

## **Dillenia suffruticosa dichloromethane root extract induced apoptosis towards MDA-MB-231 triple-negative breast cancer cells**

### **ABSTRACT**

#### **Ethnopharmacological Relevance:**

Dillenia suffruticosa is traditionally used for treatment of cancerous growth including breast cancer in Malaysia.

#### **Aim of The Study:**

Dillenia suffruticosa is a well-known medicinal plant in Malaysia for the treatment of cancer. Nevertheless, no study has been reported the cytotoxicity of this plant towards MDA-MB-231 triple-negative breast cancer cells. The present study was designed to investigate the mode of cell death and signalling pathways of MDA-MB-231 cells treated with dichloromethane Dillenia suffruticosa root extract (DCM-DS).

#### **Methods:**

Extraction of Dillenia suffruticosa root was performed by the use of sequential solvent procedure. The cytotoxicity of DCM-DS was determined by using MTT assay. The mode of cell death was evaluated by using an inverted light microscope and flow cytometry analysis using Annexin-V/PI. Cell cycle analysis and measurement of reactive oxygen species level were performed by using flow cytometry. The cells were treated with DCM-DS and antioxidants  $\alpha$ -tocopherol or ascorbic acid to evaluate the involvement of ROS in the cytotoxicity of DCM-DS. Effect of DCM-DS on the expression of antioxidant, apoptotic, growth, survival genes and proteins were analysed by using GeXP-based multiplex system and Western blot, respectively. The cytotoxicity of compounds isolated from DCM-DS was evaluated towards MDA-MB-231 cells using MTT assay.

#### **Results:**

DCM-DS induced apoptosis, G2/M phase cell cycle arrest and oxidative stress in MDA-MB-231 cells. The induction of apoptosis in MDA-MB-231 cells by DCM-DS is possibly due to the activation of pro-apoptotic JNK1 and down-regulation of anti-apoptotic ERK1, which in turn down-regulates anti-apoptotic BCL-2 to increase the BAX/BCL-2 ratio to initiate the mitochondrial apoptotic pathway. The cell cycle arrest in DCM-DS-treated MDA-MB-231 cells is possibly via p53-independent but p21-dependent pathway. A total of 3 triterpene compounds were isolated from DCM-DS. Betulinic acid appears to be the most major and most cytotoxic compound in DCM-DS.

#### **Conclusion:**

The data suggest the potential application of DCM-DS in the treatment of triple-negative breast cancer.

**Keyword:** Apoptosis; Betulinic acid; Cancer; Cell cycle arrest; Dillenia suffruticosa