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EFFECTS OF BIOSURFACTANT EXTRACT FROM ENDOPHYTIC MANGROVE BACTERIA ON RHEUMATOID ARTHRITIS INFLAMMATORY USING *IN-VITRO* MODEL

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Rheumatoid Arthritis is an inflammation of soft tissue when the synovium cell inflamed and thickened causing destruction of cartilage and joints. These will activated T-cells and B-cell followed by pro-inflammatory cytokine production. A recent study shows uncontrollable production of cytokine related to the cholinergic pathway in arthritis. It is controlled by neural input via an inflammatory reflex which involved in the central and peripheral nervous system. This study aimed to investigate the presence of biosurfactant and their anti-inflammatory and anti rheumatoid arthritis effects. All isolates bacteria were screened their biosurfactant activity and potential bacteria were identified their species by 16S rRNA. Potential bacteria were extracted and evaluated for their inhibitory effects against the Acetylcholinesterase and 5-lipoxygenase enzymes. Human synovial cell and Normal Human Articular Chondrocytes-knee cell (NHAC-kn) were used to determine their toxicity level. All four isolates (C9, I9, C7 and Ph) shows most active biosurfactant activity and were identified as *Bacillus wiedmanni*, *Bacillus toyonensis*, *Bacillus cereus* and *Bacillus amyloliquefaciens*. *Bacillus amyloliquefaciens* have higher inhibitory effects compare to other extracts against both inhibioty enzymes acetylcholinesterase (IC_{50}=50.71) and lipoxygenase (IC_{50}= 53.65). All four potential bacteria were consider as non-toxic, as their IC_{50} value were >20µg/mL. Thus, *Bacillus amuloliquefaciens* were extracted for their biosurfactant and TLC profiling reveals there is the presence of lipopeptides surfactin.

It shows that lipopeptides surfactin extract was suppressed both AChE and LO enzymatic inhibitory assay better than the crude extract. Final clinical test were tested by dose-dependent manner against induced cell with TNF-α and Interleukin-1β. One-way Anova analysis shows no significant difference with MTX as positive control which proven the effectiveness of lipopeptides biosurfactant to neutralize the activity of TNF- α and IL-1β by reducing cell apoptosis and abnormality of cell growth. Overall, surfactin possess a good potential in becoming as bioactive compound to treat Rheumatoid Arthritis, however further efforts can be done to optimum the production of biosurfactant extract by changing their carbon source used and through genetic modification of bacteria.

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KESAN EKSTRAK BIOSURFAKTAN DARI BAKTERIA ENDOFITIK BAKAU TERHADAP INFLAMASI RHEUMATOID ARTHRITIS MENGGUNAKAN MODEL *IN VITRO*

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Rheumatoid Arthritis adalah keradangan pada tisu lembut apabila sel synovia meradang dan menebal yang menyebabkan kerosakan pada tulang rawan dan sendi. Ini akan mengaktifkan T-cells dan B-cells diikuti oleh penghasilan sitokin pro-keradangan. Kajian terkini menunjukkan penghasilaan sitokin tidak terkawal berkait dengan laluan kolinergik pada arthritis. Ia di kawal oleh sel saraf input melalui tindakan refleks keradangan yang mana ia terlibat pada sistem saraf pusat dan sistem saraf periferi. Kajian ini bertujuan untuk mengkaji kehadiran biosurfaktan serta sifat anti-keradangan dan kesan anti-rheumoatid arthritis. Kesemua pencilan bakteria telah disaring aktiviti biosurfaktannya dan bakteria yang berpotensi telah dikenal pasti melalui 16S rRNA. Bakteria yang berpotensi telah dieskstrak dan dinilai kesan perencatan terhadap enzim Acetylcholinesterase dan 5-Lipoksigenase. Human synovial cell dan Normal Human Articular Chondrocytes-knee sel digunakan untuk menentukan tahap ketoksikan bakteria. Kesemua empat pencilan (C9, I9, C7 and Ph) menunjukan aktiviti biosurfaktan paling aktif dan dikenalpasti sebagai *Bacillus wiedmanni*, *Bacillus toyonensis*, *Bacillus cereus* and *Bacillus amyloliquefaciens*. *Bacillus amyloliquefaciens* mempunyai kesan perencentan tertinggi berbanding ekstrak lain terhadap kedua enzim rencatan acetylcholinesterase (IC_{50}=50.71) and lipoksigenase (IC_{50}= 53.65). Kesemua empat bakteria yang berpotensi dianggap tidak toksik, kerana nilai IC_{50} value ialah >20µg/mL. Oleh itu, *Bacillus amuloliquefaciens* telah di ekstrak untuk mendapatkan biosurfaktan dan profil Kromatografi lapisan

nipis (TLC) menunjukkan terdapat kewujudan lipopeptida surfaktan. Ia terbukti lipopeptida surfaktan telah menyekat kedua ujian AChE dan LO enzim kerencatan lebih baik berbanding ekstrak mentah. Ujian klinikal akhir telah diuji melalui teknik kebergantungan dos dengan merangsang sel normal dengan TNF-α and Interleukin-1β. Analisis Anova satu hala menunjukkan tiada perbezaan ketara dengan MTX iaitu kawalan normal yang mana terbukti keberkesanan lipopeptida biosurfaktan untuk meneutralkan aktiviti melalui pengurangan sel apoptosis dan ketaknormalan pembiakan sel. Secara kesulurahan, surfaktin mempunyai potensi yang bagus sebagai sebatian bioaktif untuk merawat Rheumatoid Arthritis, walau bagaimanapun usaha selanjutnya boleh dilakukan bagi mengoptimumkan penghasilan ekstrak biosurfaktan dengan mengubah sumber karbon dan melalui pengubahsuaian genetik.