ABSTRACT

Cancer remains one of the major world health issue after cardiovascular diseases. The area of cancer research is continually expanding with better understanding of molecular mechanism of progression of the disease, which has resulted in development of new drug targets for more efficient cancer therapy. In addition, the search for new anticancer lead compounds is a great challenge due to the development of drug resistance, deleterious side effects and unaffordable cost of current chemotherapeutic drugs. The enormous success of plant based natural products as anticancer agent represents medicinal plants as an important pool for the identification of novel drug. Darjeeling Himalayan region has a rich diversity of therapeutic plants that can be utilized for development of novel drugs. In this study some ethnomedicinally important plants of the Darjeeling Himalayan region, such as *Astilbe rivularis*, *Tupistra nutans, Zanthoxylum oxyphyllum, Bergenia ciliata, Artemesia vulgaris* and *Eupatorium cannabium* were screened for various phytochemicals, and antioxidant, antimicrobial activities, and cytotoxic potential against cancer cell lines, and finally, *Astilbe rivularis* was selected futher studies on isolation of active compound with anti-cancer potential. A steroid ester compound, spectrometrically characterized as Stigmasta-5(6), 22(23)-dien-3-beta–yl acetate, designated as A11, was isolated for the first time from the plant rhizome in a bioassay guided approach. The catalytic inhibition and structural alteration of human dihydrofolate reductase (hDHFR) by A11 was evaluated using methotrexate (MTX), a DHFR inhibitor anticancer drug as a reference. The compound was found to inhibit the *in vitro* activity of hDHFR) with IC_{50} values of 1.20 μM. A11 interacted with hDHFR as revealed by concentration dependent quenching of the tryptophan fluorescence of the enzyme suggesting its effect on structural alteration of the enzyme. Molecular docking of A11 on crystal structure of hDHFR revealed significant interaction with free energy of binding and Ki values of -10.86 kcal mol^{-1} and 11 nM, respectively. Subsequent in vitro studies at cellular level showed a relatively greater cytotoxic effect of A11 against human kidney (ACHN, IC_{50} 60 μM) and liver (HepG2, IC_{50} 70 μM) cancer cells than their respective normal cells (HEK-293, IC_{50} 350 μM and WRL-68, IC_{50} 520 μM). Scanning electron microscopy of A11 treated cells revealed the morphological feature of apoptosis, like cell rounding and surface detachment, membrane blebbing, loss of cilia and increased number of pores of decreased sizes. A11 mediated apoptosis of cancer cells was found to be correlated with induction of intracellular of reactive oxygen species (ROS) level and fragmentation of genomic DNA, which is a hallmark of apoptosis. A11 mediated induction of apoptotic feature of ACHN cells was found to be correlated with increased accumulation of cleaved active form of the pro-apoptotic proteins, like caspase 3, caspase 7, caspase 9 and PARP1. The cleaving of caspace 3 and caspase 7 was further confirmed by western blot analysis. The results thus provide an insight into the anti-tumorigenic potential of A11. The function of A11 in both inhibition of hDHFR and induction of apoptosis suggest that the compound could act via diverse signaling pathways of cancer cells without affecting normal cells. However, a possible link between hDHFR inhibition and cell cycle regulation needs to be illustrated in future studies. The outcomes of this research contribute to the growing field of natural product-based drug discovery and highlight the significance of traditional medicinal knowledge in the context of cancer treatment.