



Enzymatic Degradation: Effects of Degradation Time on the Physical Characteristics and Water Solubility Properties of Chitosan

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Abstract

Chitosan (poly- β -(1-4)-D-glucosamine) is a linear polysaccharide polymer known as a potential bioactive material which is useful in pharmaceutical applications. This is due to its bioadhesive, antimicrobial, permeability enhancing, and biodegradable properties. However, since it is poorly soluble under physiological conditions, this drawback makes chitosan difficult to formulate and is utilized in biological applications. In this study, we optimized the hemicellulase enzymatic hydrolysis of medium molecular weight chitosan (MWCS) by varying the reaction time: 2 hours, 4 hours, and 6 hours. The resulting chitosan were characterized for the change in its physical characteristics using Fourier transform infra-red (FTIR), x-ray diffraction (XRD), contact angle analyses, and scanning electron microscopy (SEM). FTIR results showed no significant changes in the functional groups present in the chitosan after enzymatic degradation. XRD diffractograms depicts the changes of chitosan internal crystal structure from amorphous to crystalline, which improves its stability. SEM images showed an increase in smoothness on the chitosan's surface morphologies. The surface contact angle analysis showed a reduced contact angle as reaction time which was increased and this result in the improvement of wetting properties of medium molecular weight chitosan, hence improved its water solubility. In conclusion, as the duration of enzymatic hydrolysis increases, the water solubility properties, as well as other physical and chemical properties of low molecular weight chitosan also improved.

Keywords: Chitosan, Hemicellulase, Enzymatic degradation, Drug Delivery, Solubility



The Use of Nata de Coco Derived Bacterial Cellulose as a Potential Excipient for Directly Compressed Tablets

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Abstract

Nata de coco is produced through the fermentation of coconut water using *Acetobacter xylinum* bacteria. Nata de coco is chemically high in fiber, consisting of bacterially derived cellulose. Bacterial celluloses present a number of unique physical properties compared with plant celluloses including a relatively high purity and crystallinity, with mechanical strength. These properties lend Nata de coco to be extremely amenable to research. The current study attempts to assess the suitability of Nata de Coco derived bacterial cellulose in direct compression tablet formulation. Nata de coco, at various states of hydration, defined here as 0%, 'low' and 'high', achieved by both oven-drying and freeze-drying is incorporated into a low-dose loratadine powder blend for direct compression into tablets. The dissolution profile of these novel tablets was evaluated to release properties. The resulting tablets reveal freeze-drying, compared with oven-drying, markedly improve the properties of Nata de Coco for inclusion in a tablet formulation, producing tablets with improved disintegration times and dissolution profiles and retaining physical stability. Oven dried Nata de Coco, resulted in physically weaker tablets, with poor friability and hardness. The upper limit for water-content in both cases, similar to HPMC, appears to be 11%. Freeze-dried Nata de Coco therefore presents considerable promise for use as a tablet excipient to produce a rapid-release formulation.

Keywords: Nata de Coco, Acetobacter xylinum, Direct compression, Disintegration, Dissolution



Homometallic Dinuclear Cu (II) and Zn (II) Complexes Derived from Salophen-type Ligand: Synthesis, Characterization and Anticancer Screening

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Abstract

A series of salophen-type Cu(II) and Zn(II) dinuclear metal complexes of a Schiff base derived from o-phenylenediamine were successfully synthesized and characterized using physicochemical and spectral techniques namely elemental analysis (C, H, N), magnetic susceptibility (MSB), molar conductivity, thermal gravimetric analysis (TGA), infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The shifting of C=N, C-O phenolic and C-O methoxy peaks and the appearance of new peaks assignable to M-N and M-O in IR spectra of the complexes indicated the coordination of azomethine N, phenolic O, and methoxy O donor atoms with metal centers. The disappearance of hydroxyl peak in ¹H NMR spectra of the Zn (II) complex supported the involvement of phenolic O upon formation of metal complex. The Cu (II) complex was paramagnetic with magnetic moment values of 1.97, close to the theoretical spin only magnetic moment, μ_{so} , for Cu (II). The compounds were screened for anticancer activity against human colon cancer cell (HCT116). The Cu (II) complex revealed the highest activity with IC₅₀ value of 21.17 ± 0.76 μM.

Keywords: Schiff base, Homometallic dinuclear, Polydentate ligand, Anticancer, Colon cancer



Biodegradation Characterization of Polystyrene Using New Isolates from Soil Sample

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Abstract

It was reported that Malaysians produced 16 427 tonne of waste per day and the landfill areas provided are not enough to accommodate the waste. Excessive usage of polystyrene can cause severe pollution problem. Plastics can be degraded by using several methods such as thermal degradation, chemical degradation, and biodegradation. Microorganisms use extracellular enzymes to carry out the biodegradation especially polymers in the environment. Long chains of polymers were converted into smaller monomers which will be absorbed and metabolized by microorganism using intracellular enzymes. The aim of this study is to investigate polystyrene biodegradation using microorganisms isolated from different soil samples. In this study, 11 waste polystyrene and surrounding soil samples from one landfill (Jeram Sanitary Landfill), two waste disposal sites (Kok Lanas and Politeknik Kota Bharu) and burial method (Kok Lanas) were collected and grown in minimal media with added polystyrene flakes. The bacteria which could grow in this screening process were isolated. Analysis of polystyrene biodegradation products were conducted using High Performance Liquid Chromatography (HPLC) to determine isolates with highest biodegradation capability. Five isolates; A1FD3, B1FD2, B1FD3, B1FD5, and B1FD6 showed highest biodegradation capability where both 1-phenyl-1, 2-ethanediol and 2-phenyl ethanol were detected in the HPLC analysis.

Keywords: Bacteria, Biodegradation, HPLC, Polystyrene



Effectiveness of Isochronic Tones on Gamma-domain Brainwave Stimulation

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Abstract

The cerebral brain cells, which produce the frequency patterns in accordance to the brain activity, can be synchronized to or resonated at a desired frequency by externally stimulating the cells using acoustical means including binaural beats (BB) and isochronic tones (IT). IT are single frequency tones that can be easily generated and perceived by the brain whereas BB are auditory brainstem responses originating from the decomposition of two tones. The states of alertness and focus can be traced to areas within the frontal lobes of the brain at gamma range of brainwave frequencies, i. e. circa 40 Hz and above. This paper presents the preliminary investigation on the effectiveness of IT over BB on stimulating the mental states of alertness and attention on human brain at the gamma domain. A maiden group of ten healthy male human subjects are chosen for this purpose. Electroencephalograph signals of the evoked potential due to resonance of the prefrontal cortex (PFC) with the induced aural stimuli are measured. It is observed that the root-mean-square values of the potentials recorded at the anterior front electrode locations namely, AF3 and AF4, after exposition to IT increased by an average of 15% over that measured post exposition to BB. Similar observation is made at the frontal right (F4) location. The results indicate that IT has an improved effect than BB on the potentials measured at the PFC locations and render a positive influence on the focus and attention mental states of the brain.

Keywords: Brainwave stimulation, Binaural beats, Isochronic Tones, Gamma brainwave, Bio-potential



Histological Observations of Adventitious Root Derived from *in vitro* Plantlet and Shoot bud of *Boesenbergia rotunda* (Zingiberaceae)

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Abstract

Boesenbergia rotunda or locally known as ‘Temu kunci’ has renowned to possess some of important bioactive compounds that are promising for pharmacological applications. It is essential to obtain structural information of the inner parts of the *in vitro* roots since growing these roots undertake various anatomical and morphological changes that influence their cells activities and nutrient uptake processes. Although root functions are thought to be significant for the growth of shoot, the morphological and anatomical knowledge of adventitious root for this plant are limited. This particular study aims to investigate and compare anatomical structures of *in vitro* adventitious root derived from *in vitro* plantlet with root derived from shoot bud of *B. rotunda*. Histological sections using resin were done to study the anatomical of adventitious root of *B. rotunda*. The root samples were fixed in a Glutaraldehyde-Paraformaldehyde-Caffeine (GPC) fixative, dehydrated, infiltrated, embedded, cut, stained, and mounted with Surgipath mounting medium for observations under light microscope. From the histological observations, adventitious roots of *B. rotunda* have showed the presence of all the three main tissue systems and have the same internal structures containing epidermis, exodermis, suberized sclerenchyma cells, cortex, and stele. Both adventitious roots derived from *in vitro* plantlet and shoot bud showing normal growth morphologically, have anatomically same normal cell structures and arrangements.

Keywords: Histological, Adventitious root, *Boesenbergia rotunda*, *in vitro*, Plantlet, Shoot bud



Reformulation of Le’Natura® Biscuit: Effects on Textural, Sensorial, Nutritional, and Glycemic Index Values

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Abstract

The purpose of this study was to reformulate Le’Natura® biscuit for textural, sensorial, nutritional, and glycemic index properties improvement. The original biscuit had a brittle and coarse texture with bitter after-taste. It also contained high carbohydrate which makes the biscuit unsuitable for diabetic patients and less desirable to the health-conscious consumers. Several ingredients from the original formulation were either eliminated, reduced or substituted in order to modify the texture, taste, to obtain a low glycemic index (GI) value and to increase acceptability. The ingredients involved in reformulation were wheat flour, milk powder, maltodextrin, atta flour, green bean flour, polydextrose, palm oil, and leavening agents. The final reformulated biscuit showed improvements in thickness, spread ratio, fracturability, and firmness properties. The improved biscuit also scored higher acceptability for appearance, texture, sweetness, after taste and overall acceptability attributes. The *in vivo* GI measured indicates that the improved biscuit was in the low GI range of 44.7 ± 4.7 compared to the original biscuit (63.5 ± 7.7 , moderate GI). The nutrients composition of total energy, fat, protein, dietary fiber, iron, and calcium content were increased while total carbohydrate and sodium were decreased. With the improvements, Le’Natura® biscuit is expected to capture the functional food market segment more competitively.

Keywords: Functional food, Biscuit, Glycemic index, High fiber



Preliminary Phytochemical Analysis of Malaysian *In Vitro* Cultured Aromatic Rice (*Oryza sativa* L. Cv. MRQ74)

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Abstract

The present study is aimed at a primary screening of the phytochemical contents in aromatic rice stems and leaves of *Oryza sativa* L. Cv. MRQ74. The plants were subjected to *in vitro* plant tissue culture techniques to ensure the plant growth condition was in sterile and controlled environments. The dehusked seeds of *Oryza sativa* L. Cv. MRQ74 were surface sterilized using 70% (v/v) clorox with two drops of Tween 20, followed by 50%, 30%, 20%, and 10% clorox. The sterilized seeds were then cultured on hormone-free MS media for 6 weeks. Stems (basal segment) of 6-week-old aseptically raised seedlings were approximately excised into 5.0 – 10.0 mm segments. The explants were cultured onto MS media fortified with 0.1 mg/L BAP and hormone-free MS media as a control. The crude extracts of stems and leaves of *in vitro* raised plantlets were prepared by a maceration process using the chloroform and methanol solvents in order to extract moderately non-polar and polar compounds, respectively. The phytochemical screenings were carried out using qualitative tests. The results revealed that the crude extracts derived from plantlets grown on MS media with and without BAP were found to be rich in saponins, terpenoids, resins, and reducing sugar. However, flavonoids, alkaloids, and tannins were absent in both crude extracts. Therefore, the results of this finding indicate that the *Oryza sativa* L. Cv. MRQ74 could be used as a source candidate for the potential applications of saponins and terpenoids in pharmaceutical, resins in furniture and reducing sugars in pesticide industries.

Keywords: *Oryza sativa*, *In vitro* cultured, BAP hormone, Phytochemical screening



Effects of erythromycin, nystatin, and their Combination on *Pseudomonas aeruginosa* and *Candida albicans* Biofilms

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Abstract

Pseudomonas aeruginosa is frequently found to coexist and interact with *Candida albicans* in the polymicrobial biofilms. Considering the pathogenicity of polymicrobial biofilms in many persistent infections, this study was aimed to evaluate the combined effects of antibiotics erythromycin and nystatin on *P. aeruginosa*-*C. albicans* polymicrobial biofilm under both aerobic and anaerobic conditions. Treatment with single (erythromycin or nystatin) and combined antibiotics caused subtle reductions in the number of viable biofilm cells as well as protein and extracellular DNA content of the biofilm extracellular polymeric substance (EPS). Protein profiling demonstrated differential expression of biofilm proteins following exposure to the antimicrobial treatments. These findings suggest that erythromycin and nystatin, either single or in combination have effects on EPS composition (protein and extracellular DNA) as well as biofilm protein expression.

Keywords: Biofilm, *Pseudomonas aeruginosa*, *Candida albicans*, Erythromycin, Nystatin



Vitexin Aggravates Morphological and Functional Abnormalities in the Kidney of Streptozotocin Induced-Diabetic Rats

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Abstract

Vitexin has been shown to possess neuroprotective and antidiabetic properties. However, we have previously demonstrated that vitexin increases the levels of serum fructose, a possible cause of acute kidney injury. Therefore, the present study was intended to investigate whether vitexin could aggravate kidney injury in streptozotocin (STZ)-induced diabetic rats. Vitexin was administered orally to STZ-induced diabetic nephropathy rats for eight weeks. Kidney function parameters, morphological changes, and oxidative stress biomarkers were then determined. The levels of apoptosis-related proteins in the kidney tissue were assessed by an ELISA assay kit while fatty acid composition of the kidney was monitored by gas chromatography using an external standard. Vitexin resulted in a significant increase in serum creatinine and uric acid as well as kidney lipid peroxidation. The kidney oleic acid, total monounsaturated fatty acid content, n-6 to n-3 ratio and caspase-8 decreased following vitexin treatment. These results suggest that vitexin aggravates morphological and functional abnormalities in the kidney of diabetic rats, resulting in lipid peroxidation and apoptosis.

Keywords: Vitexin, Kidney, Diabetes, Fatty Acid, Oxidative stress, Apoptosis



Acute Embryonic Response after *in vitro* Culture and piezo-Assisted Biopsy of Murine Embryos

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Abstract

Embryo culture and biopsy are vital procedures in Preimplantation Genetic Diagnosis (PGD). Although the use of piezo-drilling has been introduced for zona breaching in PGD, acute responses in gene expression after embryo culture and piezo-biopsy remains to be elucidated. The aim of this study was to investigate acute transcriptomic response of 8-cells murine embryos after *in vitro* culture and piezo-assisted biopsy. Embryos were harvested at the 2-cell stage and cultured *in vitro* until the 8-cell stage. Single blastomeres were removed from 8-cell embryos after piezo-drilling. The remaining blastomeres were cultured for 1 hour before RNA extraction. The cDNA were synthesized, amplified, labeled, and hybridized using microarray chip. Results indicated that a total of 82 genes were found to be significantly different at the 8-cell stage, with 27 genes up-regulated and 55 genes down-regulated. Significant differences were observed in genes involved in mitochondrial energy metabolism (Ndufa1, Ndufa12), cell cycle (Hist1h1b) and immune response (Gabarapl2) pathways. These pathways indicate their significant role in the acute recovery of cultured and biopsied preimplantation embryos.

Keywords: Gene Expression, Biopsy, Preimplantation diagnosis, Pathway analysis, Oligonucleotide array Sequence analysis, Embryonic development



Protective Effects of Adiponectin on Demineralisation Induced by oxidized HDL in Human Osteoblast cells: An *in vitro* study

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Abstract

Low mineralisation activity by human osteoblast cells (HOBs) is an indicator for abnormal bone remodeling that potentially leads to osteoporosis. Oxidation of high-density lipoprotein (HDL) could affect bone mineralisation while adiponectin is suggested to promote bone mineralisation. However, the actual role of HDL before oxidation on bone was still unclear. Therefore, this study aims to investigate the effects of HDL before and after oxidation, and the protective effects of adiponectin on demineralisation induced by oxHDL in HOBs. Mineralisation assay using alizarin red staining showed that oxHDL (10, 25, 50, and 100 µg/ml protein) significantly reduced mineralisation of HOBs, by which 100 µg/ml protein oxHDL showed the highest inhibition. This is followed by lower calcium incorporation. The gene expression study by using real time quantitative PCR (rt-qPCR) was done to measure the expression of bone mineralisation markers (ALPL, COL1 α 2, BGLAP and Runx2) in the HOBs after inducing it with HDL, oxHDL, and co-incubation of oxHDL with adiponectin. The results showed that HDL increased the gene expression of COL1 α 2 and displayed higher trend of ALPL, which suggest the protective effects of HDL in HOBs. Meanwhile, oxHDL had a significantly low ALP and COL1 α 2 compared to HDL. However, adiponectin co-incubation with oxHDL had higher ALPL, COL1 α 2, BGLAP, and Runx2 gene expression. From this study it can be concluded that oxidation reduce the protective effects of HDL and the presence of adiponectin could prevent the detrimental effect of oxHDL in HOBs especially in mineralisation.

Keywords: High-density lipoprotein, Oxidation, Adiponectin, Bone mineralization, Genes expression



Adiponectin Attenuates Inflammation Induced by Oxidised High Density Lipoprotein in Human Osteoblasts

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Abstract

Inflammation induced by presence of oxidised high-density lipoprotein (oxHDL) is suspected to disturb the normal physiological activity of human osteoblasts cells (HOBs), a bone forming cells. While, adiponectin which possess anti-inflammatory activities is postulated to has the ability in suppressing the detrimental effects of oxHDL. However, little is known regarding the effects of high-density lipoprotein (HDL) before and after being oxidised, and the potential protective effects of adiponectin towards oxHDL on inflammation. To confirm these postulations, gene expression of inflammatory biomarkers (IL-6, TNF- α , NF- κ B p65, STAT-3, CREB and PPAR- α) on the HOBs induced with oxHDL, co-incubation of adiponectin with oxHDL and HDL were measured by using real time quantitative (rt-qPCR) with GAPDH and HPRT1 served as reference genes. HOBs incubated with oxHDL had higher gene expression of pro-inflammatory markers; IL-6, TNF- α and NF- κ B (p65) in HOBs than HDL and unstimulated controls. Interestingly, co-incubation of oxHDL with adiponectin showed a significant reduction of all targeted markers including CREB gene, compared to the oxHDL groups. This suggests that oxHDL promotes inflammation and the presence of adiponectin could hinder the effects of oxHDL in the HOBs. In addition, this study also found that HDL without being oxidised shows a protective activity by exhibiting lower expression of IL-6 and TNF- α ; and expressed higher STAT-3, suggesting the protective activity of HDL in HOBs.

Keywords: Oxidised high-density lipoprotein (oxHDL), HDL, Adiponectin, Osteoblasts, Inflammation



Histological Inspection of Adventitious Roots Formation Derived from *In Vitro* Hypocotyl of *Boesenbergia Rotunda* on Solid and in Liquid Medium

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Abstract

Boesenbergia rotunda (L.) Mansf. or finger root which belongs to the Zingiberaceae family is recognized to possess a wide range of medicinal important flavonoids, highly potential for pharmaceutical applications. The flavonoids are proven to exhibit antimicrobial, antibacterial, anti-inflammatory, antioxidant, anti-tumor, and anti-cancer properties but the yields in nature are often too small for commercialization. In present years, plant tissue culture techniques comprising tissue, organ and cell cultures have been extensively used to produce various types of secondary metabolites. Organ culture, particularly adventitious root culture has been a popular and promising *in vitro* alternative to produce important secondary metabolites from various plants. This study was particularly designed to determine the effectiveness of medium types; solid and liquid medium of ½ MS medium + 0.5 mg/L NAA on the induction of *B. rotunda* adventitious roots. In addition, the cells involved and responsible in adventitious roots formation via *in vitro* hypocotyl of *B. rotunda* also were discovered. Results obtained demonstrated that the *in vitro* hypocotyls cultured in liquid medium gave significantly higher induction rate of 100% together with average number of adventitious root induced of 29.1 ± 2.9 roots and average length of adventitious root induced of 2.0 ± 0.2 cm compared to the hypocotyls cultured on solid medium which only gave 95% induction rate, 12.3 ± 2.8 of average adventitious roots induced and 0.9 ± 0.05 of average adventitious root length. Histological inspections discovered that presence of root apical meristems, responsible for adventitious roots formation were derived specifically from the pericycle cells linked at the end of phloem regions in the *in vitro* hypocotyls.

Keywords: Adventitious root, Apical meristem, Histological cross-section, *In vitro* hypocotyl, Pericycle cells, Zingiberaceae



***Azadirachta excelsa* Leaf Ethanollic Extract Ameliorates Brain Oxidative Stress and Hippocampal Microstructural Changes in Diabetic Rats**

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Abstract

Diabetes mellitus has been reported to cause oxidative stress and subsequent alterations in structural and functional aspects of the hippocampus. The consumption of antioxidant-rich plant exerts various health promoting effects. *A. excelsa* has been proven to ameliorate diabetes in rat model, but its role in alleviating the changes in brain oxidative status and microstructure remain elusive. This study aimed to ascertain the effect of *A. excelsa* leaves extract in recovering the superoxide dismutase (SOD) and malondialdehyde (MDA) levels, as well as the hippocampal microstructure affected by diabetes. Diabetes was induced among male Sprague-Dawley rats by intraperitoneal injection of 60 mg/kg body weight (bwt) streptozotocin (STZ). The experimental animals were divided into normal control (NC-saline), diabetic control (DC-saline), Metformin-treated diabetic (DMET-1000 mg/kg bwt), Quercetin-treated diabetic (DQ-40 mg/kg bwt), and *A. excelsa*-treated diabetic (DAE-250 mg/kg bwt) rats with treatment period of 8 weeks orally. Treatment with *A. excelsa* had significantly increased the SOD level and insignificantly reduced MDA level in the diabetic brain. Microscopic analysis of the hippocampal Cornu ammonis (CA) 1, CA3, and dentate gyrus (DG) regions revealed the disorganisation of neurons in all regions in the DC group. The quantitative analysis *revealed* the ability of *A. excelsa* to have the significantly larger neuron soma size and higher percentage of normal neuron in most regions. Hence, it can be concluded that, *A. excelsa* can be a potential therapeutic agent to ameliorate the brain oxidative stress and structural damage due to diabetes, with the presence of various antioxidant-rich active compounds.

Keywords: *Azadirachta excelsa*, Streptozotocin-induced diabetes, Hippocampus, Cornu ammonis, Dentate gyru



Evaluation of Antimicrobial Activity of *Cynometra cauliflora* Extracts against Foodborne Pathogens

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Abstract

C. cauliflora or Nam-nam is an indigenous tropical plant that is typically found in eastern and northern parts of Peninsular Malaysia. Presence of phytochemicals in the plants have been reported to provide protection against chronic diseases and anti-inflammatory activity in which the leaves, traditionally used to treat diabetes and hyperlipidemia are also reported to exhibit antidiarrheal and antidiabetic activities. In this study, *Cynometra cauliflora*'s whole fruits and leaves extracts from three different states in Malaysia, were screened for antimicrobial potential against several common foodborne pathogens by disc diffusion method and Minimum Inhibitory Concentration (MIC) determination. The highest yield of extract was obtained from fruits (2.39%) in diethyl ether. Results from disc diffusion method suggested that fruits from Selangor and Terengganu extracted in diethyl ether are most effective against *Streptococcus pyogenes* with inhibition zones of 33.5 ± 2.1 mm and 31.0 ± 1.4 mm, respectively. However, no significant inhibition was seen by ethyl acetate extracts of fruits. *S. pyogenes* remains the most susceptible bacteria inhibited by leaves extract in diethyl ether with 35.5 ± 0.7 mm and 30 ± 2.8 mm from Perak and Terengganu respectively. The MIC results were in accordance to the disc diffusion assay in which the strongest MIC value is exerted by diethyl ether extract of fruits (0.32 mg/ml) from Selangor and leaves from Perak (0.39 mg/ml). The different geographical area of plant sources did not show any significant trends in antimicrobial activity. In conclusion, *C. cauliflora* diethyl ether extracts from both fruits and leaves showed the best antimicrobial activity against *S. pyogenes*.

Keywords: *Cynometra cauliflora*, Fruits extract, Leaves extract, Antimicrobial activities, Foodborne pathogens



Genetic Polymorphisms of ABCA1, APOA1 and LCAT in Malaysian Population and their Association with Low Concentration of High-Density Lipoprotein

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Abstract

Coronary artery disease (CAD) is the major cause of death in the developing countries, including Malaysia. High-density lipoprotein cholesterol (HDL-c) is inversely correlated with the risks of CAD. Genetic defects that lead to HDL-c deficiency have been identified in several candidate genes including ATP-binding cassette transporter A1 (ABCA1), apolipoprotein A1 (APOA1), and lecithin cholesterol acyltransferase (LCAT). This study attempted to investigate the association of genetic variants in these three candidate genes among 70 Malaysian subjects with low concentrations of HDL-c. Genetic variations of candidate genes were PCR amplified and sequenced in 70 low HDL-c subjects (HDL-c ≤ 0.6 mmol/L and HDL-c ≤ 0.7 mmol/L for males and females, respectively) and 140 normal controls matched with respect to gender, age, ethnic, and smoking status. ABCA1 (rs4149337 and rs2066881) and APOA1 (rs12718465, rs2070665, rs5072 and rs7116797) variants were significantly associated with low concentrations of HDL-c. Linkage disequilibrium analysis revealing majority of these variants were strongly correlated with each other. Minor alleles haplotype comprised of ABCA1 rs4149337 (C) and APOA1 rs12718465 (A) variants was significantly correlated with HDL-c concentration. LCAT variant; however, was not identified in this study. Our study suggested that genetic variations of ABCA1 and APOA1 genetic variants played an important role in HDL-c deficiency amongst Malaysians.

Keywords: High-Density Lipoprotein, ABCA1, APOA1, LCAT, Single Nucleotide Polymorphism



Alterations in Osteoblastic Markers During Short-Term Spaceflight - An Explanation for Bone Loss or A Result of Multifactorial Factors?

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Abstract

Real spaceflights in contrast to simulated microgravity experiments expose cells to multiple factors at the same time. In order to understand the influence of microgravity on osteoblast cells during short-term spaceflight, we looked at the influence of mechanical stress, and temperature and their effect on the expression of osteoblastic markers. Normal Human Osteoblast (NHOst) cells were flown for 10 and 12 days for gene and protein expression in ISS during the 2007 ANGKASAWAN project. Short-term spaceflight (10 days) exposure of NHOst cells to microgravity suppressed the expression of collagen, type 1, alpha 1 (COL1A1), osteonectin (ON), beta actin (ACTB) and CD44 genes significantly and also reduced osteocalcin (OC), alkaline phosphatase (ALP), and osterix (OSX) genes. Similarly, this exposure decreased the expression of OC, CD44, and soluble intercellular adhesion molecule-1 (sICAM-1) protein after 12 days. However, interleukin-6 (IL-6) expression of gene and protein increased, which was marked as an osteoclast marker. Short-term spaceflight seems to impair the NHOst cell functions via cell-cell interaction with the extracellular matrix.

Keywords: Normal Human Osteoblast (NHOst) cells, Spaceflight, Cytodex, Bone remodeling, Microgravity



Ammonia-Nitrogen Removal and Bioplastic Reduction in Batch Culture

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Abstract

The extensive production of a polymer as single-use plastic plays an important role in the deterioration of biodiversity. The small particles of plastic waste known as micro-plastic have been detected in the food chain of the marine animals and contaminated the deep-sea sediments. Prolonging to that, research and development have been done to cater to this problem by inventing bio-plastic. This material is biodegradable and mostly made from biomass and environmentally friendly. In the bio-plastic life cycle, this waste is typically dumped in the landfill after a period of usage. Mostly bio-plastic degradation will occur in the landfill. The aim of this study is to degrade bio-plastic in landfill leachate mimicking the process on-site. The experiment was conducted using batch culture with leachate medium coming from Air Hitam Sanitary Landfill, Puchong, Selangor. The degradation of bio-plastic was observed using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM) for the morphology. The FTIR spectra of the bioplastic before the experiment displayed the presence of O-H, C-H, and C=O absorption peaks. Meanwhile, the C-H group disappeared after the experiment. It has been observed that the weight reduction of cornstarch-based bioplastic (BP) was 73.6% and cornstarch-based with chitosan (BPC) was 59.63% after the period of 14 days. The ammonia-nitrogen reduction obtained was 41% for BP and 38% for bioplastic BPC. From the outcome of this research, it can be concluded that cornstarch-based bioplastic (BP) degrade better than cornstarch-based with chitosan (BPC).

Keywords: Bio-plastic, Biodegradation, Batch culture, Leachate medium



Cytotoxic Effect of Dillapiole on Human Breast Cancer MCF-7 Cells

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Abstract

Plant secondary metabolites and their derivatives play a significant role in anticancer drug therapy, since they are effective against specific characteristics while reducing side effects. Dillapiole is a phenylpropanoid that holds several bioactivities like anti-fungal, anti-inflammatory, anti-leishmanial and anti-cancer. This study aims to investigate possible cytotoxic effect of dillapiole on human breast cancer, MCF-7 cells. MCF-7 cells were cultured in complete RPMI media and incubated at standard culture cell conditions. After the cells reached 80% confluency, the cells were treated with various concentrations (ranging from 0uM to 150uM) of dillapiole as well as tamoxifen as a control positive. The cells were later incubated at 48 and 72 hours. Under phase-contrast microscope, the morphological presentation of affected cells was observed. Using MTT assay, the cytotoxic effect was determined for both incubation times. The results demonstrated that tamoxifen inhibited the MCF-7 cells with the IC₅₀ at 75.9 uM/ml and 39.8 uM/ml for 48 and 72 hours, respectively. Parallel with positive control results, there was significant cytotoxic effect of dillapiole against MCF-7 cells at 48 and 72 hrs incubation with the IC₅₀ at 9.2 uM/ml and 63.1 uM/ml, respectively. Based on these results, dillapiole was cytotoxic against MCF-7 cells and its cytotoxic activity were both time and dose dependant. The morphological analysis supported the MTT assay. Our preliminary finding is in agreement with other similar studies, suggesting that dillapiole appears to be a promising anti-cancer agent and opens a wider possibility of downstream analysis on its underlying anti-cytotoxicity molecular mechanism.

Keywords: Cytotoxic, Dillapiole, Human breast cancer, Plant metabolite, Anti-cancer



Antioxidant Properties of Methanol and Aqueous Extract of *Rhodomyrtus tomentosa* (Aiton) Hassk. Leaves

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Abstract

Natural antioxidants are all those elements that have the potential of stabilizing and eliminating free radicals from the body. *Rhodomyrtus tomentosa* (Aiton) Hassk. is a flowering plant mainly found in Southeast Asian country which includes Thailand and Malaysia. In this study, the phytochemical properties of *R. tomentosa* leaves were assessed spectrophotometrically with absolute methanol (alcohol) and distilled water (aqueous). In TPC assay, absolute methanol leaves extract of *R. tomentosa* gave significant result compared to its aqueous leaves extract which are 448.54 ± 0.43 mg GAE/g and 263.93 ± 0.33 mg GAE/g, respectively whereas in TFC assay, the same trend occurred when absolute methanol leaves extract of *R. tomentosa* gave a significant result compared to its aqueous leaves extract which are 6495.23 ± 64.756 mg QE/g and 777.01 ± 4.053 mg QE/g, respectively. In DPPH radical scavenging activity, the IC₅₀ were found to be in the order of ascorbic acid (16.67 ± 0.199 µg/mL) > absolute methanol leaves extract (21.96 ± 0.033 µg/mL) > aqueous leaves extract (32.00 ± 0.014 µg/mL). Lastly in FRAP assay, the absolute methanol leaves extract of *R. tomentosa* showed a significantly higher value of FRAP as compared to its aqueous leaves extract which is 702.74 ± 7.361 mg GAE/g and 137.29 ± 2.252 mg GAE/g, respectively. In conclusion, absolute methanol is an ideal solvent for extraction to further assess antioxidant properties of *R. tomentosa* in the future. Further *in vivo* studies are needed aiming at the potential antioxidant ability on the leaves extract of *R. tomentosa*.

Keywords: Antioxidants, TPC, TFC, DPPH, FRAP, *Rhodomyrtus tomentosa*



N- and O-linked Glycosylation Impact Biofilm Formation and Antibiotic Resistance of Staphylococcus Epidermidis

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Abstract

Staphylococcus epidermidis is the most species of coagulase-negative staphylococci (CoNS), frequently isolated from biofilm-associated infections. Protein glycosylation was found to be one of the virulence factors involved in biofilm formation. Since the conventional antimicrobial agents are not efficient to inhibit biofilm growth, glycosylation is seeming to be a potential alternative target for effective treatment of biofilm-associated infections. Therefore, the impact of glycosylation was investigated to assess the role of N- and O-linked glycosylation in biofilm and antibiotic resistance. Tunicamycin (N-linked inhibitor) and tri-O-benzyl-D-galactal (O-linked inhibitor) caused minimal biofilm inhibition and slightly increased the MICs by one-fold against clindamycin, gentamicin, rifampicin, tetracycline, and vancomycin.

Keywords: Staphylococcus epidermidis, Antibiotic resistance, Biofilm, Glycosylation



Sufficient follow-up for Long-Term Survival among Rheumatic Heart Disease patients using Cox Proportional Hazard Cure Model

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Abstract

It is very important to examine whether the proportion of long-term survivors in the dataset has been significantly justified enough before fitting into mixture cure models. This is always neglected by many researchers. Typical standard survival model requires each subject was follow-up for until death, but it is impossible to do in practice. As a result, large undeath subject observations may appear in the dataset with no further event are observed afterward. Therefore, these observations may be considered as cured or long-term survivors. But, how long a subject's follow-up is sufficient or a long-term survivor can be considered. This paper brings a great interest into consideration on the important of sufficient follow-up test in modeling mixture cure models. The study is illustrated with an application of survival data on Rheumatic Heart Disease (RHD) dataset. The sufficiency of the follow-up was tested such as Simulation Table and Sn-tests before fitting into Cox Proportional Hazard Cure (PHMC) model. The results for both tests conclude that the length of follow-up for RHD dataset is sufficient with supported from the long-plateau tail in the Kaplan-Meier survival curve. The sufficient follow-up test is very important before modeling using mixture cure models.

Keywords: Cox Proportional Hazard Mixture Cure model, Long-term survivors, Mixture cure model, Rheumatic Heart Disease, Short-term survivors, Sufficient follow-up



***F. deltoidea* Improving Bone Histomorphometry, Oxidative Stress, and Turnover Markers in Streptozotocin-Induced Diabetic Rats**

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Abstract

The study is aimed to study the morphological changes in the femur following *F. deltoidea* treatment. *F. deltoidea* leaves extract was administrated orally to STZ-induced diabetic rats for eight weeks. The femoral bones were then removed and scanned ex vivo using micro-computed tomography (micro-CT). The images were viewed and analyzed for bone mineral density (BMD), trabecular parameters (number, TbN; separation, TbSp; thickness, TbTh) and mineralized bone volume/total bone volume (BV/TV). All samples were decalcified and histological sections were prepared to obtain high contrast 2D images. Further, assays for measures of oxidative stress and bone turnover were performed. Compared to the diabetic control group, *F. deltoidea* administration increased the density of trabecular bone and the thickness of articular cartilage. This was accompanied by a significant improvement in the BMD and trabecular bone microarchitecture parameters assessed by micro-CT. Higher TBARS in parallel with the presence of chondrocyte hypertrophy indicates the possibility of delaying osteoarthritis (OA) in STZ-induced diabetic rats. Our data also demonstrated that balance between bone formation and bone resorption together with bone stearic and myristic acid almost normalized upon *F. deltoidea* treatment. The data confirm that *F. deltoidea* extract attenuates the STZ-induced bone loss and reverses the deterioration of bone microarchitecture and strength in diabetic rats. This could be related to the equilibrium between bone formation and bone resorption activities.

Keywords: *F. deltoidea*, Diabetes, Oxidative stress, Bone loss, Micro-CT



Genetic Characterization of Three Common Malaysian *Phyllanthus* Species Using RAPD Technique

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Abstract

It is well known that there are more than 600 *Phyllanthus* species under the family of Euphorbiaceae in which twelve significant *Phyllanthus* species have been reported in medicinal fields for their phytochemistry and pharmacognostic properties. *Phyllanthus* spp. is one of the popular plants used as a folk remedy throughout the world for treating various illnesses such as dysentery, influenza, vaginitis, tumors, diabetes, jaundice, kidney stones, and many others. The objective of the study is to investigate genetic variability among three commonly found *Phyllanthus* species in Malaysia from RAPD fingerprints technique to propose a reference standard for inter genus identification. DNA was isolated from *Phyllanthus amarus* Schumach. & Thonn, *Phyllanthus niruri* Linn and *Phyllanthus urinaria* Linn by using DNeasy Plant Mini Kit from Qiagen. Thirty-six out of sixty-five primers screened showed positive polymorphism. In total, 193 marker sites were assessed, where 178 were found polymorphic revealing 92.22% polymorphism. The binary data from the RAPD banding patterns were used to generate Jaccard's similarity coefficient matrices which vary from 0.20 to 0.50. Cluster analysis by means of UPGMA was run to construct phylogenetic tree which reflects a better variation between *Phyllanthus* species within molecular level. The result of RAPD profiling was equivalent to the prominent morphological characteristics of the subjected *Phyllanthus* species. Finding obtained in this investigation may aid the identification and authentication of species used in the herbal formulation of *Phyllanthus*-based products in future.

Keywords: Phyllanthus, Genetic variability, Polymorphism, RAPD

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