Scope, objective and plan of work
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3.1 Scope

Natural products have been playing a vital role in health care for decades. Of the different sources of natural products, plants have been a source of chemical substances, which serve as drugs in their own right or as key ingredients in formulations containing synthetic drugs. The process that leads from the plant to a pharmacologically active, pure constituent is very long and tedious and requires a multidisciplinary approach. The selection of the plant species is a crucial factor for the ultimate success of the investigation. Though random selection gives some hits, targeted collection based on chemotaxonomic relationships, ethanomedical information and information derived from traditional medicine are more likely to yield pharmacologically active compounds.

The current interest in natural products as a source of therapeutic compounds is certainly expected, considering the role that medicinal plant extracts/isolated compounds have played in the development of modern day medicine. Discovery of digotal, a drug for congestive heart failure from Digitalis purpurea (Riaz and Forker, 1998), morphine, a narcotic analgesic alkaloid from Papaver somniferum (Foye, 1989), quinine, an anti-malarial agent from cinchona bark (Drulihe et al., 1988) and other agents motivated scientists to isolate and characterize other biologically active natural products. The discovery of numerous active medicinal agents in the early 19th century encouraged the continued scientific investigation of ancient medicinal formulations into the 21st century. In addition, some natural products might be useful in diseases unrelated to the traditional use of the product. For example, Cantharanthus roseua was initially used as hypoglycemic agent in folk medicine, but is now known to contain useful antineoplastic compound i.e. vinca alkaloids (Williamson et al., 1996). Therefore, screening should not be limited to application of traditional use, but should include other possible activities.

Anticancer drugs discovered from herbal medicines have a long history and plant-derived compounds have been an important source of several useful anti-cancer agents in clinical practice, such as vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, which are isolated or derived from Catharanthus roseus G. Don. (Apocynaceae), Camptotheca acuminata Decne (Nyssaceae), Podophyllum peltatum Linnaeus (Podophyllaceae) and Taxus brevifolia Nutt. (Taxaceae) (Cragg and Newman,
In recent years, traditional medicine, such as Chinese medicine, Kampo medicine, Ayurveda and so on, are popular treatment for cancer in Asian countries, and these approaches are also accepted increasingly as complementary and alternative therapies for cancer in the rest of the world. A number of scientific evidences at molecular mechanisms and clinical trials have showed their anticancer potential. Most of the medications in traditional medicine are derived from plants, so it is called as herbal medicines.

Recent research continuously focuses on clues from traditional use of herbal medicines to develop new anticancer drugs in single pure compounds. On the other hand, standardized various extracts or fractions with anticancer effects or with adjuvant therapy in cancer treatment coming from single or mixed herbs are also accepted forms as dietary supplements and botanical drug products in the US for current statutory regulations (Fang, 2006).

Another aspect which has gained attention in the past two decades is the discovery that the cytotoxic activity of various compounds is through the interference with the mitotic spindle apparatus and microtubules have become an attractive pharmacological target for anticancer drug discovery.

The Mitosis is a highly regulated process during cell division. Anti-mitotic agents, such as the vinca alkaloids and the taxanes (Arrieta, 2006; Tanaka 2004), interfere with the polymerization-depolymerization process of microtubules and lead to cell cycle arrest in mitosis. However, these agents target microtubules and display several undesirable side effects in non-dividing cells, such as neurotoxicity. Thus, it is necessary to develop anti-mitotic molecules that do not act on microtubules directly.

Based on the above statements, it has been noted that there is a greater probability of finding compounds with different biological activities form plants with reputed medicinal properties, rather than form plants collected at random. It gives an idea about the importance of natural products, especially plants in modern day drug discovery and that initiated the screening of fruits of Cucumis trigonus against its anticancer and anti-mitotic activity.
3.2 Objectives

- The main objective of the present study is to isolate the potent anticancer compound from the fruits of *Cucumis trigonus* belonging to genus Cucumis.

- Based on literature survey and their use in traditional system of medicine followed by testing the fruit extracts prepared by extraction methods- aqueous/organic solvent and test these for their anti-cancer properties by *in vitro* and *in vivo* system.

- To identify the most active and promising extract, isolate the bioactive fraction followed by compound and evaluate its *in vitro* toxicity profile by selectivity index.

- To identify the activity of the promising extract/compound by subjecting it to the preliminary *in vitro* antioxidant and survival assay.

- To assess nature of cell death induced by active extract/compounds using suitable models *viz.* nuclear staining, transcriptional gene regulation, flow cytometric analysis etc.

- To evaluate mitotic arrest accompanied by monopolar spindle through the inhibition of Kinesin Spindle Protein.

- To determine the toxic potential and safety of active compound using acute toxicity studies in mice.

- To find out if the potent extract/compounds are effective anticancer agent *in vivo* by using DLA ascites tumor model.
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3.3 Plan of Work

To achieve this, work was planned as mentioned below which includes

I. **Collection and authentication of Plant material**
   a) Identification and Collection of plant material
   b) Authentication of material and preparation of voucher specimen

II. **Preparation of plant extracts and Bio guided fractionation**
    a) Drying of plant material and preparation of powder
    b) Preparation of crude extracts by soxhlet extraction and drying
    c) Fractionation by Column Chromatography method
    d) Isolation and characterization of compound

III. **Cytotoxicity Studies**
    a) Determination of mitochondrial synthesis by Micro culture tetrazolium (MTT) assay.
    b) Determination of total cell protein content by Sulphordamine B (SRB)

IV. **Antioxidant Studies**
    a) DPPH assay.
    b) ABTS assay
    c) Nicking assay

V. **Clonogenic Assay.**

VI. **Advanced Studies:**
    Evaluate compounds for their nature of cell death
    a) Nuclear staining studies
    b) DNA fragmentation assay
    c) ATP hydrolysis assay
    d) Immunofluorescence Assay
    e) Gene expression assay
    f) Cell cycle Analysis
VII. *In vivo anticancer studies:*

a) Acute toxicity  
b) Short term studies  
c) Ascites tumor model