitable for surgery, or downstaging the disease for further treatment options. This study aims to formulate a biodegradable rod loaded with radioactive Holmium-166 (^{166}Ho) rod. Polycaprolactone (PCL) will be loaded with 10%-40% non-radioactive Holmium (III) Oxide ($^{165}Ho_2O_3$), which will be labelled as BioHol-rod. The mixture was moulded into a 0.8mm diameter rod and dried at 37°C for 48 h. The dried rods were then sent to the Malaysian Nuclear Agency for neutron activation using a research reactor (1 MW, TRIGA MARK II, General Atomics) for irradiation for 5 h. The rod's radioactivity and Holmium (III) Oxide content were analysed. Followed by the physicochemical characteristics, stability, ^{166}Ho retention efficiency, and toxicity were analysed. The BioHol-Rod achieved a specific activity of 13.29±0.26 GBq/cm. It demonstrated stability over 21 days, favourable physicochemical characteristics for internal radiation therapy, and a ^{166}Ho retention efficiency of 99.99±1.15% in PBS (pH 7.5 and 5.5) over 216 hours. Non-radioactive BioHol-Rod exhibited minimal toxicity, with a cell viability of 91.26±0.28%, toward the HepG2 cell line after 72 hours of incubation. Future studies will focus on further *in vitro* cytotoxicity tests on HepG2 cells, followed by *in vivo* animal studies.

Keywords: *Liver cancer; internal radiation therapy; Holmium-166 and BioHol-rod*

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Optimization and comparative analysis of volatile components extraction methods for sour bamboo shoots

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Abstract

Sour bamboo shoots are a fermented vegetable product with unique flavour, which has been consumed as one of the ingredients in many local specialties for hundreds of years. Volatile compounds are critical indicators for assessing the quality of sour bamboo shoots. At present, there is limited research on the extraction methods of volatile compounds from sour bamboo shoots. This study aimed to optimize and compare the extraction methods for volatile components in sour bamboo shoots a by using Solid-Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE), and Thin Film SPME (TF-SPME) through gas chromatography-mass spectrometry (GC-MS). The results revealed that the optimal extraction conditions for SPME is by using 50 µm DVB/Carboxen/PDMS fiber with a 5-fold dilution at an extraction temperature of 50°C that yielded approximately 40 volatiles with high relative content of volatile contributors. In SBSE, a 250-fold dilution was used with an extraction temperature of 50°C and duration of 90 min, resulting in approximately 90 volatiles that are significant relative content of volatile contributors. For TF-SPME, a dilution of 1250 was used with the same temperature and duration as SBSE and more than 110 volatile components were extracted. It showed that TF-SPME could detect the most volatile components of sour bamboo shoots with the highest sensitivity. Therefore, metabolomic studies of volatile compounds can be investigated in the next research by using TF-SPME to identify the metabolic pathways of volatile compounds and to understand the mechanism of flavour formation in sour bamboo shoots.

Keywords: *Solid-phase microextraction; thermal desorption unit; stir bar sorptive extraction and thin film SPME*

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Holmium-165 5 loaded folate-functionalized calcium carbonate nanoparticles for targeted radiation therapy of colorectal cancer

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Abstract

In Malaysia, colorectal cancer (CRC) is the second most common cancer and cause of cancer death as of 2022. Surgical resection is the gold standard for CRC treatment, but delayed diagnosis means 60% of patients are diagnosed in stages III and IV, limiting surgery. Chemotherapy and radiotherapy are alternatives for advanced CRC, but they suffer from poor specificity and systemic side effects. This study aims to develop folate-functionalized calcium carbonate nanoparticles (FCC) loaded with holmium-166 (Ho-166) for targeted CRC radiotherapy. Ho-166 (Emax = 1.77 MeV, half-life = 26.7 h) is a theranostic radionuclide emitting both high-energy beta particles and diagnostic gamma radiation. FCC nanoparticles were synthesised via coprecipitation, loaded with non-radioactive holmium-165 (Ho-165), and neutron-activated to Ho-166 using a TRIGA MARK II reactor at a neutron flux of 1.5 x 10^{12} n/cm^2/s for 5 h, converting Ho-165 to Ho-166 through $^{165}Ho(n,\gamma)$ ^{166}Ho reaction. Physicochemical characterization, gamma spectroscopy, and in vitro retention studies assessed the nanoparticles before and after activation. The nanoparticles ranged from 150-200 nm in size with a stable zeta potential of less than -30 mV. FTIR spectroscopy showed no changes in the chemical properties pre- and post-activation. The Ho-166-FCC nanoparticles achieved a specific radioactivity of 2.27 ± 13.3 GBq/g, with no radionuclide impurities observed after activation. Retention of Ho-166 exceeded 95% in phosphate buffered saline solutions (pH 7.4 and 5.5) up to 300 hours. The Ho-166-FCC nanoparticles demonstrated cytotoxicity in HT29 cell lines. In conclusion, the developed Ho-166-FCC nanoparticles show excellent radionuclide retention, no impurities, and unchanged physicochemical properties post-activation, while effectively displaying cytotoxicity toward HT29 cells.

Keywords: *Colorectal cancer; Holmium-166; dadionuclide therapy; theranostics, nanoparticles and anti-cancer*

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In silico and *in vitro* evaluation of allicin-amphotericin B-chitosan solution towards *Candida* and *Aspergillus* spp.

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Abstract

Antifungal resistance has been reported in various species. The limited number of available antifungals combined with the increasing prevalence of resistance has propelled the search for alternative therapeutics. Allicin and chitosan have demonstrated their broad-spectrum antimicrobial characteristics whereas, amphotericin B (AMB) is an effective antifungal with dose-dependent nephrotoxicity. A combination of the three components could serve as an ideal alternative therapeutic. *In silico* evaluation of the ability of allicin to bind to essential virulence-related proteins was evaluated through Autodock vina. Minimum inhibitory concentration (MIC) of allicin, chitosan, and AMB towards the planktonic cells of six reference *Candida* sp. and two clinical *Aspergillus* sp. was determined. The relationship between allicin, chitosan, and AMB was assessed through the checkerboard assay. Minimum biofilm inhibitory concentration (MBIC) exerted by the compounds alone and in combination was determined through crystal violet method. Cytotoxicity towards Vero and human dermal fibroblasts (HDFs) was evaluated through 3-[4,5-dimethylthiazol-2-yl]-2-5 diphenyl tetrazolium bromide (MTT) assay. *In silico* data indicate that allicin can bind to agglutinin-like sequence (ALS), sterol 14α-dimethylase (CYP51) and secreted aspartic proteinases (SAP) proteins of five *Candida* sp. with a binding affinity of-3.8 to -4.4 kcal/mol. MIC of allicin for five *Candida* sp. ranged between 1.56 to 3.125 µg/mL, whereas the MIC was 12.5 µg/mL for *Candida glabrata* ATCC MYA-2950. Meanwhile, MIC for AMB and chitosan for six *Candida* sp. was between 0.313 to 0.5 µg/mL and 250 to 500 µg/mL, respectively. MBIC of allicin for four *Candida* sp. was approximately 31.25 to 62.5 µg/mL whilst the MBIC for AMB ranged from 0.5 to 4 µg/mL. MBIC of chitosan towards four *Candida* sp. was between 250 to 1000 µg/mL. *Candida glabrata* and *C. krusei* ATCC 6258 were excluded from the biofilm assays as they are poor biofilm producers. Allicin, AMB and, chitosan demonstrated low cell cytotoxicity with 80% cell viability. It is surmised that combining the three components might be an ideal alternative therapeutic as it could lower the toxicity associated with individual compounds whilst promoting its antifungal properties.

Keywords: *Allicin; chitosan; amphotericin B; alternative therapeutic and combination therapeutic*

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Newly fabricated triple negative breast cancer (TNBC)-targeted nanoparticles polymer loaded with chemotherapeutics drugs and anti-microRNA

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Abstract

Triple-negative breast cancer (TNBC) poses a significant challenge in treatment due to the emergence of drug resistance to standard chemotherapeutics, along with associated risks to non-targeted tissues. This study is dedicated to developing a nanoparticle drug delivery system utilizing PLGA-PEG nanoparticles (NP) with the integration of A6 peptide (NPA6) as a specific targeting ligand for TNBC. Encapsulating Doxorubicin (DOX) and antisense-miR21 (AM21), these nanoparticles are designed to counter drug resistance *in vitro* and *in vivo.* Characterized by an average size of 103.5 (±8.1) nm and remarkable DOX encapsulation efficiency, these nanoparticles have been successfully engineered. Results demonstrate that NPA6.DOX.AM21 effectively mitigates drug resistance, displaying a significantly decreased 10x times lower of doxorubicin IC_{50} as opposed to free DOX (2.5 uM vs 25.7 uM respectively, $p<0.01$). In mice models, the A6 peptide-incorporated formulation (NPA6.DOX, NPA6.DOX.AM21) exhibits substantial tumor size reduction and increased doxorubicin concentration within tumors, all while minimizing non-specific doxorubicin exposure compared to non-A6 formulations (NPDOX, FREE DOX) treatments. This innovative PLGA-PEG-A6 peptide complex loaded with DOX and AM21 showcases superior efficacy in surmounting cell resistance in breast cancer cell lines, targeting tumor progression, and decreasing drug distribution in non-targeted organs in mice. These results hold promise for enhanced combat against drug resistance and precise chemotherapy, hence potentially improving patient outcomes and survival rates.

Keywords: *Triple-negative breast cancer (TNBC); doxorubicin, drug resistance; PLGA-PEG nanoparticle and A6 peptide targeted therapy.*

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Evaluation of antiviral and immune-modulatory effects of *Lactobacillus casei* and *Lactobacillus acidophilus* against respiratory syncytial virus and rotavirus *in vitro*

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Abstract

Respiratory syncytial virus (HRSV) and Rotavirus A (RVA) are leading causes of viral respiratory and gastrointestinal infections, respectively. This study investigates the antiviral and immune-modulatory effects of two probiotic strains, *Lactobacillus acidophilus* and *Lactobacillus casei*, derived from the probiotic drink Vitagen®, against these viruses *in vitro*. Infections were conducted with a multiplicity of infection (MOI) of 1. To evaluate the antiviral properties of the probiotics, both viruses were preincubated with *L. acidophilus* and *L. casei* ($3x10^7$ CFU/mL) for 1 hour at 37°C. Following this, the virus-probiotic mixtures were applied to host cells, resulting in a reduction of cytopathic effects (CPE) in both probiotic-treated samples, RVA-induced cell lysis in MA104 cells and syncytium formation by HRSV in HEp-2 cells. A plaque assay demonstrated that *L. acidophilus* and *L. casei* reduced RVA plaque counts by approximately 30 PFU/mL. However, plaque counts for HRSV could not be determined due to the syncytium formation characteristic of HRSV infection in HEp-2 cells. Viral infectivity was further assessed using the endpoint dilution assay ($TCID_{50}$). Both probiotics reduced the infectivity of RVA and HRSV by approximately 1 log compared to untreated controls. However, *L. casei* did not significantly inhibit HRSV in HEp-2 cells, as its $TCID_{50}$/mL value (4.52×10^4) was comparable to that of the untreated sample (4.43×10^4). Cytokine profiling of infected HEp-2 cells using a multiplex bead array revealed significant upregulation of pro-inflammatory cytokines and chemokines, including IL-6, IL-1β, IL-17A, and IFN-γ, in HRSV-infected cells treated with L. acidophilus. This suggests that L. acidophilus triggers a strong immune response during HRSV infection, potentially aiding in viral clearance. In conclusion, *L. acidophilus* and *L. casei* exhibit antiviral activity against RVA and partial activity against HRSV *in vitro*. *L. acidophilus* also promotes immune-modulatory effects in HRSV-infected cells. Despite these promising results, limitations include the use of non-human (MA104) and cancerous cell lines (HEp-2), suggesting the need for further studies with human and non-cancerous cell models. Additionally, animal and clinical studies are necessary to confirm the probiotics' protective effects *in vivo*.

Keywords: *Respiratory syncytial virus; Rotavirus A; Lactobacillus acidophilus; Lactobacillus casei and probiotic*

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Microbial succession and diversity in bamboo shoots fermentation under varying conditions using 16S RNA sequencing

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Abstract

The fermentation of bamboo shoots, a common ingredient in Chinese cuisine, involves a rich microbial ecosystem. However, there is limited knowledge on microbial diversity, particularly how the microbial changes in response to different fermentation conditions. Thus, this study aimed to investigate the microbiota succession over a 38-day fermentation using 16S rRNA high-throughput sequencing, with a focus on the effects of varying initial pH levels, bamboo shoot parts, types of fermentation water, and inoculated microbial strains. The results indicated that the variation in fermentation water and initial pH levels did not show significant differences on microbiota diversity. However, inoculation microbial strains with DANISCO (DS) and *Lactobacillus plantarum* (LP) exhibited significant differences in both Shannon and Simpson indices. Beta diversity analysis indicated significant differences among different bamboo shoot parts, as well as between fermentation using mountain spring water and sterile water. When fermented with different parts of bamboo shoots, *Lactiplantibacillus, Weissella, Pediococcus,* and *Lactococcus* were dominant during initial 8 days of upper parts of bamboo shoots fermentation, and dominated by *Lactiplantibacillus* after day 32. Unlike the middle and bottom parts, which initially exhibited *Weissella* and *Lactococcus* as dominant, then dominated by *Lactiplantibacillus* and *Weissella* in the late fermentation. Throughout fermentation, *Lactiplantibacillus* remained dominant across various initial pH levels and inoculated strains, but *Lactococcus,* and *Weissella* accounted for a small percentage, suggesting that environmental pH and inoculated microorganisms inhibit the growth of acid-sensitive genera. This study revealed the dominant role of *Lactiplantibacillus* in bamboo shoot fermentation, underscored the inhibitory effect of environmental pH and inoculated microbes on acid-sensitive genera, and emphasized the impact of fermentation parameters on the microbial diversity and succession throughout bamboo shoots fermentation. Future study on the correlation of microbial community and flavour of fermented bamboo shoots is highly recommended.

Keywords: *Fermented bamboo shoots; fermentation conditions; microbiota dynamics and optimization*

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Value of multi-parameter magnetic resonance imaging in guiding left bundle branch pacing and evaluating clinical prognosis

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Abstract

This study aimed to investigate the impact of preoperative cardiac MRI in evaluating myocardial fibrosis of patients with bradyarrhythmia and heart failure on left bundle branch pacing (LBBP), so as to promote its application and alleviate the burden of family medical expenses. Starting in March 2023, a total of 96 consecutive patients who underwent LBBP treatment were enrolled and divided into two groups. Among them, 48 patients in the experimental group underwent cardiac MRI before LBBP surgery, while the other 48 patients in the control group proceeded with LBBP surgery without MRI. The results showed that cardiac magnetic resonance (CMR) could significantly reduce the surgical and fluoroscopy times. It could also assist in precisely locating different regions within the left bundle branch for pacing. Pacing at the main trunk of the left bundle branch resulted in shorter QRS durations and superior QRS areas, and the proximal area 1 exhibited better ventricular synchrony compared with the more distant area 2. This study verified the effectiveness of the technique of using cardiac MRI to guide LBBP, including shortening the operation time and so on. Although no long-term echocardiographic benefits of left bundle branch trunk pacing (LBTP) were observed, determining the optimal ventricular pacing site remains crucial for patients with biventricular desynchronization in heart failure. LBTP may potentially provide a better prognosis for these patients.

Keywords: *Pacemaker, artificial; arrhythmias, cardiac; magnetic resonance imaging and bundle of His*

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Target prediction of pomegranate polysaccharide for anti-colorectal cancer treatment and mechanism

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Abstract

Colorectal cancer is a common malignant tumor in the digestive system, and its morbidity and mortality are increasing year by year. The pathogenesis of this disease is complex, and the treatment options are diverse. However, the existing chemotherapy methods have certain drawbacks, so it is still an important task to seek effective and healthy chemotherapy drugs. Pomegranate not only has rich nutrition, but also has medicinal health value. Meanwhile, polysaccharide is one of the bioactive components with research value and application prospects. In this study, pomegranate polysaccharide was used as a potential drug to analyze its possible targets and mechanisms in the treatment of colorectal cancer. According to the effective chemical components of pomegranate polysaccharide recorded in the literature, Pubchem and Swisstarget databases were used to analyze the potential therapeutic effects of pomegranate polysaccharide. OMIM (Online Mendelian Inheritance in Man), GeneCards and DisGeNE database in colorectal cancer pomegranate polysaccharide active ingredient of potential targets. The polysaccharide composition-target-disease network and protein-protein interaction network (PPI) were constructed using String, Cytoscape3.10.1 software and David database, and the interaction targets were then used for GO enrichment analysis and KEGG pathway enrichment analysis. The analysis identified 11 interaction-targets between pomegranate polysaccharide and colorectal cancer, and their signaling pathways mainly involved PI3K-Akt signaling pathway and the chemical carcinogenic-receptor activation signaling pathway. These signaling pathways provide the theoretical basis for future research and the probable direction of drug design for the use of pomegranate polysaccharides in the treatment of colorectal cancer.

Keywords: *Target prediction; pomegranate polysaccharide; anti-colorectal cancer treatment and mechanism*

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Formulation, characterization and functional efficacy of hyaluronic acid-oleic acid (HA-OA) ester dissolvable microneedles for the improvement of skin antioxidant

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Abstract

Skin ageing characterised by elasticity loss, hyperpigmentation and dryness is accelerated by oxidative stress from reactive oxygen species (ROS) primarily due to extensive UV exposure. However, due to the limitations of organisms' antioxidant defence system, outsource antioxidants are necessitated. Hyaluronic acid (HA) is known for skin hydration and anti-aging with emerging antioxidant properties. Nonetheless, common single hydrophilic HA with relatively high molecular weight (>500kDa) is inefficient to penetrate the stratum corneum (SC) to decelerate the ageing process. Microneedle (MN) technology offers a promising minimal invasive approach of bypassing the SC to enhance drug permeation. Among the various types of MNs, dissolving microneedles have emerged as patient-friendly system with excellent biocompatibility and biodegradability, enabling the effective delivery of encapsulated biopharmaceuticals across the skin barrier regardless of molecular weight. Recent advances in both modified HA and microneedle delivery systems offer promising solutions to impede skin ageing. This research aims to develop HA-oleic acid (OA) ester dissolving microneedles for improved skin penetration and antioxidant effects. OA, a monounsaturated fatty acid with significant antioxidant properties is utilised to form an amphiphilic ester with HA, enhancing amphiphilicity and antioxidant activity. Different HA-OA ester formulations will be evaluated for antioxidant activity. The modified HA-OA will be characterised using NMR and UV-vis spectroscopy then formed into microneedles using centrifugal lithography, a method requiring no complex tools. The microneedles will be characterised for morphology, skin penetration, antioxidant and cytotoxicity properties. Skin penetration will be assessed using *in vitro* models, and antioxidant activity will be evaluated through DPPH and ABTS assays, while cytotoxicity will be measured using the CCK-8 WST assay. The HA-OA-loaded dissolving microneedles are anticipated to exhibit greater skin penetration and antioxidant effects with non-toxic than the native HA, making them suitable for various cosmeceutical and pharmaceutical applications.

Keywords: *Skin aging; hyaluronic acid; microneedle; oleic acid and antioxidant*

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Therapeutic efficacy and safety of curcumin, piperine, and silver nanoparticles in a vaginal rat model

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Abstract

Infections produced by *Candida glabrata* build significant obstacles due to their resistance to present antifungal treatments. This research aims to evaluate the therapeutic potential and safety profile of a novel formulation combining curcumin, piperine, and silver nanoparticles in a vaginal rat model. Curcumin possesses a wide range of antifungal properties, including the ability to affect inflammatory responses, damage fungal cell membranes, and prevent fungal cells from growing. Piperine is known to increase curcumin bioavailability by inhibiting drug-metabolizing enzymes and influencing fungal biofilm development and cellular integrity. Silver nanoparticles will be incorporated for their natural antibacterial activities, as well as their ability to enhance the transport and retention of therapeutic substances, thereby avoiding early degradation and ensuring extended release at the target location. Six groups of female SD rats will be studied, including control, infected, and uninfected rats receiving either the new formulation or existing antifungal therapies. The efficacy of the formulation will be examined by measuring decreases in fungal load and performing histological examinations of vaginal tissues. Local tissue toxicity and systemic inflammatory responses will also be assessed during safety evaluations. Initial findings suggest that the combination of curcumin, piperine, and silver nanoparticles significantly lowers fungal load with minimal adverse effects. This combination is expected to show promise as an alternative method for treating resistant vaginal Candida infections, potentially offering a safer and more effective approach than existing treatments.

Keywords: *Curcumin; piperine; Candida glabrata and silver nanoparticles*

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Production, optimization, and functional characterization of fermented *Aquilaria sinensis* (Agarwood) leaves tea using *Lactobacillus plantarum*

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Abstract

Aquilaria leaves are an important resource of the Aquilaria tree. To date, Aquilaria leaves have been found rich in bioactive ingredients. However, Aquilaria leaves are still underutilized as few studies have been conducted to develop Aquilaria leaves into products. Additionally, some products cannot be easily and directly absorbed and utilized *in vivo*. The objectives of the present study are to develop a new approach for fermenting *Aquilaria sinensis (A. sinensis)* leaves tea using *Lactobacillus plantarum*. The experiment will be conducted in four phases. In the first phase, a new approach will be explored to develop A. sinensis leaves into fermented tea and the optimal fermentation conditions will also be investigated to acquire high quality fermented *A. sinensis* tea product by testing the sensory characteristics, physicochemical and biological properties. Then, the phytochemical composition of *A. sinensis* leaves, unfermented *A. sinensis* leaves tea and fermented *A. sinensis* leaves tea will be identified and quantified using LC-MS and UPLC-Q-TOF/MS approaches. Next, the biological properties of fermented *A. sinensis* leaves tea will be evaluated by testing the antioxidant, anti-inflammatory, and hypolipidemic activities. Finally, the effects of different brewing conditions on the quality of tea infusions will be investigated. This study will significantly reduce the waste of Aquilaria leaves resources and improve the economic value and application prospect of Aquilaria leaves. Furthermore, these findings can also systematically illustrate the relationship between the phytochemical composition and biological activity, and provide theoretical reference for further development of functional beverages using plant-based by-products.

Keywords: *Aquilaria sinensis leaves; fermented tea; Lactobacillus plantarum; phytochemical compounds and biological activities*

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Ultrasonic-assisted extraction of bioactive compounds from *Aquilaria Sinesis* (Agarwood) flower and evaluation of their biological activities

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Abstract

The limited research on agarwood flowers *Aquilaria sinensis* leaves unknown key properties such as potency, stability, biological activity, and cytotoxicity. Studies on agarwood leaves, resin and oils have demonstrated that they have antibacterial, anticancer and antioxidant properties. However, the biological activities of agarwood flowers, such as their potential antioxidant, anti-inflammatory, and antimicrobial effects, have yet to be thoroughly investigated. This research is motivated by the potential for agarwood flowers to have similar or greater biological activities compared to the leaves and essential oils, expanding agarwood's usage in medicine and finding a use for the flowers which are otherwise just a byproduct of the agarwood tree. To investigate this, agarwood flowers will first be ground and extracted using various solvents. A total phenolic content test will then be conducted to determine the most effective solvent for evaluating the flowers' biological activities. The antibacterial, cytotoxicity, and antioxidant activities of the agarwood flower extracts obtained using selected solvents will be evaluated.

Keywords: *Aquilaria sinensi; total phenolic content; antibacterial; cytotoxicity and antioxidant*

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Characterization and formulation of *Chlorella sorokiniana* extract-infused nanoemulsion creams: Assessment of antioxidant and anti-aging efficacy for cosmeceutical applications

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Abstract

In recent years, consumer expectations for skincare products have increased, particularly regarding functionality, safety, naturalness, and absorption efficiency. Skin oxidation, a common phenomenon, can lead to wrinkles, inflammation, pigmentation, loss of elasticity and other problems. Therefore, skin anti-oxidation and anti-aging are particularly important. Natural antioxidants are increasingly popular in skincare, with *Chlorella sorokiniana* emerging as a focal point of current research. *Chlorella sorokiniana* is rich in bioactive compounds, easy to cultivate on a large scale, and presents a promising raw material for cosmetic applications. In addition to functional ingredients, enhancing the skin's ability to absorb these compounds is crucial for achieving optimal effects. Nanotechnology offers a solution, improving the stability and solubility of natural products while enhancing the skin's absorption due to the "nano-size effect", leading to better efficacy in skincare. Therefore, this study aims to optimize the extraction method for efficiently obtaining antioxidant active ingredients from *Chlorella sorokiniana*, analyze the composition of the extract, and evaluate its antioxidant activity, cytotoxicity, skin whitening and anti-aging effect. The extract will then be incorporated into a nanoemulsion cream, followed by quality analysis and clinical efficacy evaluation. The most effective method for extracting *Chlorella sorokiniana*'s active antioxidants will be identified, alongside the determination of its antioxidant and anti-aging properties. The development of a *Chlorella sorokiniana* extract-infused nanoemulsion cream with enhanced efficacy holds significant potential for both *Chlorella sorokiniana*'s application in cosmeceuticals and the broader skincare industry.

Keywords: *Chlorella sorokiniana; nanoemulsion; antioxidant and anti-aging.*

Taylor's University- Xi'an Jiaotong Liverpool Unversity-Mae Fah Luang University Graduate Research Symposium 2024

Molecular interactions and mechanical performance of crosslinked nanocellulose incorporating gentamicin for antibacterial and wound healing application

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Abstract

Despite substantial progress in the development of wound dressings, wound management remains a significant challenge, which places a huge burden on patients and healthcare systems. In particular, wounds with bacterial loads pose substantial challenges to traditional wound dressings due to their complex pathophysiology. Thus, new and more effective wound healing modalities need to be developed. Therefore, the purpose of this study is to design a novel hydrogel membrane composed of chitosan and nanocellulose, while loading gentamicin to improve the antibacterial properties of the hydrogel membrane and accelerate wound healing. This paper will elaborate on the fabrication process of chitosan (CS)- based hydrogels combined with cellulose nanocrystals (CNC), the characterization methods to evaluate the physicochemical properties, biocompatibility, and antibacterial properties of the hydrogels. By precisely documenting the experimental methods and techniques used, this study aims to provide a comprehensive and reproducible protocol for the fabrication optimization of fabrication and application of CNC/CS hydrogel antibacterial dressings in skin injuries, thereby promoting the development of CS-based hydrogel antibacterial dressings.

Keywords: *Wound dressing; chitosan; cellulose nanocrystals; gentamicin and antibacterial effect*

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Investigation of the depigmenting potential of polar chlorella strains through α-MSH and MITF induced melanogenesis pathways

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Abstract

Photoaging-associated pigmentary disorder is a common cosmetic concern among the Asian demographic. Overexposure to ultraviolet (UV) radiation can lead to accumulation of reactive oxygen species (ROS) in the skin which can exacerbate skin hyperpigmentation disorder. Common skin-whitening agents have been reported to exhibit undesirable effects on the skin, making treatment options limited. Currently, there is increasing attention and demand for skin-whitening substances derived from natural sources in the cosmetic sectors due to their diverse range of natural bioactive compounds. Microalgae have been considered as alternative sustainable resources of bioproducts due to their availability, diversity, and versatility. Polar microalgae, in particular, are promising due to their adaptation to extreme environments, resulting in the production of unique metabolites with potential therapeutic benefits. Despite their recognized antioxidant and anti-inflammatory activities, the anti-melanin mechanisms of polar microalgae remain under-researched. This study, therefore, aims to investigate the physiological characteristics of polar microalgae *Chlorella* UMACC 234 and *Chlorella* UMACC 237 and their potential anti-melanin properties, contributing to the development of innovative cosmeceutical formulations. Their physiological characteristics will be assessed by measuring their biomass, protein, carbohydrate, and lipid content. The harvested cultures will undergo extraction and compound identification, followed by antioxidant quantification. The anti-melanin activities and cytotoxicity effects of the extracts will be evaluated using human keratinocytes (HaCaT) and mouse melanocytes (B16-F0) cell lines. To elucidate their depigmentation mechanisms, protein identification and gene expression analysis will be conducted to examine the effects on key regulators in the α-MSH and MITF-induced melanogenesis pathways. The results from both *Chlorella* sp. strains will be compared to suggest a potential natural depigmentation agent.

Keywords: *Polar microalgae; chlorella; melanogenesis and depigmentation*

Taylor's University- Xi'an Jiaotong Liverpool Unversity-Mae Fah Luang University Graduate Research Symposium 2024

Unravelling mechanisms of glycolysis pathway-driven mannose uptake from necrotic debris as fuel for breast cancer growth and survival under nutrient deficiency and hypoxia

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Abstract

Hypoxia is a prominent feature of solid tumor which is frequently found at tumor core. Typically occurs alongside nutrient deficiency and tissue necrosis as a result of insufficient blood flow, cancer hypoxia and nutrient deficiency act as stressors and are able to induce multiple changes in cancer to assist their survival in noxious conditions. Imperatively, alternative nutrient source was required to support the proliferation of cancer. Recently, extracellular necrotic debris was postulated to provide alternative fuel for the cancer via nutrient scavenging pathway. Furthermore, our preliminary data demonstrated that cancer cells might utilize mannose as alternative fuel in hypoxic and glucose free environment. Therefore, this study aims to explore how breast cancer cells can obtain, utilize mannose and the release of mannose from necrotic debris in hypoxic and nutrient deficient condition. Two macropinocytic cell lines, MCF-7 and MDA-MB-231, and one non-macropinocytic cell line, HCC1569, was selected for our study. Baseline macropinosome formation will be performed to establish the macropinocytic activity of cell lines. HIF, PMI and PMM2 expression will be evaluated in normoxic and hypoxic condition with cells supplemented with glucose or mannose. Lysosomal activity evaluation with LysoTracker Red will be conducted in cell lines supplemented with or without necrotic debris in hypoxic and normoxic condition. Lysosomal mannosidase expression will be evaluated in normoxic and hypoxic condition with cells supplemented with or without necrotic debris. MTT assay will also be performed to evaluate the role of PMI, lysosome and lysosomal mannosidase in mannose metabolism and cancer cell survival in hypoxic and low nutrient environment. Overall, this project seeks to investigate cancer mannose metabolic pathway in hypoxic and nutrient-deficient setting. Deeper enquiry in hypoxic metabolic pathway could enhance our understanding of how these cells adapt to challenging environments and potentially reveal new therapeutic targets for hypoxic cancer region

Keywords: *Cancer biology; cancer metabolism and breast cancer*

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Investigation of the roles of Forkhead Box O1 (FOXO1) transcription factor in regulating cell proliferation, apoptosis, cell cycle dynamic and metabolic activity of leukemia

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Abstract

The development of effective therapies against relapsed cancer, especially leukemia, has been a longstanding focus in cancer research for several decades. However, the implication of the role of the transcription factor, Forkhead Box O1 (FOXO1) in leukemic progression and metabolism remains underexplored. By silencing FOXO1 using a small molecule inhibitor, this research aims to clarify whether FOXO1 functions as a tumor suppressor or oncogene by analyzing its effects, as current literature presents conflicting views as their role depends on cell type context. To achieve this, multiple assays such as cell-based assays and immunoassays will be employed to analyze several human leukemic cell lines representing different leukemia subtypes. Both quantitative and qualitative data will be analyzed using respective analysis tools. This study seeks to provide insights into FOXO1's mechanistic roles in leukemia *in-vitro*, contributing to the ongoing discussion and understanding of its influence on cancer progression, apoptosis, cell cycle and metabolic regulation. Uncovering the important pathways modulated by FOXO1 could highlight its potential as a therapeutic target, offering new strategies to address treatment resistance and reduction of leukemia relapse. The findings are expected to deepen our understanding of FOXO1's role in leukemic cell behavior and therapeutic interventions, ultimately contributing to the efforts aimed at reducing leukemia relapse cases.

Keywords: *FOXO1; tumor suppressor; oncogene and cancer*

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Green synthesis of silver nanoparticles of *Adhatoda vasica* whole plant extract and evaluation for its biological activities

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Abstract

Plants have been used in the indigenous medicine systems of many cultures for a long time. *Adhatoda vasica* has been used in the Asian subcontinent as a medicinal source for many years. In this study, five parts of the *Adhatoda vasica* plant; root, leaf, flower, fruit, and bark will be extracted using methanol as a solvent to obtain crude extract. Crude methanol extract will be fractioned using polar, moderately polar and non-polar solvents such as methanol, acetone, and n-hexane fractions, respectively. These fractions will be then be subjected to antimicrobial activities. Anti-microbial studies will then be performed via disc diffusion method, agar well diffusion method, and minimum inhibitory concentration methods. The fraction with the best anti-microbial activity will be synthesized into silver nanoparticles (AgNPs). The anti-microbial studies will be repeated on the AgNPs and anti-inflammatory studies will be conducted using albumin denaturation inhibition and anti-proteinase activity. The active compounds present in the AgNPs will be identified, characterized and standardized using High-Performance Liquid Chromatography analysis. The phytoconstituents which are responsible for the biological activity will be isolated using column chromatography and then it will be characterized using spectroscopic data.

Keywords: *Anti-microbial; anti-inflammatory; Adhatoda vasica; silver nanoparticles and chromatography*

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Effect of plant growth regulators on in vitro propagation of strawberry (*Fragaria* sp.)

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Abstract

This study explores the in vitro propagation technique as an effective method for strawberry (*Fragaria* sp.) cultivation, because *in vivo* propagation of strawberry is insufficient to cater the increasing demand for a year-round supply of high quality strawberry. The objective of this study is to investigate the effect of Murashige and Skoog (MS) media supplemented with different concentrations of cytokinin's, namely Kinetin (KN) and Benzylaminopurine (BAP) (0.1, 0.25, 0.5 and 1.0 mg/L) with or without 0.1 mg/L of auxin (IAA or IBA) on strawberry leaf, shoot and root development. Clean cultures of strawberry cultivar Yotsuboshi F1 hybrid established from seeds in MS media with 30 g/L sucrose and 3.2 g/L Gel-rite were used for micropropagation under fluorescent light of 1500 lux with 12 hours photoperiod at 25±1°C for 6 weeks. The data (sample size, n = 8) was taken every two weeks and the highest significant rate of survival, leaf and shoot multiplication and root formation was recorded. Based on the preliminary analysis, the MS media supplemented with 0.1 mg/L KN and 0.1 mg/L IAA has recorded highest number of leaf (mean ± SD = 13.00 ± 3.87), whereas 0.1 mg/L BAP in combination with 0.1 mg/L IBA recorded the highest number of shoot (mean ± SD = 2.75 ± 1.16). MS media supplemented with 0.25 mg/L KN and 0.1 mg/L IAA has recorded the highest number of root (mean ± SD = 3.67 ± 3.01). The findings will hope determine the best combination of plant growth regulators in MS media for strawberry shoot and root multiplication after prolonged subculture.

Keywords: *In vitro propagation; strawberry and plant growth regulator*

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