Summary and Conclusion
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Despite the recent interest in molecular modeling, combinatorial chemistry, and other synthetic chemistry techniques by Pharmaceutical companies and funding organizations, Natural products, particularly medicinal plants, remain an important source of new drugs, new drug leads, and new chemical entities. It is evident that, natural products have played a vital role in drug discovery, by contributing to a wide variety of phytochemicals for the treatment of cancer, cardiovascular diseases, infections related with viral and microbial origin and other health disorders. A number of Indian traditional medicinal plants have been a source for a variety of new phytochemicals with diversified biological properties. This encouraged the researchers to investigate the traditional medicinal plants for their various biological properties.

Hence, in this work, an attempt has been made to study the in vitro and in vivo anticancer activity of different extracts of the fruits of Cucumis trigonus, a herb belonging to the family Cucurbitaceae. The experimental data demonstrated that the non-polar chloroform extract is highly promising in the management of malignant disorders. The active fractions (Fraction 7 & 8) form the chloroform extract were identified on the basis of the cytotoxic potential of different fractions fractionated from chloroform extract. The two compounds (compound I - Cucurbitacin A and compound II- Cucurbitacin B mono hydrate) were isolated from fraction 7 & 8 and assessed for nature of cell death using suitable molecular models. The results were further validated by ascites tumor animal models.

The results are summarized as follows;

The five extracts (Pet. Ether, chloroform, ethyl acetate, methanol and water) were selected for detailed phytochemical analysis in order to identify the compounds responsible for the activity. The phytochemical studies confirmed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids & sterols and triterpenoids in different extracts.

In vitro cytotoxicity studies were carried out with MTT and SRB methods because in vitro cytotoxicity methods are important tools to enhance the understanding of hazardous effects caused by chemicals or bioactive components, which avoids usage of animals (Broadhead and Combes, 2001). These tests provide useful and necessary information in defining basal
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cytotoxicity, which is commonly used as a starting point in an integral assessment of potential *in vivo* toxicity of chemicals or active components in foods. The endpoints frequently used in cytotoxicity testing are based on the breakdown of the cellular permeability barrier, reduced mitochondrial function, changes in cell morphology, and changes in cell replication (Eisenbrand et al, 2004).

The *in vitro* cytotoxicity was performed for extracts on seven cancer (HeLa, HEp-2, A-549, MCF-7, MDA-MB 231, HT-29 and C-6) and two normal (Vero and L-929) cell lines. Amongst the extracts tested, the significant cell growth-inhibitory potency was observed with the chloroform extract against A-549 cell line (0.60±0.76 µg/ml). The chloroform extract was fractionated by column chromatography and a total of 10 pooled fractions were obtained. The cytotoxicity was performed on these 10 fractions and fraction 7 & 8 showed CTC<sub>50</sub> at 0.073 µg/ml and 0.080 µg/ml respectively against A-549. The re-column chromatography of these two compounds produced two individual steroid triterpenes *viz.*, Cucurbitacin A and Cucurbitacin B mono hydrate. The structures of the compounds were elucidated by means of UV spectroscopy, FTIR and advanced NMR experiments, complete <sup>1</sup>H and <sup>13</sup>C, Mass spectroscopy analysis.

Antioxidant activity of extracts/compounds was tested in various *in vitro* models and the cytotoxicity of the extract/compounds identified above were found to have antioxidant activity. The DNA nicking assay also confirmed the same.

In advanced study, the compounds were further evaluated for their mechanism of cytotoxic activity. Morphological studies using microscopy, nuclear staining with dual stains *viz.*, Acridine orange – Ethidium bromide combination revealed that the extracts inhibited cell proliferation and induced cell death via apoptotic pathway. Morphological changes of apoptotic cell death were observed such as membrane blebbing, cell shrinkage, condensed nuclei and fragmented nuclear material.

The apoptotic cell death was further confirmed by nuclear fragmentation assay which showed typical laddering pattern of inter nucleosomal cleavage due to apoptotic cell death. The flow cytometric analysis of cells treated with compounds showed accumulation of cells in sub G<sub>0</sub> phase and G<sub>2</sub>/M phase on lung cancer cell line. The compounds may inhibit the production and inactivate the proteins regulating cell cycle which is responsible for the cell cycle arrest.