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Anti-tumor activity from stems of *Zanthoxylum schinifolium* toward human acute leukemia Jurkat T cells is attributable to apoptosis induced via mitochondria-dependent activation of caspase cascade regulated by Bcl-xL

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To isolate novel compounds with anti-tumor activity from stems of *Zanthoxylum schinifolium*, cytotoxicity of various organic solvent extracts from stems of *Z. schinifolium*on were investigated toward human acute leukemia Jurkat T cells. Among these extracts such as methanol extract (SS-7), methylene chloride extract (SS-8), ethyl acetate (SS-9), n-butanol extract (SS-10), and residual fraction (SS-11), the methylene chloride extract appeared to contain the cytotoxic activity against Jurkat T cells. The methylene chloride extract was able to induce apoptotic cell death of Jurkat T cells at the concentration of 50~100 μg/ml. The methylene chloride extract-induced apoptosis of Jurkat T cells accompanied various apoptotic events including mitochondrial cytochrome c release, activation of caspase-9 and -3, and degradation of poly (ADP-ribose) polymerase (PARP), leading to apoptotic DNA fragmentation. The ectopic overexpression of Bcl-xL, which is known to prevent apoptotic cell death by blocking mitochondrial cytochrome c release, completely abrogated these apoptotic events. The GC-MS analysis of the methylene chloride extract identified fifteen components of SS-8 as the major ingredients; 9,12-octadecadienoic acid (22.3%), 3H-pyrazol-3-one, 2,4-dihydro-5-methyl-4- (1-methylethylidene)-2-(4-nitrophenyl)-(9CI) (15.0%), hexadecanoic acid (14.2%), Benzofuran, 5,6-dimethoxy-2-methyl-(9CI) (11.0%), and 4-methoxy-2-methylcinnamic acid (5.4%). These results demonstrate that the antitumor activity of the methylene chloride extract from stems of *Zanthoxylum schinifolium* is attributable to apoptotic cell death induced through mitochondria- dependent activation of caspase cascade regulated by Bcl-xL.

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E_2F and Sp1 mediate the expression of the human MCAK gene: Characterization of promoter activity of the 5′-flanking region

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Mitotic centromere-associated kinesin (MCAK) is a novel kinesin with central motor domain, which has been implicated in coordinating the dynamics of kinetochore microtubes during M phase. In this study, we have cloned and characterized the 5′-flanking region of the human MCAK gene in order to investigate the transcription regulation mechanism of the gene expression during the cell cycle. The 1838-bp fragment of up stream of the transcription start site was cloned and the nucleotide sequence was determined. Functional analysis of the 5′-flanking region by deletion mutagenesis, and transient transfection and luciferase activity assay revealed that -270/+52 region is the minimal promoter of the human MCAK gene in HEK 293T and HeLa cells. Within this region, there are three putative binding sites for Sp1 (-92/-84, -174/-165, -194/-183), two putative binding sites for egr-1 (-96/-86, -194/-183), and a putative binding site for E_2F (-58/-51). Site-directed mutagenesis, transient transfection and luciferase assay, and electrophoretic mobility shift assay confirmed that the MCAK promoter is positively regulated by Sp1 and is repressed by E_2F_1.