

Anti-angiogenic effect of *Ardisia crispa* root invitro and in vivo and its potential pathway(s)

ABSTRACT

Ardisia crispa Thunb. A. DC (Myrsinaceae), locally known as “hen’s eyes” in Malaysia, is a traditional medicine plant with anti-inflammatory and anti-tumor promoting properties. This study aims to investigate the anti-angiogenic effect of the hexane extract of the plants root (ACRH) and its isolated benzoquinonoid (AC2), using HUVE cells, in vitro and zebrafish angiogenic assay, in vivo. In cell invasion assay, both ACRH and AC2 at lowest concentration (0.1 ug/ml) significantly reduced the number of invaded cell about 30% ($p < 0.001$), both ACRH and AC2 also started to inhibit the tube formation assay at 1 ug/mL, respectively. The gelatinases activity of ACRH and AC2 was determined in gelatine zymography assay. The results demonstrated the potential inhibitory effects of ACRH and AC2 on pro MMP-2 activity with a concentration dependent manner, while anti-MMP-9-activity-of the treatment was not apparent. Milliplex® Map Human Angiogenesis/Growth Factor Magnetic Bead Panel 1 was used to further elucidate the possible molecular pathway of their-inhibitory action. Both ACRH and AC2-treated HUVECs concentration-dependently, downregulated Angopoeitin-2, HgH, VEGF-c and VEGF-D. Interestingly, only ACRH significantly downregulated Follinstatin in concentration-dependent manner, but not AC2. Whilst, FB-EGF was significantly downregulated at higher concentration of ACRH and AC2. Last but not least, in transgenic zebrafish assay, only treatment with 5ug/ml of ACRH showed apparent reduction of ISV sprouts arising from the dorsal aorta. These findings validated the anti-angiogenic effects of both ACRH and AC2 potentially mediated via several angiogenic activators, by downregulating HGH, Angopietin-2, VEGFVEGF-D., respectively.

Keyword: Anti-angiogenic effect; *Ardisia crispa* root; *Ardisia crispa*; Anti-angiogenic.