Development of a natural anti-cancer formulation which can target triple negative breast cancer stem cells.

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Cancer stem cells (CSC) are responsible for the initiation, extensive proliferation and metastasis of cancer. CSCs including breast cancer stem cells (bCSCs) have a capacity to generate chemo and radiotherapy resistance heterogeneous population of cells. Over expressed ABCB1 has reported as a main reason for drug resistance of CSCs via activating drug efflux pumps by creating pores in the cell membrane. Overall efficiency of chemotherapeutic agents might be enhanced by blocking the ABCB protein efflux pump in CSC membrane. There is an urgent need to search persuasive natural drugs which can target CSCs. Anti-cancer properties of *Hylocereus undatus* on cancer CSCs have not yet been studied. In the present study, anti-cancer effects of peel and flesh of *H. undatus* fruit on bCSCs were evaluated with the aim of developing a marketable anti-cancer nutraceutical formulation. The flesh and peel of *H. undatus* were freez-dried and sequentially extracted in to four different solvents (hexane, chloroform, ethyl acetate and ethanol). All extracts (eight extracts) were dried under reduced pressure and different concentrations (12.5-400 µg/mL) were treated on bCSCs isolated from a triple negative chemo-resistant breast cancer phenotype (MDA-MB-231 cells). Anti-proliferative effects of all extracts and paclitaxel (positive control) were determined by a colorimetric assay (WST-1 based). Since peel-chloroform (IC_{50}= 54.8 µg/mL) and flesh-ethyl acetate (IC_{50}= 150.5 µg/mL) extras exerted a potent anti-proliferative effects at 72 h post-incubation, a combinatorial formulation (CF) was developed with the most active peel-chloroform extract and 20 µg/mL of verapamil (a known ABCB1 drug efflux pump blocker) first time in the world. Anti-proliferative effects and pro-apoptotic effects of CF were confirmed by estimating activated caspase3 and caspase7 levels and apoptotic morphological features in the CF treated bCSCs compared to untreated and only verapamil (20 µg/mL) treated bCSCs and CF treated normal mammary epithelial cells (MCF-10A). The anti-proliferative effects of CF (16.4 µg/mL) is greater than paclitaxel (19.2 µg/mL) and three folds greater than peel-chloroform extract (IC_{50}= 54.8 µg/mL) on bCSCs while exerting a less effects on normal cells (> 400 µg/mL). Collectively, CF can be considered as a potential initiative of a nutraceutical formulation which can target CSCs.