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Metabolic Respond of Synacinn™ In Rats

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Abstract

Diabetes mellitus is a chronic systemic disorder characterized by hyperglycemia and metabolic abnormalities. Synacinn™ is a traditional herbal medicine standardized against Curcumin, Andrographolide, Rosmarinic Acid, Gallic Acid and Catechin; were used traditionally for maintaining healthy blood glucose. The compounds have been reported to possess antidiabetic properties. This research was carried out to evaluate the metabolic changes in Streptozotocin(STZ)-induced diabetic rats treated with Synacinn™. Synacinn™ was administered orally to the rats after the induction of streptozotocin (STZ) and the plasma samples of STZ-induced diabetic rats were analyzed using Fourier-transform infrared spectroscopy (FTIR) after the oral administration of Synacinn™ and then multivariate data analysis was performed using MetaboAnalyst 3.0. Chemometric analysis of the blood samples shows there was metabolic recovery in diabetic rats treated with Synacinn™. It can be concluded that Synacinn™ contained active phytochemicals that have assist in the improvement of metabolic profiling. The results also proved that Synacinn™ have an effect in the recovery of metabolism disorders in STZ-induced diabetic rats.

Keywords--- **Diabetes, Traditional Herbal Medicine, Fourier-Transform Infrared Spectroscopy (FTIR)**

Immune Characterization of Lymphoid Organs in Brown-marbled Grouper

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Abstract

Aquaculture in Malaysia has been expanded and developed to be one of the economic potential to the country. Like other aquaculture species, the development of sustainable aquaculture of grouper however has been hampered with serious challenges associated with infectious diseases. Therefore, the understanding in immunology on this commercially valuable species is vital. In the present study, tissue leukocytes were isolated from brown-marble grouper (*Epinephelus fuscoguttatus*) lymphoid organs such as head kidney, spleen and gut to be used in immunological functional studies. Functional studies like respiratory burst assay, phagocytosis assay, and flow cytometric scattering profile were performed to characterize the leukocytes populations. Based on these study, we compared mean of respiratory burst activity of three different organs, and the result showed that, gut resident leukocytes recorded the highest response upon simulation of zymosan which was 0.96 ± 0.06 followed by head kidney 0.72 ± 0.07 and spleen 0.69 ± 0.12. In addition, flow cytometric phagocytosis assays showed that gut resident leukocytes possessed highest percentage of reactive phagocyte population which was 1.87 ± 0.35%, followed by spleen 1.60 ± 0.29% and head kidney 0.96 ± 0.11%. Grouper leukocytes cultured with mitogen stimulation such as phytohaemagglutin (PHA), lipopolysaccaride (LPS) and concanavalin A (Con A) also showed a significant growth of lymphocytes. PHA stimulated more tissue lymphocyte growth than those of the Con A and LPS. In the present study, grouper gut showed stronger immune responses than the other lymphoid organs. As one of the mucosa-associated lymphoid tissues, gut immunity constantly interacts with the resident microbiota and it lies in immediate vicinity with the external environment. The robustness of the immune responses makes the organ ideal for immunoprophylactic approaches.

Keywords--- **respiratory burst assay, phagocytosis assay, flow cytometry, mucosa-associated lymphoid tissue, *Epinephelus fuscoguttatus***

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Phytochemical Screening and *In vitro* Cytotoxicity for Potential Anti-Arthritic effect of Two Malaysian Mangrove Species

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Abstract

Phytochemical screening and cytotoxicity assessment of two mangrove species (*Brugueira sexangula* and *Heritiera littoralis*) for leaves and barks were studied. The objective this study was to investigate the toxicity of these two mangrove plants against Human Fibroblast-like Synoviocytes (HFLS) and Normal Human Articular Chondrocytes Knee Cell (NHAC-kn) in preparation for future anti-arthritic study. Both of the samples were collected, dried, grinded which later extracted with methanol (MeOH). Result of the MeOH extraction were then proceed for semi fractionation via liquid-liquid separation using hexane, dichloromethane (DCM), ethyl acetate and n-butanol sequentially according to polarity. Phytochemical screening process was conducted to detect presence of terpenoids, phenols, tannins, alkaloid, glycoside and steroids for all extracted samples. The assessment of cell viability of the extracts were conducted using NHAC-kn and HFLS cell line which both of the cell line involve in the arthritic disease inflammatory process. The results of cell viability on NHAC-kn cell line using extracts from both species shows non-toxicity at IC_{50} > 30µg/ml except for n-Butanol fraction extract from *B. sexangula* bark and also ethyl acetate fraction extract from *H. littoralis* bark which exhibit IC_{50} < 20 µg/ml. While, for HFLS cell line viability test shows that extracts from both species shows non-toxic concentraion < 25µg/ml except for hexane leaf extract and ethyl acetate bark extract of *B. sexangula* and both ethyl acetate and n-butanol extract of *H.littoralis* bark which show toxic on cell line with concentration < 50µg/ml. Hence, further studies can be conduct to discover potential anti-arthritic effect of mangroves metabolite agents against pro-inflammatory factors.

Keywords--- ***Brugueira sexangula, Heritiera litoralis,* phytohemical screening, anti-inflammatory, cytotoxicity**

The Potential of Aaptamine in Downregulating Hepatic PCSK9 mRNA Expression

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Abstract

Aaptamine, a marine natural compound isolated from marine sponges Aaptos *aaptos* has been demonstrated to possess various important bioactive properties such as antimicrobial, antifungal, antiretroviral activities with anticancer effect being the most frequently reported. However, its anti-atherosclerotic potential is yet to be explored. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a newly identified gene associated with familial autosomal dominant hypercholesterolemia. PCSK9 blocked the recycling process of low density lipoprotein receptor (LDLR) and promotes its degradation in lysosome. The inhibition of this gene is able to significantly reduce the plasma low-density lipoprotein cholesterol (LDL-C) levels especially in patients with high cholesterol, suppressing or slowing the development of atherosclerosis and reducing mortality cause by cardiovascular disease. In this study, the anti-atherosclerotic potential of aaptamine as inhibitor of PCSK9 was investigated. Dual-luciferase assay using PCSK9 promoter transfected liver hepatocellular carcinoma cell line (HepG2) was carried out to evaluate the effect of different doses of aaptamine ranging from 3.125-50 µM to the expression of PCSK9 promoter at transcriptional level. Results showed that only aaptamine concentration at 50 µM has significantly reduced the transcriptional activity of PCSK9 promoter by 20% compared to non-treated cell. These results were further confirmed with quantitative real-time polymerase chain reaction (qPCR) where the PCSK9 mRNA expression was also downregulated at concentration 50 µM. This study indicates that aaptamine has the potential to reduce the expression of PCSK9 but only at higher concentration. Therefore additional studies will be required to elucidate the mechanism involved that coordinates the role of aaptamine in regulation of PCSK9 expression.

Keywords--- **Aaptamine, PCSK9, dual-luciferase assay, HepG2, qPCR**

Screening of Anti-inflammatory Properties and Enzyme Inhibitory activities in R-38 Herbal Medicine for Gout Treatment

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Abstract

Gouty arthritis is a type of inflammatory arthritis caused by uric acid crystals deposited within the joints. First line of treatment for gout attack using NSAIDs are very effective to treat inflammation but with undesirable side effects. Herbal medicine formulated from five herbs, known as R-38, consists of *Alpinia galanga*, *Curcuma xanthorrhiza*, *Orthosiphon stamineus*, *Curcuma zedoaria*, and *Elephantopus scaber*. The herbs used in this formulation are well known in traditional medication systems and by the modern scientific communities to possess important phytochemicals that active in managing conditions related to arthritis. Cytotoxicity test on R-38 water extract using HSF cells treated with LPS induced inflammation showed that this herbal medicine is non-toxic and promote survival of the inflamed cells even at highest concentration (10^5 µg/ml). R-38 also able to reduce the production of inflammatory mediators in joints by lowering the level of TNF-α and IL-6 concentration in LPS-induced HSF cells. In the paw edema study, treatment with R-38 extract in monosodium urate (MSU) crystals-induced gouty arthritis mice showed significant reduced paw thickness within 24 hours after MSU injection. Recent studies on R-38 extracts revealed two contributing phytochemicals which are curcumin and quercetin that inhibit the activities of xanthine oxidase (XO) and cyclooxygenase-2 (COX-2) in gout. Acetone extract of R-38 had showed the highest inhibition activity in both assays followed by ethanol, water and petroleum ether extracts. Water and ethanol extracts of R-38 showed good inhibition activity in both assays, suggesting that these R-38 extracts are suitable and fulfil the requirement for human consumption. These results provided important insights into the safety and protective effect of R-38 against gout in dose-dependent manner. Further investigation are needed to identify other potential phytochemicals in R-38 besides quercetin and curcumin. Also, *in vivo* study may be suggested to confirm R-38 role as therapeutic agent to treat gouty arthritis.

Keyword--- ***herbal medicine, R-38, inflammatory mediators, gout***

Discovery and Testing Of Heat Shock Protein 70 (Hsp70) Stimulating Factor for Immune Enhancement and Disease Control In *Penaeus Vannamei* (The White Leg Shrimp)

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Abstract

Disease outbreak due to Vibriosis caused severe losses of the white-leg shrimp *P. vannamei* in many countries, impeding both economic and social development. Several bio-control alternatives have been suggested to battle Vibriosis in shrimp culture and these include application of microalgae (i.e.greenwater), probiotic bacteria, immunostimulants and bio-active compounds, the latter usually derived from edible plant or herbs. In this study, the efficacy of the naturally occurring compounds from methanol extracts of Pandan, *Pandanus tectorius* to safeguard *P vannamei* larvae against pathogenic *V. parahaemolyticus* was investigated, work which included verification of the extracts' toxicity on shrimps upon exposure to varying concentrations in a time dependent manner. The trial to test with *Pandanus tectorius* fruit and leaf extracts showed protective effect of the plant extracts against acute concentration of *Vibrio parahaemolyticus* (10^6 CFU/ml) when the post larvae were treated with the different concentration of plant extract for 24 h. The protein profiles compared between exposed and non-exposed shrimp revealed massive up-regulation of a single polypeptide of approximately 70 kDa. Which was later confirmed to be heat shock protein 70 (Hsp70) by western blot. Administration of immune stimulants is known to alters the expression of wide range of cellular and immune related genes by upregulation of Hsp70, crustin, and prophenoloxidase. The real time -PCR results reveals the upregulation of these genes. Based on these observations, the enhanced protection against Vibrio in *P. vannamei* might be associated with the accumulation of this anti-microbial peptide and/or other immune proteins.

Genome Study of *Serratia marcescens* subsp. *sakuensis* Strain K27, a Marine Bacterium Isolated from Sponge (*Haliclona amboinensis*)

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Abstract

Serratia marcescens subsp. *sakuensis* strain K27, a marine bacteria isolated from sponge proven produced amylase and lipase. This species is the first *Serratia* spp. that produces endospore where other *Serratia* spp. doesn't. Previous study had proposed that either this bacteria species encoded the endospore forming gene but never been reported or it may due to gene transfer. However, there is lacking of the whole genome sequence information until this study was done. Thus, this study was carried out to clarify and identify the endospore forming gene, amylase and lipase encoding gene(s) through whole genome sequence using computational method. Total DNA was extracted using Qiagen DNeasy tissue kit and sent to Monash University Malaysia for sequencing. Generated raw FASTQ paired-end reads were *de novo* assembled using Velvet version 1.2.09. Prodigal version 2.60, RNAmmer version 1.2 and tRNAscan-SE version 1.3.1 were used to annotate the genome for open reading frame (ORF), rRNAs and tRNAs respectively. Protein functions were predicted using InterProScan 5. Results show that K27 has generated a total length of 5,325,727 bp genome sequence with N_{50} of 67,510 bp. It contains 5,140 ORF, 10 rRNAs and 67 tRNAs. Interestingly, K27 has encoded gene for spores coat protein (PIN 55133.1) which involved in spore formation. In the same time, K27 genome sequence had encoded one amylase and seven lipase forming genes. K27 had also containing gene that involved in protein synthesis however, preliminary test show negative on protease production. In general, K27 encoded the genes for targeted proteins in theoretically. However, further confirmation in the present of the protein in laboratory-based is needed to study the real function of the genes encoded by this strain of bacteria.

Keywords--- ***Serratia marcescens* subsp. *sakuensis*, spore coat protein, amylase, lipase**

In Vivo Study on Anti-Hypercholestrolemia Activity of *Pandanus tectorius* Fruit Extract

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Abstract

Hypercholesterolemia is a condition in which the blood cholesterol exceeds its normal limits. Some studies reported that current drugs for the treatment of hypercholesterolemia have the side effect to the patient if they used for long period. Thus, an alternative treatment from a plant which has no side effects was used to reduce the hypercholesterolemia. *Pandanus tectorius* fruit was chosen in this study since this part is still wasted and not utilized as a source of food as well as for research. Besides that, *P. tectorius* fruit is also rich in bioactive compounds such as phenolics, flavonoids, and vitamins. Some studies reported that these compound groups have potency as antioxidant, antibacterial, and anti-hypercholesterolemia activity. Thus, in the study, *P. tectorius* was used as an alternative treatment for anti-hypercholestrolemia in in vivo on *Sprague Dawley* rats. Fruit sample was extracted successively using hexane, ethyl acetate, and methanol to obtain tree kind of fruit extracts, namely hexane (PHK), ethyl acetate (PEK), and methanol (PMK). Based on our preliminary study, the PEK was chosen for in vivo study since this extract showed the highest activity in increasing the SR-B1 gene expression in vitro. The rats were divided into 3 groups, namely standard food group (A), high cholesterol food group (B), and treatment group: feed by high cholesterol food and PEK extract (C). The in vivo study was done for 8 weeks. The result showed that PEK has reduced total blood cholesterol levels and increased the HDL cholesterol levels by 33.4 % and 72%, respectively compared to the cholesterol group. This results found that *P. tectorius* fruits have a good potency as an alternative for reducing hypercholesterolemia in vivo.

Keywords--- ***Pandanus tectorius*, anti-hypercholestrolemia, *in vivo***

Ankistrodesmus gracilli is Susceptible to Heterologous Expression of *Streptococcus pneumoniae pezT* Toxin Genes

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Abstract

PezT is a toxin originated from *Streptococcus pneumoniae* which will inhibit the bacterial cell wall synthesis. The functionality of bacterial toxin pezT has been studied in various eukaryotic organism such as zebrafish, *Arabidopsis thaliana*, yeast as well as human cell line. However, it is lack of research regarding the functionality of toxin pezT in microalgae which currently limited to *Chlorella vulgaris*. In view of that, this project demonstrated the effect of bacterial toxin pezT in *Ankistrodesmus gracilli*. The activator vector pMDC150 encoded with XVE transcription cloned with CaMV 35S promoter and responder vector, pMDC221 cloned with *pezT-GFP (Green fluorescent protein)* fusion gene were co-transformed into *Ankistrodesmus gracilli* through *Agrobacterium*-mediated transformation. During the induction of 17-β-estradiol, the activator vector readily initiates transcription of pezT-GFP fusion which eventually cause the cell lysis of microalgae. Upon the induction of 17-β-estradiol, GFP signals were detected in transgenic *Ankistrodesmus gracilli* which showed signs of cellular demage and lysis. The expression of toxin pezT also greatly affected the cell viability of transgenic *Ankistrodesmus gracilli* cells. The viability of *Ankistrodesmus gracilli* was significantly reduced by 71 % after treatment of 17-β-estradiol. The mechanism of toxin pezT toward microalgae will be further elucidate through transcriptomic and metabolomic analysis. This study demonstrate the possibility of develop novel means to harvest cellular content of microalgae efficiently through lysis of transgenic microalgae cells triggered by toxin activation upon induction with the appropriate signal.

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Systemic Vaccination against *Mannheimia haemolytica* A2 with Adjuvant from Microalgae Species

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Abstract

Pneumonic diseases in ruminants are the main cause of economic losses to producers worldwide. The rate of pneumonia incidence in goat varies from 10 to 40%, whereas mortality exceeds 20%, which is higher in young goat. In Malaysia, the goat industry considered as small-scale enterprise, which geared mainly for mutton production. The population of goats in Malaysia is about 350,000 that is found mainly in Kedah, Perak and Kelantan. Pneumonic pasteurellosis is a complex multifactorial disease causing a severe form of pneumonia caused by *Mannheimia haemolytica* as the predominant virulence factor. *M. haemolytica* is a gram-negative bacterium that typically lives as a resident in the tonsils of healthy cattle. However, *M. haemolytica* acts as an opportunistic bacterium that compromises the alveolar epithelium. Antibiotics are widely used in the feedlot industry, including prevention and treatment, but their efficacy differs due to inconsistent diagnostic and treatment protocols and the development of antibiotic resistance. Although traditional vaccines have failed to prove their protective effects. Modern vaccines use culture supernatants containing Lkt and other soluble antigens or bacterial extracts. These vaccines have 50-60% efficacy in preventing hemolytic pneumonia. In the present work, we attempt to increase the efficacy by incorporating adjuvants to enhance the body's immune response to the vaccine and to prove their protective effects. For that we use EPS as adjuvant that we extract it from microalgae using ion-exchange chromatography on DEAE-cellulose columns. We selected three species of freshwater and marine microalgae before the extraction of EPS to be developed as adjuvant. In the present work we start massive culture of microalgae species and we got the data for more than one species until now, we will show it in the conference.

Keywords--- **microalgae, vaccine, EBS, immunology**

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Phenotypic and Molecular Identification of Marine Sponges from Order Suberitida Off Pulau Bidong,Terengganu, Malaysia

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Abstract

Marine sponges are one of the primitive multicellular animals in the world that have important role in the ocean and to the other marine life. Considerable research and development has been done for the purpose of discovery and sustainable supply of pharmaceutical products from sponges. Being an important resource for natural products, there are still lacks in clarifications of sponges taxonomic position. The purpose of this research is to identify the five sponge samples collected at Pulau Bidong using morphological characteristics and molecular identification technique with comparison to NCBI database. The morphological characteristic for five samples studied exhibit similarity in coloration, shape and surface structure but differences were found in the type of spicules between the samples. The major spicule found were megasclere spicule and only one sample have the microspicule with all samples having strongyloxea spicule. The different in the types of spicules might be the indicator of the sponges to belong in different genus or species. *Hymeniacidon sp*. and *Rhizaxinella sp* were identified for samples studied using cytochrome oxidase subunit I gene (COI) technique. Although two samples were found to be belonged to *Hymeniacidon sp*., they might from different species due to the different percentage of similarity when compared in the NCBI. Therefore, this study showed that the molecular identification is importance to completion the key taxonomy using morphological data. Further detailed taxonomic studies should be taken to identify the samples to species level.

Keyword--- **sponges, COI, taxonomic**

Enzyme Inhibitory and Cytotoxic Properties of *Acanthus ebracteatus* and *Lumnitzera racemosa* as Potential Suppresser on Pro-inflammatory Cytokines in Rheumatoid Arthritis

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Abstract

The present work report investigation on cytotoxicity activity and enzyme inhibitory activity (15-lipoxygenase, 15-LOX and Acetyl-cholinesterase, AChE) of two Malaysian mangrove plant extracts; *Acanthus ebracteatus* and *Lumnitzera racemosa.* Acetylcholine and Lipoxygenase inhibits inflammation by suppressing the production of pro-inflammatory cytokines through a mechanism on α7 nicotinic acetylcholine receptor and reactive oxygen species (ROS) regulation respectively. Plant materials including leaf and bark were collected, dried, powdered and extracted using Methanol (MeOH). The MeOH extract was undergo semi-fractionation via liquid-liquid separation technique using hexane, dichloromethane (DCM), Ethyl acetate (Ea) and n-Butanol sequentially. The dried fractions were then screened for, the AChE inhibitory effects using the microplate assay based on Elman's colorimetric method with some modifications, and LOX inhibitory effects using the LOX inhibitor screening assay kit. The extracts were then tested for cytotoxicity activity using chondrocytes and synovial cell lines. This test were conducted for further investigation in application on cell arthritis. The results indicated that stem and leaf of *A. ebracteatus* DCM fraction possessed the highest activity with (respectively: IC_{50} = 57.80 ± 0.01 and 26.20 ± 0.05 µg/ml respectively, when tested at a concentration of 2µg/ml in the assay. Inhibition activity obtained by the DCM bark fractions of *L. racemosa* against the enzyme were IC_{50} = 43.30 ± 0.03. Activity recorded for the Galanthamin (positive control) was IC_{50} = 1.20 ± 0.03 µg/ml at a concentration of 0.1 mg/ml in the assay. DCM extract result for both *Acanthus* and *Lumnitzera* bark/stem showed a potent inhibitory rate on 5-Lipoxygenase enzyme with percentage inhibition between 73% and 69% respectively. Both sample shows low lethality on both cell lines on >50µg/ml concentration. The result suggests that the tested plant species may provide a substantial source of secondary metabolites with AChE and LOX inhibitory effects. Thus, further studies are needed on the effects of these metabolites against other pro-inflammatory factors by in-vitro for treatment of Rheumatoid Arthritis.

Keywords---**anti-inflammatory cytokines, Acetylcholinesterase, 15-Lipoxygenase, mangrove, rheumatoid arthritis**

Production of Microalgae for Feeding of Oyster Larvae in an Outdoor Closed Photobioreactor System

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Abstract

Microalgae are substantially important in aquaculture especially in the rearing of bivalves. Bivalve hatcheries rely heavily on cultured microalgae as the main food sources for feeding in early life stages. Recent technology in microalgae production is giving way to the development of photobioreactors to enhance biomass yield and improve biomass qualities by controlling the operational conditions. For the production of high quality oyster larvae, it is essential to develop a microalgal culturing system that produces high quality microalgae biomass constantly. The present study proposes the setup of a photobioreactor in outdoor conditions with the aim to increase microalgae production for hatchery rearing of oyster larvae. The photobioreactor which consisting of four 0.01 m^3 transparent glass tubes will be built outdoor under a roof with natural sunlight and photoperiod. The temperature of cultures will be regulated by sparging water over the surface of the culture tubes. Aeration with 1 % carbon dioxide will be supplied through a tube extending to the bottom of the photobioreactor while the filtered seawater will be sterilised using an integrated ozone generator before the inoculation of microalgae. The reactor performance in terms of total biomass, cell density, and the quality of microalgae including pigment and fatty acid contents will be evaluated on continuous cultures of selected diatom and dinoflagellate microalgal species. The effects of selected microalgae on the growth, survival and body composition of oyster larvae will also be assessed to establish an optimal feeding strategy for oyster larviculture. Comparing the performances of different microalgal species and culture conditions would offer significant improvements to the viability of photobioreactors in bivalve hatcheries.

Keywords--- **microalgae, photobioreactor, oyster larvae**

Screening of Marine Microalgae as Potential Natural Source of Antioxidant

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Abstract

In recent years, attention for microalgae has been growing rapidly because of the applicability of their bioactive chemical constituent such as antioxidant, lipid and omega-3-fatty acids for commercial use including food, pharmaceutical, cosmetics and aquaculture. Particularly, screening of suitable strain for mass culture is of interest because the properties in bioactive compounds and growth usually vary between species or strain. Although there are already some species that has been introduced to commercial use for antioxidant and bioactive compound production, there are many more species yet to be found in the field. Therefore, we aim to search for more strains with high growth and high-value compounds production potential. In this study, a total of 30 marine microalgae isolated from Malaysia were screened for its antioxidant properties via ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. In addition, carotenoid content of each strain was also analyzed to examine its correlation with their antioxidant potentials. Some of the screened strains showed high growth rate which is also an important factor that determines its potential for future application. The further results of the screening will be presented with a discussion regarding the application of the selected species as a potential strain for a production of antioxidant.

Keywords--- **microalgae, antioxidant, carotenoid, ABTS, DPPH**

Potential of Marine Metabolites in Reducing the Progression of Atherosclerosis

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Abstract

Marine environment is a source of biological diversity and potential for discovery of novel drugs. Several marine metabolites have been successfully developed as drugs, mostly in the treatment of cancer, pain and hyperlipidemia. In search a potential alternative drug for the treatment of atherosclerosis, two species of marine invertebrates, *Acaudina molpadioides* and *Acanthaster plancii* were chosen. Phytochemical screening was used to analyze the chemical constituents content on the methanol extracts of both samples. The methanol extracts were fractioned by using MPLC technique to get the enhance fractions (EF). All the 10 enhanced fractions obtained were tested for cytotoxicity and luciferase assay. The cytotoxicity was performed by using MTS assay on HEPG2 cell line. Anti atherosclerotic activity on the enhanced fractions was carried out by treating onto HEPG2 cells transfected with PCSK9 (in various concentrations ranging from 0 to 50 µg/mL). Fatty acid methyl esters (FAMEs) were determined for both *Acaudina molpadioides* and *Achanthater plancii* extract samples . Result on phytochemicals screeing revealed that saponin, steroid and terpenoid were present in *Acaudina molpadioides* crude extract. Cytotoxicity assay revealed that *Acaudina molpadioides* enhanced fractions showed no cytotoxic activity on HEPG2 cells with no IC_{50} value more 30 µg/mL. For Achanthaster plancii, there are no IC50 value less than 25µg/mL. For antiatherosclerotic potential agent, result showed that EF 7 and EF 8 from *Acaudina molpadioides* and EF 2, 4 of *Achanthaster plancii* were was demonstrated to reduce the transcriptional activity of PCSK9 promoter. EF 7 and 8 of *Acaudina molpadioides* reduced the transcriptional activity of PCSK9 promoter as the result show 0.08 fold change at 6.25 µg/mL and 0.04 fold change at 3.13 µg/mL respectively. EF 2 and 4 of *Achanthaster placii* reduced the transcriptional activity of PCSK9 promoter as the result show 0.3 fold change at 6.25 µg/mL and 0.4 fold change at 25 µg/mL respectively. The predominant fatty acids in Acaudina molpadioides methanol extracts is palmitic (32.73%) and stearic (31.89%) while in Achanthaster plancii hexane extracts is arachidonic (26.66%) and palmitic (7.15%). This study found that secondary metabolite from *Acaudina molpadioides* and *Acanthaster plancii* possess a potential activity in inhibiting the progression of atherosclerosis by reducing the transcriptional activity of PCSK9.

Keywords---**Natural products, Marine invertebrates, atherosclerosis, PCSK9, hyperlipidemia**

Cytotoxicity of Lysates from *Acanthamoeba* sp. Isolated from Setiu Wetland (SW) Water against MCF-7 Cell Lines.

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Abstract

Acanthamoeba is a free-living organism and can be found in fresh, brackish and sea waters. Previous studies shown the pathogenic free-living *Acanthamoeba* extract displayed morphological features of apoptosis such as cell shrinkage and nuclear condensation in tumor cells. The purpose of this study was to investigate anti-cancer activities in lysate from environmental isolate of *Acanthamoeba*, namely *Acanthamoeba* sp. (SW isolate) against MCF-7 cells. The death mechanism was determined by measuring the percentage of half inhibition concentration (IC_{50}), DNA laddering and morphology changes by inverted light microscopy. The activities of *Acanthamoeba* sp (SW isolate) were compared to the clinical isolate, *Acanthamoeba castelleanii* (IMR isolate). The results obtained for both *Acanthamoeba* was comparable. The IC_{50} for *Acanthamoeba* sp. (SW isolate) was 17.56µg/ml and IC_{50} for *Acanthamoeba castelleanii* (IMR isolate) was 18.52µg/ml. The lysates also induced apoptosis in MCF-7 and was confirmed by DNA fragmentation. The morphology of lysed MCF- 7 cells was examined by inverted light microscopy. The MCF-7 cells displayed morphology featured characteristic of apoptosis such as cell membrane blebbing, and cell shrinkage. The result suggests that lysates of *Acanthamoeba* spp have potential to act as anti-cancer in the future.

Keywords--- ***Acanthamoeba,* MCF-7, cytotoxicity, apoptosis.**

The Potential of *Acanthaster plancii* Fractions in Reducing the Progression of Atherosclerosis via the Inhibition of PCSK9

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Abstract

Atherosclerosis which is highly associated with the elevated levels of low density lipoprotein is gravely threatening the world population. It has become a crucial issue as current common drug treatment, statin therapy has left detrimental effects like rhabdomyolysis, atrial fibrillation, liver disease, abdominal and chest pain. Interestingly, the discoveries of proprotein convertase subtilisin-kexin type 9 (PCSK9) have paved a new way in the treatment of atherosclerosis. This serine protease is believed to involve in the regulation of LDL- uptake by LDL-receptor by the degradation of LDL-receptor. Based on the previous preliminary screening, enhance fraction of *Acanthaster plancii* reduced the expression of PCSK9 promoter. Therefore, this study was conducted to determine the transcription factors that involve in the downregulation and minimal elements of the PCSK9 promoter that mediate the inhibitory actions. Analysis using luciferase assay with seven different promoter fragment (D1-D7) revealed that PPAR may involves as the transcription factor as fragment that possess PPRE site gave the lowest expression of PCSK9 gene. fragment D1 was mutated to eliminate the binding sites and designated as D1-1, D1-2, D1-3 and D1-4. Luciferase assays of transfected mutated PPRE plasmid revealed that the inhibitory action of the sample was abated and the luciferase activity was higher than to that of untreated sample. It is suggested that further analysis to unlock the mechanism on how PCSK9 regulate the cholesterol homeostasis should be done to validate the potential of marine compound from *Acanthaster plancii* in combating atherosclerosis.

Keywords--- **Cardiovascular diseases, Crown of Thorn, PCSK9, PPRE, Reporter Gene Assay**

Screening of Biosurfactant Activity and Species Identification of Endophytic Bacteria Isolated from Setiu Mangrove Wetland, Terengganu

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Abstract

Biosurfactants are biologically surface-active molecules or chemical compounds produced by various microorganisms such as bacteria, yeasts and fungi. It has great application in environmental, oil, food, and pharmaceutical industries. Sustainable biosurfactant sources from endophytic microorganism isolated from mangrove plant is explored as alternative sustainable resources in this study. Endophyte is a mutualistic organism which provides protection to their host by producing secondary metabolite such as biosurfactant. The aims of the study to screen for biosurfactant activities and species identification of surfactant producing endophytic bacteria. Drop collapse method was used to select endophytic bacteria according to their biosurfactant activity. Bacteria species identification was determined using 16S RNA analysis. The study selected four bacteria with most active biosurfactant activity to lowering the surface tension of water. The 16S RNA analysis confirms the four bacteria as *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus manliponensis* and *Bacillus toyonensis*.

Keywords--- **biosurfactant, *Bacillus sp.* endophytes, mangrove, Drop collapse method**

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