ABSTRACT

Cancer is a large group of diseases that arises from malfunction in biological cells and characterized by uncontrolled cell proliferation (abnormal cell cycle regulation), and resistance to cell death (apoptosis resistance) ultimately disrupting the organization of tissue. Despite significant investments in capital, manpower, and intellectual innovations for the development of cancer therapies over the past several decades, cancer still remains a powerful threat with high mortality rates across the globe. Tumor initiation begins with the accumulation of multi-gene mutations, which are the result of the interaction between genetic host factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism) and external agents, which may act together or in sequence to initiate or/and then promote carcinogenesis. Cancer treatment includes surgery, radiation, chemotherapy, hormone therapy, biological therapy, and targeted therapy, however each therapy has its own disadvantages like reoccurrence, adverse side effects, pain, killing of adjacent normal cells etc. Over the years, chemotherapy has emerged as a significant and promising anti-cancer treatment modality that reduces the mortality of cancer and generally exerts their activity through inhibiting tumor cell growth generally by induction of apoptosis of cancerous cells.

Despite significant advances in the treatment of breast cancer, this disease not only remains the second most frequent cause of cancer death but also one of
the most commonly diagnosed cancers among women. Similarly, prostate cancer is the most commonly diagnosed neoplasm and the second leading cause of male death. These grim medical statistics underscore the need for development of novel strategies to better manage and control breast and prostate cancers.

Apoptosis is defined as an active physiological process of cellular self-destruction, with characteristic morphological and biochemical changes. It reflects the operation of an intracellular death process that silently eliminates unwanted or aged or abnormal cells, without an inflammatory response. Based on the various stimuli that induce apoptosis and the initiator caspases involved, two major pathways have been reported; the death receptor pathway involving caspase-8 and the mitochondrial pathway, in which various signals can trigger the release of mitochondrial proteins into the cytoplasm, leading to activation of various caspases ultimately leading to cell death.

Natural products have been used in traditional and folk medicine for therapeutic purposes. They provide one of the most important sources of promising leads for the development of novel therapeutics. Active ingredients from natural sources, mostly plants such as Paclitaxel, Etoposide, Vinblastine, etc. are used for treating various types of cancer. It is thus considered important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them.
In light of these trends and developments, the present study focuses on the screening, identification and biological validation of anti-proliferative agents from the structures available in natural products. Dried materials of *Piper longum* fruits *Ficus racemosa* leaves and whole plant of *Cissus quadangularis* were screened for anti-proliferative property in MDA-MB-231, PC-3, and K562 cells. *Piper longum* was prioritized based on the initial anti-proliferative assays in MDA-MB-231 cells and PC-3 cells.

The genus *Piper*, belonging to the Piperaceae, has received considerable attention in recent years because of its chemico-biological properties. Various *Piper* species including *Piper longum*, widely distributed in the tropical and subtropical regions of the world, have been used as a spice and also as a folk medicine for various ailments. Understanding the mode of action of these compounds should provide useful information for their possible application in cancer therapy.

In this study, *Piper longum*, was evaluated for its anti-proliferative potential *in vitro* and after confirming its apoptotic induction in MDA-MB-231 breast adenocarcinoma and PC-3 prostate adenocarcinoma cells, the ethyl acetate extract of *Piper longum* was subjected to column fractionation and the pure compound showing anti proliferative activity was isolated and its structure elucidated. Both the *Piper longum* ethyl acetate extract and the pure compound (1-C 3’ 4’-Methylenldioxy phenyl dodec-1-en-10-one) induced apoptotic cell death in human breast cancer MDA-MB-231 cells and PC-3 cells in a time and dose-dependant manner. In MDA-MB-231 cells, 25 µg/ml of *Piper longum* ethyl acetate extract and MDPD (1-C 3’ 4’-Methylenldioxy
phenyl dodec-1-en-10-one) showed significant antiproliferative activity after 36 hours. In PC-3 cells, 25 µg/ml of *Piper longum* ethyl acetate extract and 15 µg/ml of MDPD induced 50% cell death at 24 hours.

Cell death induced by *Piper longum* in MDA-MB-231 and PC-3 cells was further confirmed by intranucleosomal cleavages and propidium iodide staining. A good correlation between loss of mitochondrial membrane potential followed by cytochrome c release and apoptotic cell death was apparent indicating the participation of mitochondria-related mechanism in MDA-MB-231 and PC-3 cells. Crude extract and active molecule from *Piper longum* in both the cell lines enhanced bax up-regulation and also induced the activation of initiator caspase-9, and executor caspase-3 that was accompanied by an apparent down-regulation of Bcl-2, suggesting the involvement of intrinsic apoptosis pathway. However in MDA-MB-231, cell regulation on G2/M arrest was shown from the propidium iodide staining and from protein levels of cell cycle proteins upon treatment with crude extract and active molecule from *Piper longum*. In prostate cancer cells, increase in the levels of Reactive Oxygen Species (ROS) leading to apoptosis was observed upon treatment with active molecule of *Piper longum*. 