Synergistic anticancer potential of *Andrographis paniculata* and *Datura metel* combined with Doxorubicin in inhibiting breast cancer cells

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Breast cancer remains one of the prevalent malignancies affecting women worldwide. This study investigates the synergistic anticancer potential of triple-combinatorial phytotherapeutic agents, *Andrographis paniculata* and *Datura metel*, along with the chemotherapeutic drug doxorubicin (DOX), on estrogen-receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast carcinoma cells and normal breast epithelial cells (MCF-10A). The objective was to identify a synergistic drug–natural product combination that enhances cytotoxic efficacy while minimizing off-target toxicity. Sequential ultrasound-assisted extractions were performed using hexane, ethyl acetate, methanol, and aqueous-ethanol. SRB-based cytotoxic screening revealed that ethyl acetate extract of *A. paniculata* (AP) and methanolic extract of *D. metel* (DM) were most potent, demonstrating IC_{50} values at 168.2 µg/mL (MCF-7) and 90.26 µg/mL (MDA-MB-231) for *A. paniculata*, and 0.23 µg/mL (MCF-7) and 21.59 µg/mL (MDA-MB-231) for *D. metel* after 72 h, with relatively low cytotoxicity toward MCF-10A. Bioactivity-guided fractionation of *A. paniculata* ethyl acetate extract was performed using normal-phase column chromatography with a 10% gradient solvent system consisting of hexane and ethyl acetate. Methanolic extract of *D. metel* was subjected to Kupchan's solvent-solvent partitioning to isolate active constituents. Fraction C8 of *A. paniculata* (AP-C8) exerted denotable cytotoxicity with IC_{50} values at 32.99 ± 15.27 µg/mL on MCF-7 and 4.674 ± 0.73 µg/mL on MDA-MB-231 at 48 h post-treatment. The chloroform fraction of *D. metel* (DM-F2) demonstrated the highest cytotoxicity with IC_{50} at 16.04 ± 0.33 µg/mL and 2.535 ± 0.09 µg/mL on MCF-7 and MDA-MB-231, respectively. Triple-combination treatments (DOX + AP-C8 + DM-F2) were assessed in various fixed dose ratios using the SRB assay. CompuSyn software analysis confirmed that the highest synergistic interactions were observed at a ratio of 1:64:256 on MCF-7 (combination index [CI]=0.71846) and at 1:1.25:10 on MDA-MB-231 (CI=0.77291). Both combinations demonstrated higher dose reduction index (DRI) values in a favorable range, especially for DOX, indicating potential for therapeutic dose minimization. Mainly, the combinations exhibited minimal cytotoxic effects in MCF-10A cells, supporting their selective anticancer potential in the selected combination ratios.

Keywords: *Synergism, Breast cancer, Andrographis paniculata, Datura metel, Doxorubicin*