STAT5A-related anticancer molecular mechanisms of *Drimia calcarata* inbreast cancer cells

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Drimia calcarata (*D. calcarata*) is one of the plants used by BaPedi people in South Africa to treat a plethora of ailments. However, its anticancer therapeutic use is less understood. Thus, this study was aimed at evaluating the potential anticancer activities of *D. calcarata* extracts against human breast cancer cells. The potential anticancer activities of the *Dc* plant extracts were investigated using the MTT assay, fluorescence microscopy, the Muse® Cell Analyser and gene expression analysis. Both *D. calcarata* water extract (WE) and methanol extract (ME) displayed cytotoxic effect against T47D and MDA-MB31 breast cancer cells; however, T47D cell line exhibited higher sensitivity to the methanol extract. Fluorescence microscopy and Annexin V showed that both *D. calcarata* extracts induced apoptosis in breast cancer cells; but ME significantly (***p<0.0001) induced a caspase-dependent apoptosis in T47D cells, while the WE significantly (***p<0.0001) induced a caspase-dependent apoptosis in MDA-MB-231 cells. Reverse Transcription Polymerase Chain (RT-PCR) showed that the *D. calcarata* extracts induced p53-dependent apoptosis where the ME upregulated p53 splice variant 1 and down-regulated splice variant 2 while the WE had an opposite effect on the p53 splice variants. Additionally, the *D. calcarata* methanol extracts downregulated Bcl-2. The *Drimia calcarata* extracts upregulated the expression of STAT3 and downregulated the expression of STAT5A in both breast cancer cell lines. Our data suggest that *D. calcarata* extracts do not target STAT3-related pathway. Importantly, *D. calcarata* extracts effectively induced growth inhibition and p53-related apoptosis in human breast cancer T47D and MDA-MB-231 cells through STAT5A regulation. The *D. calcarata* plant can therefore be targeted for new therapeutic drug targets and development.

Keywords: *Drimia calcarata*, breast cancer, apoptosis, p53, STAT5A.