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CYTOTOXIC ACTIVITY OF ANNONA MURICATA L. LEAF EXTRACT

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ABSTRACT
Cancers are abnormal uncontrolled cell growth with the potential to invade or spread to other parts of the body. Conventional treatment of cancer includes chemotherapy, radiotherapy and surgery. All these treatments induce severe side effects. To reduce the side effects caused by these treatments, the use of alternative medicine which includes herbal drugs in the treatment of various diseases has expanded rapidly in both developed and developing countries. Herbal medicine is one of the most commonly used complementary and alternative therapies (CAM) by people with cancer. In the present work a plant selected for screening of anticancer properties which has been using long back for curing different ailments is Annona muricata belongs to the family Annonaceae. The leaves of the plant are tested for its cytotoxicity on L929 cell lines. The ethanol extract of leaf showed cytotoxic effect against L929 cells and further screening on different cell lines is in progress.

KEYWORDS: 1. Annona muricata, 2. Cell culture, 3. MTT assay, 4. Adjuvant.

INTRODUCTION
Cancer drug discovery and development have been the major research endeavor around the globe. Several studies have shown that plant-derived compounds scavenge free radicals that modulate oxidative stress-related effects. Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects of anticancer drugs, cancer can be a cause of death. Therefore, efforts are still being made to search for effective naturally occurring anticarcinogens that would lessen or prevent the cancer development. Treatment of cancer includes surgery, chemotherapy and radiotherapy and all these treatments significantly induce severe side effects. There is an urgent need for the development of novel anti-tumor agents that are cheaper, stable, can selectively target cancer dependent pathways without affecting normal cells. Medicinal plants have a special place in the management of cancer. It is estimated that plant-derived compounds in one or the other way constitute more than 50% of anticancer agents.[1,2] Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents. Taking into consideration the above facts, an attempt has been made to evaluate the anticancer activity of the selected plant extract in selected cell lines through a systematic study by invitro method. Indeed, the struggle to combat cancer and increase in longevity of the cancer patients is one of the greatest challenges of mankind.[3] Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects.[4] The isolation of the vinca alkaloids, vinblastine and vincristine from Catharanthus roseus introduced a new era in the use of plant material as anticancer agents.[5] Over 50% of the drugs in clinical trials for anticancer properties were isolated from natural sources.[6] Several natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anticancer agents derived from plants are podophyllotoxin, taxol, vincristine, vinblastin and camptothecin.[7] Plants had been screened for its phytocompounds which are therapeutically effective. Among the different plants studied, Annona muricata L. is one which has gained much importance in recent years as therapeutically active. Traditionally the plant is used to cure different ailments from different parts of the tree. Literature revealed the Cytotoxicity and apoptosis inducing activities on T47D breast cancers.[8] Based on the traditional literature and methods of treatment, the plant is selected for evaluation of cytotoxic activity.

Annona muricata L *(A. muricata*) commonly known as graviola or Lakshman phal belongs to the Annonaceae family. It is a typical tropical tree with heart shaped edible fruits and widely distributed in most of the tropical countries. All parts of *A. muricata* tree are used in natural medicine including the twigs, leaf, root, fruit

and seeds. Previous scientific reports have demonstrated that the leaf, bark, root, stem, fruit and seed extracts of Annona muricata showed anti-bacterial activity.[9, 10] Its leaves extract were also found to possess antioxidant[11] and recently, it has also been reported to exhibit antiinflammatory and analgesic effects.[12] A. muricata leaves and fruits have been subjected to numerous numbers of investigations on human diseases, including different types of cancers.[13] As a part this study, the leaves of the plant is screened for cytotoxic effect on selected cancer cell line (Lung).

MATEIRALS AND METHODS

Sample preparation

Fresh leaves obtained are shade dried up to two weeks and the dried leaves were powdered. About 50g of leaf powder was extracted using Soxhlet apparatus up to 48hrs. The filtered ethanol extract was evaporated to dryness under reduced pressure at 40°C and a stock solution was then prepared by dissolving the extract powder in distilled water and the experimental concentrations were diluted in basal medium.

Cell line used is L929. ATCC- L-929 is an established and well characterized mammalian cell line that has demonstrated reproducible results.

MTT assay

The cell viability was measured by using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). Test sample was sterilized by steam at 121ºC for 20 minutes. Extract was prepared by incubating 15mg of test material AMLO1SR in 1 ml sterile distilled water at 37±1ºC for 24± 2h.

Negative control: Poly Ethylene
Preparation of negative control: Negative control was prepared by incubating 1.25 cm2 High.
Density Poly Ethylene with 1ml culture medium containing serum at 37 ±1ºC for 24± 2h.

Positive control: Dilute phenol.
Preparation of positive control: Positive control was prepared by diluting phenol stock solution (13mg/ml) to 1.3 mg/ml with culture medium containing serum.

Procedure

The MTT assay was performed to measure the metabolic activity of cells to reduce yellow colored tetrazolium salt 3-(4, 5-Dimethyl thiazol -2-yl)-2, 5- diphenyltetrazolium bromide to purple colored formazan. Extract was prepared by incubating 15mg of test material (AMLO1SR) in 1 ml sterile distilled water at 37±1ºC for 24± 2h. After extraction the pH changed to acidic and was adjusted to 7.2 to 7.4 with 7.5% Sodium bicarbonate. The extract was mixed with MEM2X (1 part of extract and 1 part of MEM 2X) medium to get 7.5mg/ml extract. This was further diluted with culture medium to 7.5mg/ml, 3.75mg/ml, 1.87mg/ml and 0.93mg/ml extract. Cells cultured in normal medium were considered as cell control. Equal volume (100µl) of various dilution of test samples, extract of negative control, cell control and positive control were placed on sub confluent monolayer of L-929 cells.

After incubation of cells with various concentration of test sample and controls at 37 ±1□C for 24 ± 2 h, extract and control medium was replaced with 50µl MTT solution (1mg/ml in medium without supplements), wrapped with aluminium foil and were incubated at 37± 2°C for 2 h. After discarding the MTT solution 100 µl of Isopropanol was added to all wells and swayed the plates. The color developed was quantified by measuring absorbance at 570 nm using a spectrophotometer. The data obtained for test sample were compared with cell control.

RESULTS AND DISCUSSION

The MTT assay of L929 cells after contact with 7.5mg/ml, 3.75mg/ml, 1.87mg/ml and 0.93mg/ml extract of the selected plant showed 17.77%, 18.69%, 40.30% and 69.04% metabolic activity respectively. Positive control showed 14.55% and negative control showed 94.72% metabolic activity. The extract tested inhibited the proliferation of L929 cells in a concentration dependent manner.

Extracts of this medicinal plant are believed to contain a wide array of polyphenolic compounds which might possess wide therapeutic properties.[14] Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world.[15] [16] The present evaluation is to determine whether the extracts of these plants exerted an inhibitory effect on cancer cell proliferation and caused cell death. The results of our studies suggest that ethanol extract of A. muricata possess the strongest cytotoxic effects on both human cancer cells. In the present study, we observed that the extract of A. muricata caused marked cell growth inhibition in the L929 cell line in a dose dependent manner. This study provides an important basis for further investigation into the isolation, characterization and mechanism of cytotoxic compounds from the Annona muricata plantL. The present findings justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent against cancer.

In addition, further studies are required to elucidate the precise molecular mechanisms and targets for cell growth inhibition which will allow the rationale design for more effective molecules for the eventual use as cancer chemo preventive and/or as an adjuvant to chemotherapy.

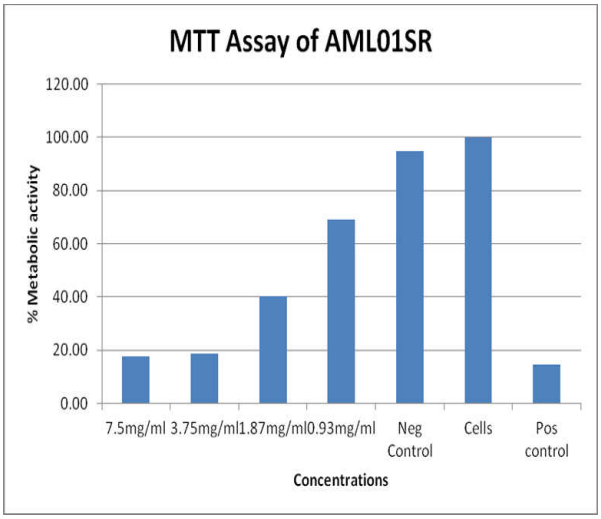
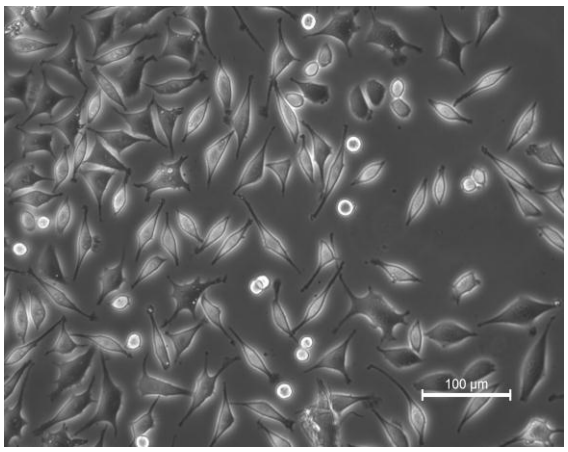
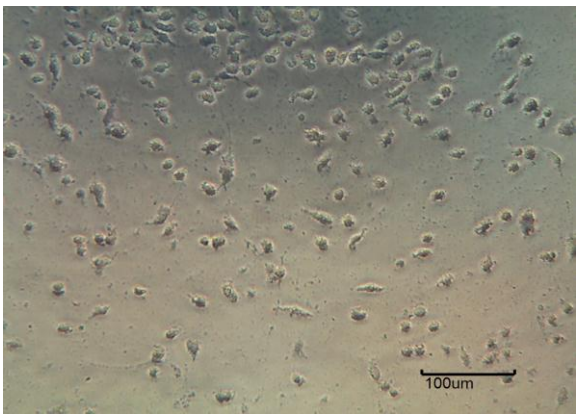


Fig. 1. Ethanol leaf extract of Annona muricata L. showing the metabolic activity on different concentrations.

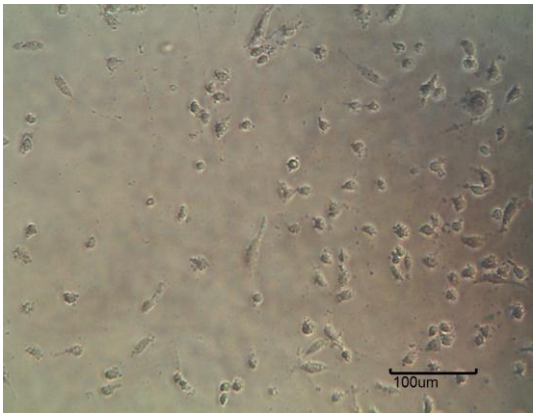


a. Normal cells

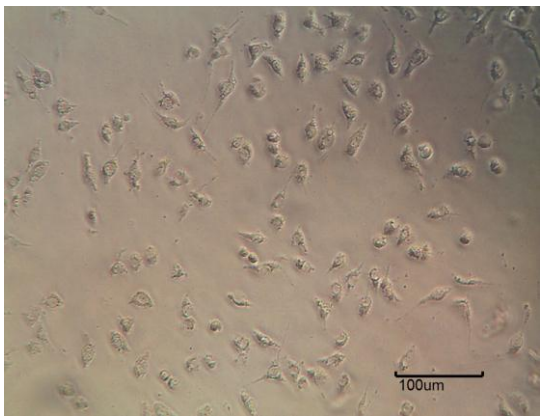


b. 1.87mg/ml dose conc. Showed 40.30% metabolic activity

Fig. 2. Effect of ethanol leaf extract of Annona muricata L. on the selected cell lines



c. 3.75mg/ml dose conc. Showed 18.69% metabolic activity.



d.7.5mg/ml conc. Showed 17.77% metabolic activity.

Conclusion and Scope

Large numbers of herbal species have been used traditionally or as folk medicines against cancer. Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. In medicine, particularly in the field of cancer, the use of herbs is increasingly enhanced especially with the excessive use of synthetic drugs and awareness of their toxicity, which contributed in oncology, leading to a favorable reconsideration of the medicinal practices made from natural herbal. A better understanding of the characteristics of tumor cells has recently led to the development of more targeted treatments, and therefore generally less toxic. In conclusion, the use of naturally occurring herbal medicines and their isolated biomolecules in the treatment of cancer and other disease has greatly contributed to the improvement of the therapeutic efficacy of drugs. Further screening of the leaf and fruit extract on different available cell lines is in progress.

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