Synergistic effects of triple combination therapy with thymoquinone, *Datura metel* and *Andrographis paniculata* extracts on the proliferation of human lung cancer cells

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Lung cancer remains one of the leading causes of cancer-related mortality worldwide. Conventional therapeutics often exhibit limitations in providing complete cure for cancer and occurrences of drug resistance is evidenced in solid tumors. Combination therapies that target multiple oncogenic pathways have emerged as a promising strategy to overcome chemo-resistance and minimize treatment-associated toxicity. Thymoquinone (TQ), a bioactive compound derived from *Nigella sativa*, has been reported for its potent anti-cancer properties. *Datura metel* (DM) and *Andrographis paniculata* (AP) are medicinal plants, containing several compounds with anti-cancer properties. Therefore, this study investigated the synergistic anti-proliferative effects of TQ in combination with *D. metel* and *A. paniculata* extracts against human lung cancer cells (NCI-H292). Using sequential ultrasound-assisted extraction, aerial parts and leaves were extracted with hexane, dichloromethane, ethyl acetate, and methanol. SRB-based cytotoxic screening revealed that ethyl acetate extract of *A. paniculata* and dichloromethane extract of *D. metel* were most potent, demonstrating IC_{50} values at 53.46 µg/mL and 63.04 µg/mL at 24 h exposure, respectively. Triple combination treatments in nine different combination ratios (from 1:1:1 to 16:16:1) were assessed using SRB assay. Synergistic interactions were quantified using CompuSyn and SynergyFinder software. The combination 2:2:1 of TQ:DM:AP demonstrated prominent cytotoxicity (IC_{50} values at 2.411 µg/mL) and strong synergistic effects with combination index values of 0.466 (CI < 1) at fractional affected (Fa) 0.5. This synergistic combination showed remarkable drug reduction index (DRI) values of 2.793, 65.234, and 25.684 for TQ, DM, and AP, respectively, demonstrating substantial dose reduction potential while maintaining cytotoxic efficacy. SynergyFinder analysis further confirms these findings, with all four reference models yielding negative scores with synergism (ZIP: -11.52, Loewe: -1.45, HSA: -0.62, Bliss: -10.96), indicating prominent synergistic interactions. This finding demonstrates the potential of the selected triple combination to be developed as an improved therapeutic agent against lung cancer.

Keywords: *Synergism, Lung cancer, Thymoquinone, Andrographis paniculata, Datura metel*