Abstract

The current study investigated cytotoxicity and apoptotic activities selected indigenous medicinal plant extracts belonging to *Withania somnifera* of the family Solanacea and *Tinospora cordifolia* of the family Menispermacea in human breast cancer cells. Results revealed that ethanolic extracts of *Withania somnifera* and *Tinospora cordifolia* possessed dose-dependent cytotoxicity, induced apoptosis and cell cycle arrest in human breast cancer cells. The current study also investigated anti-cancer stem cells activity of selected medicinal plants. First, we established a side population (SP) analysis-based bioactivity guided assay for the isolation of phytochemicals targeting cancer stem cells. Treatment with doxorubicin, a widely used cancer chemotherapeutic drug, enriched for SP. This is consistent with the cancer stem cell hypothesis that predicts that current chemotherapeutic drugs reduce the burden of tumor, but leave behind the cancer stem cells population. However, we found that the ethanolic extracts of *Tinospora cordifolia*, significantly inhibited the SP phenotype. Bioactivity (based on anti-SP activity) guided isolation of *Tinospora cordifolia* extracts lead to the isolation of four compounds viz., TCD5-F2-C (TC-A), TCD5-F3-B (TC-B), TC-D4-A2 (TC-C) and TC-D3-A2 (TC-D) having potential anti-cancer activity. Elucidation of mechanisms of action revealed that these compounds inhibited cancer cell proliferation and induced apoptosis in breast cancer cells. The compounds also found to have cancer stem cell specific anticancer activity mediated via inhibition of side population, CD44<sup>high</sup> CD24<sup>low</sup> population, breast cancer spheres and cancer stem cell enriched cell line (NBLE CD44+/CD24-). Moreover, these compounds inhibited multidrug resistant (MDR) transporters, ABCB1, ABCG2 and ABCC1 suggesting that it may work as MDR modulators. Among all the four compounds investigated for anticancer activity, TC-B was found to have most potent activity. These phytochemicals will however have to be subjected to preclinical evaluations (*in vivo* mouse models), clinical trials, and toxicological studies for future therapeutic use as a potential chemotherapeutic drug for the effective treatment of breast cancer.