

OP 4

Inhibition of cell proliferation in leukemia cancer cells by mediating caspase-3 apoptosis from Thebu leaf, *Costus speciosus* (Koen.) Sm.

Samarakoon KW^{1*}, Jayasooriya RGPT², RanasingheP³, Peiris LDC⁴

¹Institute for Combinatorial Advanced Research and Education (KDU-CARE), General Sir John Kotelawala Defence University, Sri Lanka, ²Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, ³Industrial Technology Institute, Halbarawa Gardens, Malabe, Sri Lanka, ⁴Department of Zoology, University of Sri Jayewardenepura, Sri Lanka.

Background: Medicinal plants are prolific sources that have a great demand for screening of plant products with chemical and therapeutic medicinal properties. *Costus speciosus* (Koen.) Sm. is a tropical herbaceous plant found in home gardens but the medicinal applications of its leaf extract has not been extensively studied.

Objective: To assess *in vitro* anticancer potential of *C. speciosus* leaf extracts, including methanol (T-ME), hexane (T-HE), chloroform (T-CE), ethyl acetate (T-EA) and aqueous (T-WE) fractions against cancer cells.

Method: Anticancer activity was assessed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using cancer cell lines including human leukemia (HL-60), mouse melanoma (B16F10) and human lung carcinoma (A549), while apoptotic activity was examined against HL-60 cells. Western blot assay assessed the expression of apoptosis-related proteins by potent fraction.

Results: Among the *C. speciosus* leaf extracts, T-EA extract showed potent cancer cell growth inhibitory activity against all cancer cell lines tested. The determined mean IC₅₀ values of T-EA extract for anticancer effects were 26.1±1.2, 31.0±0.3 and 30.5±0.8 µg/mL against HL-60, A549 and B16F10, respectively. T-EA suppressed the HL-60 cell proliferation dose-dependently over the Annexin-V fluorescein isothiocyanate (V FITC⁺) /Propidium iodide (PI) staining under the flow cytometric analysis. z-DEVD-fmk. Evaluation of apoptosis in leukemia cancer cells through the caspases-3 dependent pathway showed that, a caspase-3 inhibitors significantly inhibited cell cytotoxicity and apoptotic bodies induced by T-EA. The activation of Bax and suppression of Bcl-xL through apoptotic inducing control of pro- and anti-apoptotic proteins were determined and confirmed by up-regulation of caspase-3 proteins dose-dependently from western blot assays. It is evident that activation of these pro-apoptotic proteins is associated to release of cytochrome *c* into the cytosol while lead to mitochondrial apoptotic pathway.

Conclusion: *C. speciosus* leaf extracts offer chemotherapeutic potentials against leukemia cancers and might be used in remedies for preventing cancers.