

## Effects of *Payena dasyphylla* (Miq.) on hyaluronidase enzyme activity and metalloproteinases protein expressions in interleukin-1beta stimulated human chondrocytes cells

### ABSTRACT

**Background:** Hyaluronidases have been found as the target enzymes in the development of osteoarthritis (OA) disease. While there is still no curative treatment for this disease, recent studies on the treatment of OA were focused on the effectiveness of natural products which are expected to improve the symptoms with minimal side effects. The aim of this study was to screen selected Malaysian plants on their anti-hyaluronidase activity as well as to evaluate the active plant and its derived fractions on its potential anti-arthritic and antioxidant activities. **Methods:** A total of 20 methanolic crude extracts (bark and leaf) from ten different plants were screened using a colorimetric hyaluronidase enzymatic assay. The active plant extract (*Payena dasyphylla*) was then studied for its hyaluronidase inhibitory activity in the interleukin-1 $\beta$  (IL-1 $\beta$ ) stimulated human chondrocytes cell line (NHAC-kn) using zymography method. The *Payena dasyphylla* methanolic bark extract was then fractionated into several fractions in where the ethyl acetate (EA) fraction was evaluated for its inhibitory effects on the HYAL1 and HYAL2 gene expressions using reverse transcription-polymerase chain reaction (RT-PCR) technique. While the MMP-3 and MMP-13 protein expressions were evaluated using western blot method. The phenolic and flavonoid contents of the three fractions as well as the antioxidant property of the EA fraction were also evaluated. **Results:** Bark extract of *Payena dasyphylla* (100  $\mu$ g/ml) showed the highest inhibitory activity against bovine testicular hyaluronidase with 91.63%. The plant extract also inhibited hyaluronidase expression in the cultured human chondrocyte cells in response to IL-1 $\beta$  (100 ng/ml). Similarly, treatment with *Payena dasyphylla* ethyl acetate (EA) fraction (100  $\mu$ g/ml) inhibited the HYAL1 and HYAL2 mRNA gene expressions as well as MMP-3 and MMP-13 protein expression in a dose dependent manner. *Payena dasyphylla* EA fraction has demonstrated the highest amount of phenolic and flavonoid content with  $168.62 \pm 10.93$  mg GAE/g and  $95.96 \pm 2.96$  mg RE/g respectively as compared to water and hexane fractions. In addition, the *Payena dasyphylla* EA fraction showed strong antioxidant activity with IC<sub>50</sub> value of  $11.64 \pm 1.69$   $\mu$ g/mL. **Conclusion:** These findings have shown that *Payena dasyphylla* might contained potential phenolic compounds that inhibiting the key enzyme in osteoarthritis development, which is the hyaluronidase enzyme through interruption of HYAL1 and HYAL1 gene expressions. The degradation of cartilage could also be inhibited by the plant through suppression of MMP-3 and MMP-13 protein expressions. We also reported that the inhibitory effect of *Payena dasyphylla* on hyaluronidase activity and expression might be due to its antioxidant property.

**Keyword:** Hyaluronidase; MMP-13; MMP-3; Osteoarthritis; *Payena dasyphylla*.