

Cancer metastasis is a primary cause of cancer death. *Antrodia cinnamomea* (*A. cinnamomea*), a medicinal mushroom in Taiwan, has been shown antioxidant and anticancer activities. In this study, we first observed that ethanol extract of fruiting bodies of *A. cinnamomea* (EEAC) exerted a concentration-dependent inhibitory effect on migration and motility of the highly metastatic CL1-5 cells in the absence of cytotoxicity. The results of a gelatin zymography assay showed that *A. cinnamomea* suppressed the activities of matrix metalloproteinase (MMP)-2 and MMP-9 in a concentration-dependent manner. Western blot results demonstrated that treatment with *A. cinnamomea* decreased the expression of MMP-9 and MMP-2; while the expression of the endogenous inhibitors of these proteins, *i.e.*, tissue inhibitors of MMP (TIMP-1 and TIMP-2) increased. Further investigation revealed that *A. cinnamomea* suppressed the phosphorylation of ERK1/2, p38, and JNK1/2. *A. cinnamomea* also suppressed the expressions of PI3K and phosphorylation of Akt. Furthermore, treatment of CL 1-5 cells with inhibitors specific for PI3K (LY 294002), ERK1/2 (PD98059), JNK (SP600125) and p38 MAPK (SB203580) decreased the expression of MMP-2, and MMP-9. This is the first report confirming the anti-migration activity of this potentially beneficial mushroom against human lung adenocarcinoma CL1-5 cancer cells.