

Abstract

Cancer is one of the leading causes of mortality worldwide. About 44% of all cancer morbidity and 53% of all cancer mortality occur in countries with a low to medium Human Development Index (HDI). Thus, cancer is rapidly emerging as a serious threat to public health in Africa and most especially, sub-Saharan Africa. The International Agency for Research on Cancer (IARC) projects that there will be 1.28 million new cancer cases and 970 000 cancer deaths in Africa by the year 2030 owing to the increase in economic development associated lifestyles. The dominant types of cancer in Africa are those related to infectious diseases such as Kaposi's sarcoma and cervical, hepatic and urinary bladder carcinomas. The main challenge to cancer treatment in Africa is the unavailability of efficacious anticancer drugs. This is because most developing countries can only afford to procure the most basic anticancer drugs, which are also frequently unavailable due to intermittent supplies. This results in patients progressing to more advanced cancer states. One way of combating this African problem is to focus on research that aims at discovering efficacious and cost effective cancer therapies from available natural resources within the African continent. This study investigated the potential anti-proliferative activity (against HeLa cervical cancer cells) of four plants (*Adansonia digitata*, *Ceiba pentandra*, *Maytenus senegalensis* and *Drimia altissima*) commonly used in the African traditional treatment of malignancies. After in vitro bio-assay screening using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, *M. senegalensis* root extract (MS-R) and *D. altissima* bulb extract (DA-B) showed anti-proliferative activity against HeLa cervical cancer cells with IC₅₀ values of 25 µg/mL and 1.1 µg/mL respectively. By possessing the strongest anti-proliferative activity among the tested extracts, *D. altissima* was selected for further studies. Liquid-liquid partitioning of the *Drimia altissima* bulb extract with n-hexane, ethyl acetate, and n-butanol, yielded partitions 79a – d, with the n-butanol fraction, 79d, exhibiting the strongest cytotoxic activity (IC₅₀ = 0.497 µg/mL). Through High Content Analysis (HCA) screening, fraction 79d was found to induce marked early mitotic cell cycle arrest. Fractionation of 79d using Diaion® HP-20 open column chromatography and a stepwise gradient of reducing polarity (water-methanol-ethanol-ethyl acetate) yielded cytotoxic fractions 82b, 82c, 82d and 82e, all with significant anti-proliferative activities at the tested concentrations of 0.1, 1.0 and 10 µg/mL. Bio-assay guided fractionation of 82c (the most effective fraction at the lowest tested concentration of 0.1 µg/mL) using Sephadex® LH-20 open column chromatography and 50% MeOH led to the isolation of compound 3.17. After structural elucidation using 1D and 2D Nuclear Magnetic Resonance spectroscopy (NMR), High resolution Mass spectrometry (HRMS), Fourier-Transform Infrared spectroscopy (FTIR), ultraviolet spectroscopy (UV) and Circular Dichroism (CD), compound 3.17 was identified as a novel C-glucosylflavonoid-O-glucoside, 6-C-[apio- α -D-furanosyl-(1 $\rightarrow$ 6)- β glucopyranosyl]-4', 5, 7-trihydroxyflavone (Altissimin, 3.17). Compound 3.17 exhibited a dose dependant anti-proliferative activity with an IC₅₀ of 2.44 µM. The mechanism of action for compound 3.17 was investigated through cell cycle arrest, phosphatidylserine translocation (PS), caspase activation and mitochondrial membrane depolarization. The mechanism of cell death elicited by compound 3.17 in HeLa cells was found to involve the induction of M phase cell cycle arrest with consequent activation of apoptotic cell death which was evident from annexin V staining, mitochondrial membrane potential ($\Delta\Psi$ m) collapse and the activation of caspases -8 and -3. In silico computational techniques were employed to virtually determine potential biological targets of compound 3.17. Target fishing using the Similarity Ensemble Approach (SEA) target prediction gave human aldose reductase (hAR, AKR1B1) the highest ranking with a p value of 2.85×10^{-24} , a max Tc

of 0.35 and a Z-score of 41.8217. Using AutoDock4 and the AutoDock tools suite (ADT), molecular docking of compound 3.17 in the hAR binding pocket was successfully achieved with a lower ΔG free energy binding (-9.4 kcal/mol) than that of positive control ligand 393 (-8.7 kcal/mol). In conclusion, this study identified the genus *Drimia* and particularly *D. altissima* as a potential source for novel cytotoxic compounds. The discovery of altissimin (3.17), the first flavonoid glycoside to be isolated from *D. altissima*, enquires into the possible existence of similar compounds within the species. In addition to the observed in vitro cytotoxic activity against HeLa cells, the potential of altissimin (3.17) as a hAR enzyme inhibitor opens up the possibility of its use as an adjunct to increase cancer cell sensitivity to chemotherapy. Thus, altissimin (3.17) shows promise as a potential anticancer agent.