



# Effects of *Acanthopanax senticosus* extracts on inducing apoptosis on B16F10 melanoma cell

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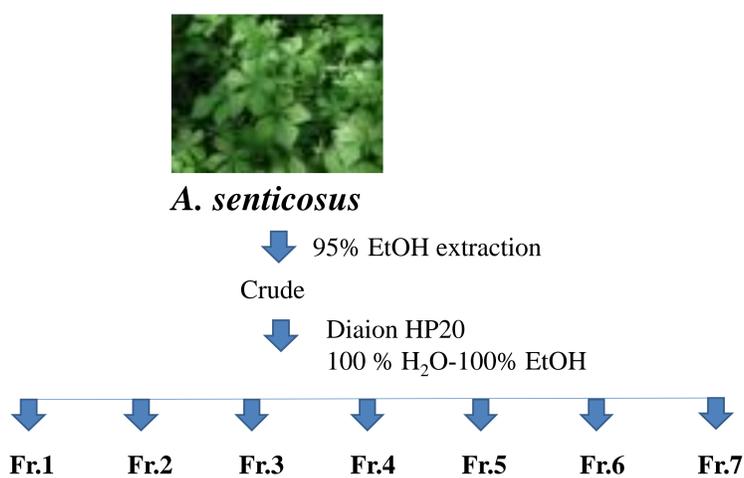
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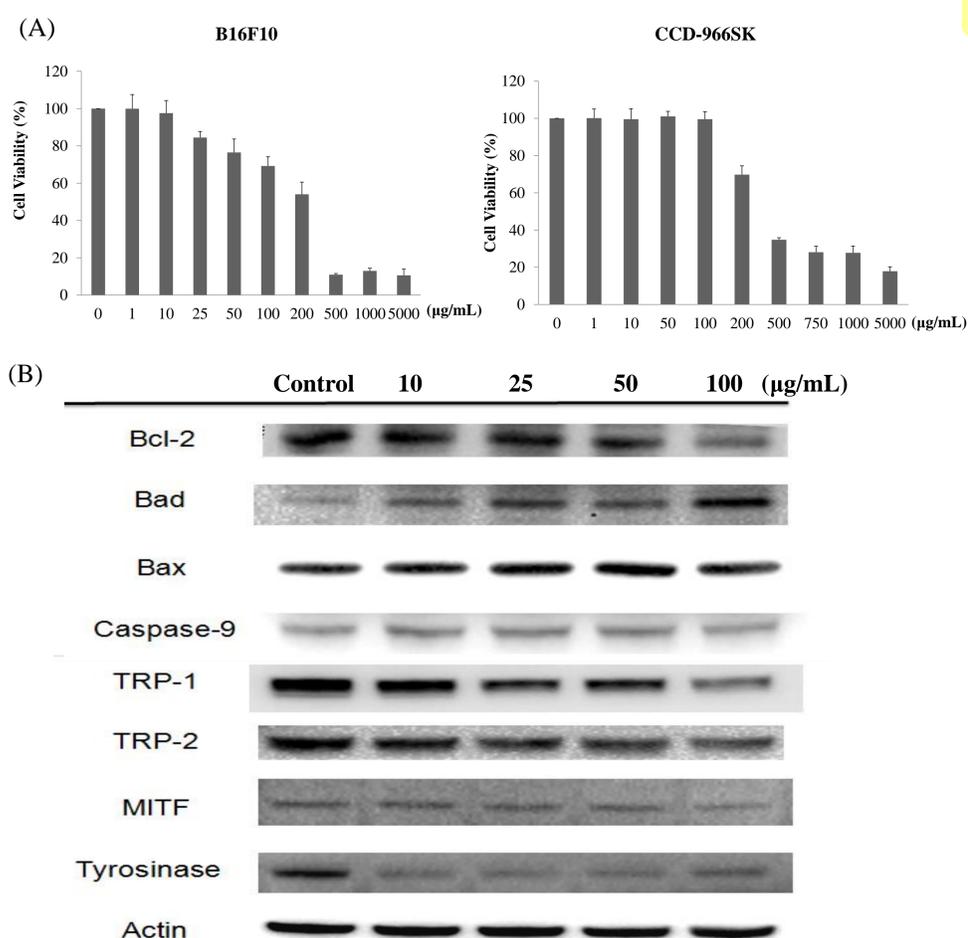
## Abstract

In the United States, Malignant melanoma is the sixth most common cancer, which is the most serious type of metastatic skin cancer. In the theory of Traditional Chinese Medicine, *Acanthopanax senticosus* is a commonly used traditional Chinese herb for invigorating qi, enhancing the spleen, and nourishing the kidney, which is also known as Siberian Ginseng and Ciwujia in Chinese. Evidences are accumulating that extract of *A. senticosus* has anti-inflammatory, anti-cancer and anti-tyrosinase activities effects. However, further researches and studies still need to be conducted on melanoma. In the present study, 80% of EtOH extract (ACE) from *A. senticosus* was tested for its *in vitro* anti-tumor activity by using B16-F10 murine melanoma cells. On the other hand, the aqueous extract did not show any activity. The result showed that ACE suppresses B16-F10 cell growth via down-regulation the BCL-2 and up-regulation BAD protein expression. In addition, the ACE-5 and 6 isolated 80% EtOH extract on inhibitor melanogenesis in highly metastatic B16-F10 cells in the dose of 25  $\mu\text{g}/\text{mL}$ . And, the examination of melanogenic protein expression showed that tyrosinase, tyrosinase-related protein (TRP)-2, and Microphthalmia-associated transcription factor (MITF) protein by ACE-5 and 6. In the end, these results suggest that ACE-5 and 6 could have therapeutic potential for melanoma and melanoma-associated depigmentation.

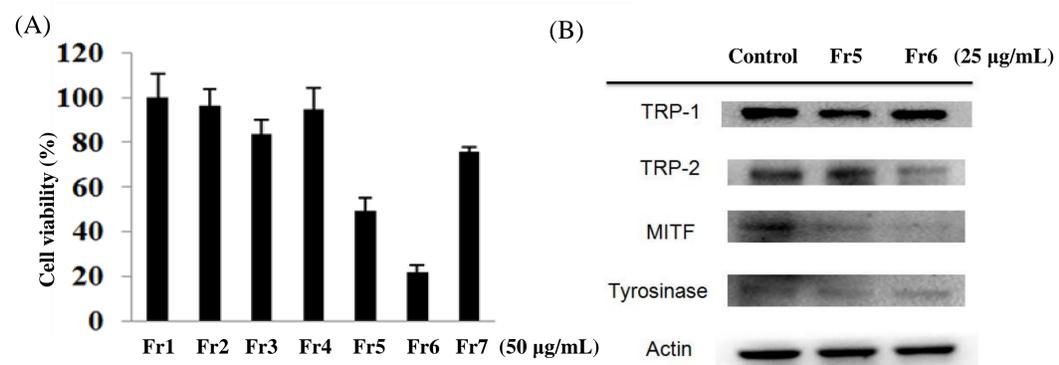
## Results



**Figure 1. Extraction and isolation.** The fresh whole plants of *A. senticosus* were extracted three times with 80% EtOH at room temperature. The 80% EtOH extract was chromatographed on Diaion HP20 with H<sub>2</sub>O-EtOH gradient to yield seven fractions.



**Figure 2. Inhibitory effect of *A. senticosus* on growth and melanogenesis.** (A) Effect of *A. senticosus* on the cell viability of B16F10 and CCD-966SK cells. Cells were treated with various concentrations of *A. senticosus* for 24 h, and cell viability was determined by 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. (B) Expressions of apoptosis and melanogenesis proteins in B16F10 cells with *A. senticosus* treatment. Western blotting data show the changes in Bcl-2, Bad, TRP1, TRP2, MITF and Tyrosinase expressions in B16F10 melanoma cells treated with *A. senticosus* at different concentrations (0–100  $\mu\text{g}/\text{mL}$ ) for 24 h.



**Figure 3. Inhibitory effect of fraction 5 and 6 on growth and melanogenesis from *A. senticosus*.** (A) Effect of *A. senticosus* on the cell viability of B16F10 cells. Cells were treated with 50  $\mu\text{g}/\text{mL}$  of *A. senticosus* for 24 h. (B) Expressions of melanogenesis-related proteins in B16F10 cells with *A. senticosus* treatment. Cells were treated with various concentrations of *A. senticosus* for 24 h.

## Conclusions

Inhibitory effect of *A. senticosus* on the viability of B16F10 melanoma cells have been evaluated. The results showed that ACE-5 (Fr5) and 6 (Fr6) possess the highest cytotoxic activity and the ability to inhibit the process of melanogenesis among the tested fractions. Moreover, Fr5 and Fr6 significantly decreased melanogenesis of B16F10 melanoma cells, suggesting that Fr5 and Fr6 may mediate through the tyrosinase, tyrosinase-related protein (TRP)-2, and Microphthalmia-associated transcription factor (MITF) pathway.

