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Research Article

EVALUATION OF IN-VITRO ANTI-CANCER ACTIVITY OF FRUIT *LAGENARIA SICERARIA* AGAINST MCF7, HOP62 AND DU145 CELL LINES

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Abstract

To evaluate the in vitro anti cancer activity of *Lagenaria siceraria* fruit of petroleum, chloroform and methanol extracts against human cancer cell lines viz. human breast cells (MCF7), lung cancer cells (Hop62) and prostate cancer cells (DU145) are used. In vitro anti cancer tests was performed against the human cancer cells, cultured for 48h in presence of different concentrations *Lagenaria siceraria* fruit extracts and percentage of cell viability, was evaluated using the sulforhodamine-B—(SRB), Adriamycin was used as a reference standard. The 50 % Growth of Inhibition (GI50), concentration resulting in total growth inhibition (TGI), concentration of drug causing lethality to 50 % of the cells (LC50) was calculated. In the literature survey the present plant Lagenaria siceraria is describe as anti hyperlipidemic, Analgesic, Antidibetic and Antiinflammatory, Anthelmintic. Researchers able to report few compounds from the plants and done pharmacological study on the fruit of Lagenaria siceraria reach to the final conclusion of their uses. Like other members of the cucurbitaceae family, it contain Cucurbitacins that are known to be cytotoxic. A toxin called tetracyclic triterpenoid cucurbitacins compound, present in fruits and vegetables of the cucurbitaceae family is responsible for the bitter taste and can cause ulcers in the stomach.

Key words: anticancer activity, *Lagenaria siceraria*, Adriamycin, CytoScan – SRB Assay.

Introduction

Cancer

The word cancer means excessive proliferation of cells, also termed as neoplasm, tumor. Neoplasm means the new plasm i.e. the neuclic core is un-identical means change in base pairing and they

goes for mitosis and rate is very fast. Tumor means the neoplasm or cancerous cell when accumulate form a definite structure called as Tumor.

Cancer is still a dangerous disease, which accounts for 9% of the deaths throughout the world. Cancer can occur in any organ of the body and the cancer cell will tend to resemble their normal tissue of origin. Cancers of various organs are quite different from one another and even within the organs. [2]

Modern cancer chemotherapy originated in the 1940s with the demonstration that nitrogen mustard possessed antitumor activity against human lymphomas and leukemia's. Approximately 10 types of human cancer have 40 to 80% "cure" rates using chemotherapy alone or chemotherapy plus surgery or radiation .For this purpose cure is defined as the disappearance of any evidence of tumor for several years and a high actuarial probability of a normal life span. Patients with other types of unrespectable cancer also may benefit from chemotherapy, as evidenced by prolongation of life, shrinkage of tumor, and improvement in symptoms. Notable among these are ovarian epithelial and breast carcinomas, oat cell (small cell undifferentiated) carcinoma of the lung, and acute myelocytic leukemia. Cancers that are for the most part resistant to today's agents include melanoma, colorectal and renal carcinomas, and non–oat cell cancers of the lung. [3]

Concepts in tumor cell biology

The normal cell cycle

The normal cell cycle consists of a definable sequence of events that characterize the growth and division of cells and can be observed by morphological and biochemical means. Two of the four phases of the cell cycle can be studied directly: the M-phase, or mitosis, is easily visible using light microscopy because of chromosomal condensation, spindle formation, and cell division. The S-phase is the period of DNA synthesis and is observed by measuring the incorporation of tritiated thymidine into cell nuclei.

The mitotic index is the fraction or percentage of cells in mitosis within a given cell population. The thymidine labeling index is the fraction of cells incorporating radioactive thymidine. They represent cells in M-phase and S-phase and define the proliferative characteristics of normal and tumor cells. [4]

The Tumor Cell Cycle

The duration of the S-phase in human tumors is 10 to 20 hours.This period is followed by the G2-phase, or period of preparation for mitosis, in which cells contain a tetraploid number of chromosomes. The G2-phase lasts

only 1 to 3 hours for most cell types, with mitosis itself lasting approximately 1 hour. The two daughter cells then enter the G1-phase, whose duration varies from several hours to days.The G1-phase also can give rise to a resting state, termed G0, in which cells are relatively inactive metabolically and are resistant to most chemotherapeutic drugs.

The generation time, or Tc, is the time required to complete one cycle of cell growth and division. The Tc will vary with the duration of the G1-phase. The factors that influence daughter cells to enter the G0, or resting stage, are not well understood. The ability to cause such resting cells to reenter the cell cycle would be quite useful, since proliferating cells generally are more sensitive to chemotherapy than are resting cells. [5]

Drugs and the Cell Cycle

Various classification schemes have been proposed to describe the effects of drugs on the cell cycle. One such classification divides the anticancer drugs into three categories:

1. Class 1 agents (e.g., radiation, mechlorethamine, and carmustine) exert their cytotoxicity in a nonspecific (i.e., non–proliferation dependent) manner. They kill both normal and malignant cells to the same extent.
2. Class 2 agents are phase specific and reach a plateau in cell kill with increasing dosages. Only a certain proportion of cells are sensitive to the toxic effects of these drugs. For example, hydroxyurea and cytarabine kill only cells in the S-phase. Similarly, bleomycin is most toxic to cells in G2- and early M-phases. Because they affect only a small fraction of the cell population at any one time, it has been suggested that these drugs should be given either by continuous infusion or in frequent small doses. Such a dosage regimen would increase the number of tumor cells exposed to the drug during the sensitive phase of their cell cycle.
3. Class 3 agents kill proliferating cells in preference to resting cells. It has been recommended that these proliferation-dependent but non–phase-specific agents be administered in single large doses to take advantage of their sparing effect on normal cells that may be in G0.

Unfortunately, many human cancers have a large proportion of cells in the resting phase, and these cells are also resistant to the class 3 agents, which include cyclophosphamide, dactinomycin, and fluorouracil.

Tumor Growth and Growth Fraction: The rate of growth of human and experimental cancers is initially quite rapid (exponential) and then slows until a plateau is reached. The decrease in growth rate with

increasing tumor size is related both to a decrease in the proportion of cancer cells actively proliferating (termed the growth fraction) and to an increase in the rate of cell loss due to hypoxic necrosis, poor nutrient supply, immunological defense mechanisms, and other processes. The rate of spontaneous cell death for some human tumors is thought to be a significant factor in limiting growth. However, the growth fraction, or percentage of cells in the cell cycle, is the most important determinant of overall tumor enlargement. The doubling times of human tumors have been estimated by direct measurement of chest radiographs of lesions or palpable masses to be 1 to 6 months.

The growth fraction indicates dividing cells that are potentially sensitive to chemotherapy; thus, it is not surprising that tumors with high growth fractions are the ones most easily curable by drugs. Among human tumors, only Burkitt's lymphoma and trophoblastic choriocarcinoma are readily curable by single-agent chemotherapy; both of these tumors have growth fractions close to 100%.

As tumors grow larger, the growth fraction within the tumor decreases, and the greater the distance of cells from nutrient blood vessels, the more likely they are to be in the G0-, or resting, phase. The growth fraction is less than 10% for slow-growing cancers of the colon or lung.

A number of factors must be considered before chemotherapy is instituted for a human cancer that has a low growth fraction. For instance, the larger the tumor, the more cells will be present in the nonproliferating, relatively resistant state. Therefore, the earlier chemotherapy is instituted, the greater the chance of a favorable response. Debulking of tumors by surgery or radiation therapy may be a means of stimulating the remaining cells into active proliferation. Small metastases may respond to drugs more dramatically than will large primary tumors or a larger metastasis in the same patient. Several cycles of treatment may be necessary to achieve a substantial reduction in tumor size. The chemotherapeutic regimen, especially when one is dealing with large, solid tumors, probably should includeagents that have cytotoxic activity against resting cells. [7]

Cancer Therapy and the Immune System

Although manipulation of the host immune response in animal tumor models has at times yielded impressive therapeutic results, attempts to extend these results to human cancers generally have been

disappointing. Several proteins that stimulate subsets of lymphocytes involved in various aspects of the immune response are now produced by recombinant DNA techniques.

The pharmacology of these "lymphokines" as potential anticancer agents is being investigated. Interleukin (IL) 2, originally described as a T-cell growth factor, induces the production of cytotoxic lymphocytes (lymphokine-activated killer cells, or LAK cells). IL-2 produces remissions in 10 to 20% of patients with melanoma or renal cell carcinoma when infused at high doses both alone or with lymphocytes that were previously harvested from the patient and incubated with IL-2 in vitro. The ability of certain anticancer agents to suppress both humoral and cellular immunity has been exploited in the field of organ transplantation and in diseases thought to be caused by an abnormal or heightened immune response. In particular, the alkylating agents cyclophosphamide and chlorambucil have been used in this context, as have several of the antimetabolites, including methotrexate, mercaptopurine, azathioprine, and thioguanine. Daily treatment with these agents rather than high-dose intermittent therapy is the preferred schedule for immune suppression. [9]

Why the herbals ?

By studying all the aspects of cancer and its recent therapy, it is very essential to screen the new molecule or entity possessing the anticancer activity, with less toxicity. Most of the plants possessing number of medicinal properties and today number natural products are in market with very good results, hence myself trying to find out the plant gives the anticancer activity.

Anticancer plants used in the India [11]

Acronychia barberi	Mappia foeida
Allanthus malabarica	Cissamepos pareira
Maytenus serrata	Plumbago zeylanica
Podophyllum emodi	Podophyllum peltatum
Solanum dulcamarra	Solanum triobatum
Tylophora asthmatica	Xanthium strumarium

Development of Anticancer Drugs from Plants

There are several drugs at present available for the treatment of cancer and out of that 25 to 30% are from plant origin. These compounds have a variety of novel structure and mechanism of action. [12]

Some of the important anticancer compounds [13]

Active principle	Plants
Baccharin	Baccharin megapotamics
Bruceantin	Brucea antidycentrica
3-Deoxycolchicine	Colchicum speclosum
Taxol	Taxus brevifolia
Triptolide, Tripdiolide	Tripterygium wildfordii
Vincristine,Vinblastine	Vinca rosea

Materials and methods

Materials used:

SR. NO.	MATERIALS USED	MFG. BY
1	Petroleum Ether	RLCC
2	Chloroform	RLCC
3	Methanol	RLCC

Methods:

Pharmacognostic investigation:

Collection, authentication, processing and storage were done according to standard procedure for the plant material.

Procurement of drug:

Lagenaria siceraria Stand (cucurbitaceae) has taken for dissertation work and on the basis of literature survey of plant had selected. Part of plant Lagenaria siceraria was collected from the region of farms of Kalwan (Nashik).

Authentication of drug:

It was authenticated from Botanical survey of India, Koregaon road, pune. The voucher specimen no KAK179 (reference no. BS/WRC/Tech./2011 dated 28.1.2011)

Drying and Size Reduction:

It was cleaned, cut into pieces and dried at room temperature in shade and away from sunlight and reduced to coarse powder using mechanical grinder.

Macroscopic evaluation:

Features and organoleptic characters of dried fruit powder of Lagenaria siceraria were studied.

Phytochemical investigation:

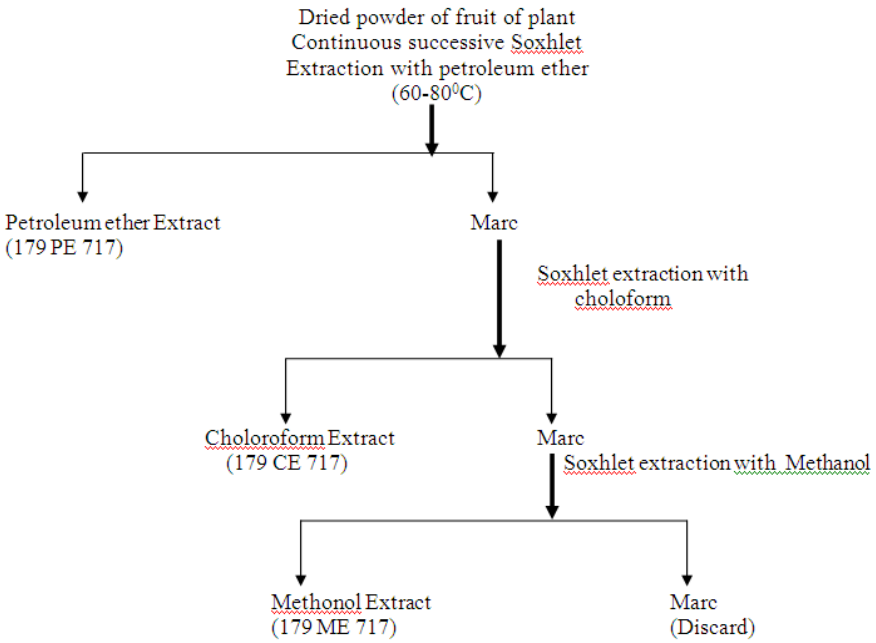
Extraction of crude drug:

The crude drug contains the different types of chemical constituents, which can be isolated by using various methods of extraction. The extraction of powdered fruit material was done by Soxhlet apparatus.

Successive Extraction By Using Soxhlet Apparatus

In successive solvent extraction, a dried material is extracted with different solvents, starting from solvent of low polarity. After extraction by one solvent, material is removed from thimble, dried and re-extracted with solvent of successively high polarity (Figure 01).

Figure-1: Flow Chart for Extraction Methodology.



Successive extraction was done by using following solvents

1. Petroleum ether(60-80^{0}C)
2. Chloroform
3. Methanol

250 gm of powdered of fruit of Lagenaria Siceraria was extracted with pet ether in several batches by using Soxhlet apparatus. For extraction, the powder was packed in thimble containing filter paper and extracted with pet ether in Soxhlet apparatus for the period till the active constituents were extracted after complete extraction, the extract was filtered and solvent was distilled off in a distillator. The extract was concentrated to dry residue, in a desiccators over anhydrous sodium sulphate. The percentage yield of the extract was calculated with reference to air dried powder. In the same manner chloroform and methanol extracts were prepared and the percentage yield of the extract was calculated with reference to air dried powder. All part of the total extract was used for pharmacological screening.

CytoScan – SRB Cell Cytotoxicity Assay

Introduction: The sulforhodamine B (SRB) assay is used for cell density determination, based on the measurement of cellular protein content. The method described here has been optimized for the toxicity screening of compounds to adherent cells in a 96-well format. After an incubation period, cell monolayers are fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye is removed by washing repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye is dissolved in 10mM Tris base solution for OD determination at 510 nm using a microplate reader. The results are liner over a 20-fold ranger of cell numbers and the sensitivity is comparable to those of fluorometric methods. The method not only allows a large number of samples to be tested within a few days, nut also requires only simple equipment and inexpensive reagents. The SRB assay is therefore an efficient and highly cost-effective method for screening.

CytoScan – SRB Cell cytotoxicity Assay is an accurate and reproducible assay based upon the quantitative staining of cellular proteins by sulforhodamine B (SRB). This assay has been used for high-throughput drug screening at e National Cancer Institute (NCL) (1). Sulforhodamine B is an anionic aminoxanthene sye that forms an electrostatic complex with the basic amino acid residues of protein under moderately acid conditions, which

provides a sensitive linear response. The color development acid conditions, which provides a sensitive linear response. The color development is raid and stable and is readily measured at absorbances between 560 and 580nm. The kit components are sufficient for performing up to 1000 assays.

KIT COMPOUNDS cat 786-213

SRB Dye (Sulforhodamine B) 0.4gm
Fixative Reagent 60ml
Dye Wash Solution 10X 100ml
SRB Solubilization Buffer 200ml

Storage condition:

Store all kit components at room temperature, protected from light. The kit components are stable for one year when stored as recommended.

Items needed but not supplied with the kit:

- ✓ Microplate reader capable of readout between 550 and 580nm
- ✓ 96 well tissue culture plates

Preparation before use:

1. Dilute the Dye Wash Solution by adding 1 part 10X Dye Wash Solution to 9 parts distilled water. You require ~0.8ml per well to sufficiently wash the wells.
2. In a clean glass container, add 100ml 1X Dye Wash Solution. Remove 1ml 1X Dye Wash Solution and add to the SRB Dye vial, pipette up and down to resuspend and transfer the entire contents to the 100ml 1X Dye Wash Solution. Stir to mix. The SRB Dye Solution can be stored at room temperature protected from light. Crystals may form during storage, remove by filtering with a syringe filter prior to use in the assay.

Protocol

The optimal conditions for monitoring cytotoxicity are to have the cells in the log phase of growth and not to exceed 106cells/cm2. We recommend that each test is performed in a final volume of 200µl and includes a 200µl control sample of cell free medium to be used to blank absorbance readings.

Dilution of the Extract Use for Testing

Each drug i.e. extract is tested at 4 dose levels $1x10^{-7}$M, $1x10^{-6}$M, $1x10^{-5}$M, $1x10^{-4}$M, or 10,20,30,40 microgram/ml.

Appropriate positive control is run in each experiment: Extraction of fruit powder was done with solvents Petroleum Ether, chloroform, methanol, and the extracts were coded as Petroleum Ether extract- 179PE717, Chloroform extract- 179CE717, Methanol extract- 179ME717.

These extracts submitted to TATA MEMORIAL CENTER for screening the activity with cell line MCF-7 BREAST CANCER, HOP-62 LUNG CANCER, DU145 PROSTATE CANCER.

Result and Discussion

Currently chemotherapy is regarded as one of the most efficient cancer treatment aproach. Although chemotherapy significantly improves symptoms and the quality of life of patients with lung cancer, only modest increase in survival rate can be achieved. Faced with palliative care, many cancer patients use alternative medicines, including herbal therapies. (1)

Phytochemicals are secondary metabolites produced by plants. These secondary metabolites are not essential for plant growth, however they possess certain protective value, enhancing the survival of the plant, and they may also possess medicinal properties. (3)

More extensive phytochemical and pharmacological studies further identified steroid saponins as the main antitumor active components. (4)

Lagenaria siceraria, have shown the presence of phytochemicals alkaloids (Draggendorff's), flavonoids (Shibata's reaction), saponins(Frothing test), tannins(10% ferricchloride),terpenoids (2,4-dinitrophenylhydrazine), glycosides (fehling's solution), steroids (Liebermann's Burchard test).(5)

The immunosuppressant activity of *Lagenaria siceraria*, an important plant in indigenous medicinal practice was also explored. Administration of *Lagenaria siceraria* was found to decrease total WBC count and spleen leukocyte count significantly indicating that the extract could suppress the non-specific immune system.(6)

Lagenin, a novel ribosome inactivating protein (RIP) obtained from dried seeds of bottle ground and some other Curcubitaceous plants possess antiproliferative, antitumour, immunosuppressive, antifertility, antiviral and anti- HIV activities. (7)

The identification of a novel therapy that is effective against recurrent tumor could substantially impact the morbidity and median survival of patients with this disease. . (2) The purpose of this study was to investigate

the anticancer effects of plant secondary metabolites from *Lagenaria siceraria* against human breast cancer cell line MCF7, human prostate cancer cell line DU145 and human lung cancer cell lineHop62.

Petroleum ether, methanol and chloroform extract of *Lagenaria siceraria*, were evaluated in the concentration of 10^{-7} M, 10^{-6} M, 10^{-5}M and 10^{-4}M .

On human breast cancer cell line MCF7, drug 179PE717 shown % control growth in concentration dependent manner i.e. 10^{-7} M has shown 100, 10^{-6} M shown 94, 10^{-5}M shown 87.5, and 10^{-4}M shown 75.7. Drug 179ME717 also shown % control growth in concentration dependent manner i.e. 10^{-7} M has shown 100, 10^{-6} M shown 98.8, 10^{-5}M shown 92, and 10^{-4}M shown 87.8. Drug 179CE717 also shown % control growth in concentration dependent manner i.e. 10^{-7} M has shown 99.2, 10^{-6} M shown 97.5, 10^{-5}M shown 88.1 and 10^{-4}M shown 84.6. But all these extracts shown LC50, TGI, G150 values are more than 100 as shown in result. But LC50, TGI, G150 values of standard drug Adramycin are 52.2, 19.5 and < 0.1 respectively which is considered to be active (Table-01, 04)

Table-1: Results of SRB Assay against MCF7 cell line.

	Human Breast Cancer Cell Line MCF7 % Control Growth					
	Molar Drug Concentrations					
	Conc.	**179PE717**	**179ME717**	**179CE717**	**PAR05**	**ADR**
Experiment 1	**10^{-7} M**	100.0	100.0	98.5	99.9	-11.7
	10^{-6} M	100.0	98.4	97.8	91.7	-36.5
	10^{-5} M	98.5	94.5	95.0	90.0	-55.8
	10^{-4} M	85.0	89.9	92.4	87.4	-56.6
Experiment 2	**10^{-7} M**	100.0	100.0	100.0	100.0	-16.5
	10^{-6} M	91.0	100.0	100.0	100.0	-41.0
	10^{-5} M	85.7	94.8	88.5	97.6	-69.6
	10^{-4} M	69.4	83.5	81.6	92.4	-72.6
Experiment 3	**10^{-7} M**	100.0	100.0	98.9	100.0	-24.7
	10^{-6} M	91.1	97.9	94.6	100.0	-46.9
	10^{-5} M	78.3	86.7	80.6	90.9	-65.6
	10^{-4} M	72.7	90.0	79.9	84.9	-66.3
Experiment 4	**10^{-7} M**	100.0	100.0	99.2	100.0	-17.6
	10^{-6} M	94.0	98.8	97.5	97.2	-41.5
	10^{-5} M	87.5	92.0	88.1	92.9	-63.7
	10^{-4} M	75.7	87.8	84.6	88.2	-65.1

MCF7	LC50	TGI	GI50*
179PE717	>100	>100	>100
179ME717	>100	>100	>100
179CE717	>100	>100	>100
PAR05	>100	>100	>100
ADR	52.2	19.5	<0.1

*GI50 ≤ 1 µMolar is considered to be active

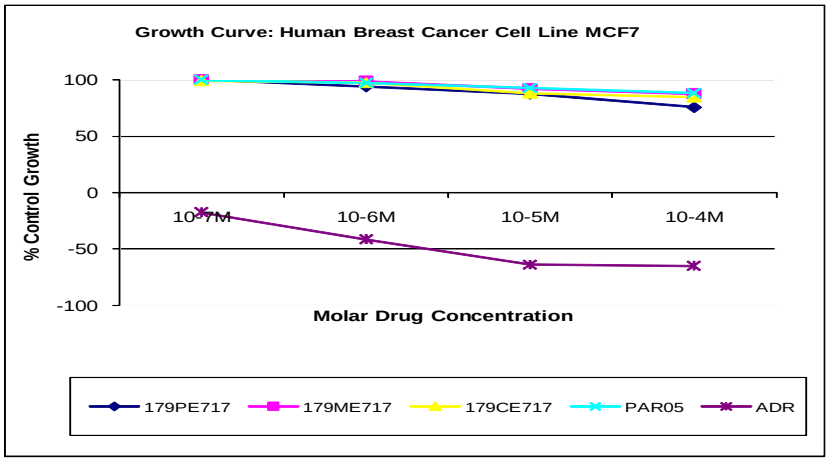


Table 4: % Control growth of various extracts of Lagenaria siceraria fruit on human breast cancer cell line MCF7.

	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
L.s. Petroleum extract	100±0	84.03±2.985	89.27±5.89	75.70±4.747
L.s. Methanol extract	100±0	98.77±0.63	92.00±2.65	87.80±2.150
L.s. Chloroform extract	99.13±0.4485	97.47±1.568	88.03±4.163	84.63±3.914

Values are mean ± SEM, number of experiment in each group=3

L.s - Lagenaria siceraria

LC50 is concentration of drug causing 50% cell kill, G150 is concentration of drug causing 50% inhibition of cell growth, and TGI is concentration of drug causing total inhibition of cell growth. G150 value < 1 µmolar or < 10 µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, G150 value < 20 µg/ml is considered to demonstrate activity.

On human prostate cancer cell line DU145, drug 179PE717 and 179ME717 shown % control growth 100 in all the concentrations used. Drug 179CE717 shown % control growth as 10^{-7} M has shown 99.3, 10^{-6} M shown 98.6, 10^{-5}M shown 100 and 10^{-4}M shown 99.9. But all these extracts shown LC50, TGI, G150 values are more

than 100 as shown in result. But LC50, TGI, G150 values of standard drug Adramycin are >100, 61.2 and < 0.1 respectively which is considered to be active (Table-02, 05)

Table-2: Results of SRB Assay against DU145 cell line.

	Human LUNG Cancer Cell Line Hop62 % Control Growth					
	Molar Drug Concentrations					
	Conc.	**179PE717**	**179ME717**	**179CE717**	**PAR05**	**ADR**
Experiment 1	**10^{-7} M**	100.0	100.0	100.0	100.0	-25.4
	10^{-6} M	100.0	100.0	100.0	99.1	-43.5
	10^{-5} M	96.0	93.4	93.9	91.7	-50.4
	10^{-4} M	93.0	91.0	89.6	90.0	-50.9
Experiment 2	**10^{-7} M**	100.0	100.0	100.0	100.0	-23.3
	10^{-6} M	100.0	100.0	100.0	100.0	-39.6
	10^{-5} M	100.0	100.0	100.0	100.0	-43.3
	10^{-4} M	99.7	89.9	97.6	97.6	-53.5
Experiment 3	**10^{-7} M**	100.0	100.0	100.0	100.0	-21.7
	10^{-6} M	100.0	100.0	100.0	100.0	-40.1
	10^{-5} M	100.0	100.0	100.0	100.0	-42.7
	10^{-4} M	100.0	99.5	100.0	95.8	-49.5
Experiment 4	**10^{-7} M**	100.0	100.0	100.0	100.0	-23.5
	10^{-6} M	100.0	100.0	100.0	99.7	-41.1
	10^{-5} M	98.7	97.8	98.0	97.2	-45.3
	10^{-4} M	97.5	93.5	95.7	94.4	-51.3

DU145	LC50	TGI	GI50*
179PE717	>100	>100	>100
179ME717	>100	>100	>100
179CE717	>100	>100	>100
PAR05	>100	>100	>100
ADR	>100	61.2	<0.1

*GI50 ≤ 1 µMolar is considered to be active

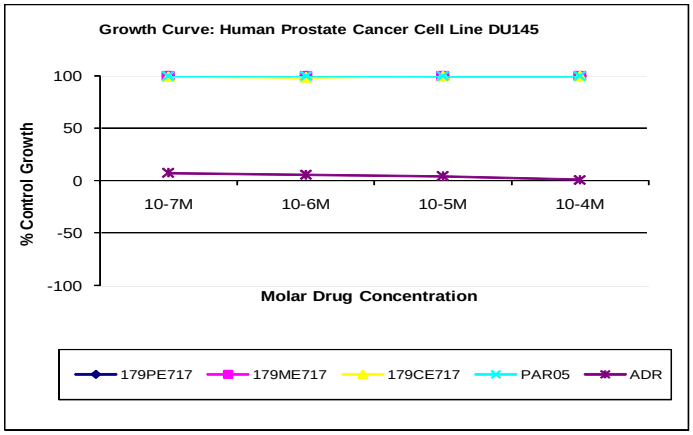


Table 5: % Control growth of various extracts of Lagenaria siceraria fruit on human prostate cancer cell line DU145.

	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
L.s. Petroleum extract	100±0	100±0	100±0	100±0
L.s. Methanol extract	100±0	100±0	100±0	100±0
L.s. Chloroform extract	99.27±0.73	98.57±1.43	100±0	99.93±0.066

Values are mean ± SEM

On human lung cancer cell line HoP62, drug 179PE717 and 179ME717, 179CE717 shown % control growth 100 in the concentrations 10^{-7} M, 10^{-6} M and 10^{-5}M, 10^{-4}M has shown % control growth in concentration dependent manner. But all these extracts shown LC50, TGI, G150 values are more than 100 as shown in result. But LC50, TGI, G150 values of standard drug Adramycin are 60.4, 21.47 and < 0.1 respectively which is considered to be active (Table-03, 06)

Table-3: Results of SRB Assay against Hop62 cell line.

	Human Prostate Cancer Cell Line DU145 % Control Growth					
	Molar Drug Concentrations					
	Conc.	**179PE717**	**179ME717**	**179CE717**	**PAR05**	**ADR**
Experiment 1	**10^{-7} M**	100.0	100.0	100.0	100.0	1.2
	10^{-6} M	100.0	100.0	100.0	100.0	0.8
	10^{-5} M	100.0	100.0	100.0	100.0	0.5
	10^{-4} M	100.0	100.0	100.0	100.0	-3.8
Experiment 2	**10^{-7} M**	100.0	100.0	100.0	100.0	5.2
	10^{-6} M	100.0	100.0	100.0	100.0	5.1
	10^{-5} M	100.0	100.0	100.0	100.0	4.3
	10^{-4} M	100.0	100.0	100.0	100.0	-0.1
Experiment 3	**10^{-7} M**	100.0	100.0	97.8	100.0	15.7
	10^{-6} M	100.0	100.0	95.7	100.0	10.6
	10^{-5} M	100.0	100.0	100.0	100.0	7.5
	10^{-4} M	100.0	100.0	99.8	100.0	6.4
Experiment 4	**10^{-7} M**	100.0	100.0	99.3	100.0	7.3
	10^{-6} M	100.0	100.0	98.6	100.0	5.5
	10^{-5} M	100.0	100.0	100.0	100.0	4.1
	10^{-4} M	100.0	100.0	99.9	100.0	0.8

Hop62	**LC50**	**TGI**	**GI50***
179PE717	>100	>100	>100
179ME717	>100	>100	>100
179CE717	>100	>100	>100
PAR05	>100	>100	>100
ADR	60.4	21.47	<0.1

*GI50 ≤ 1 µMolar is considered to be active

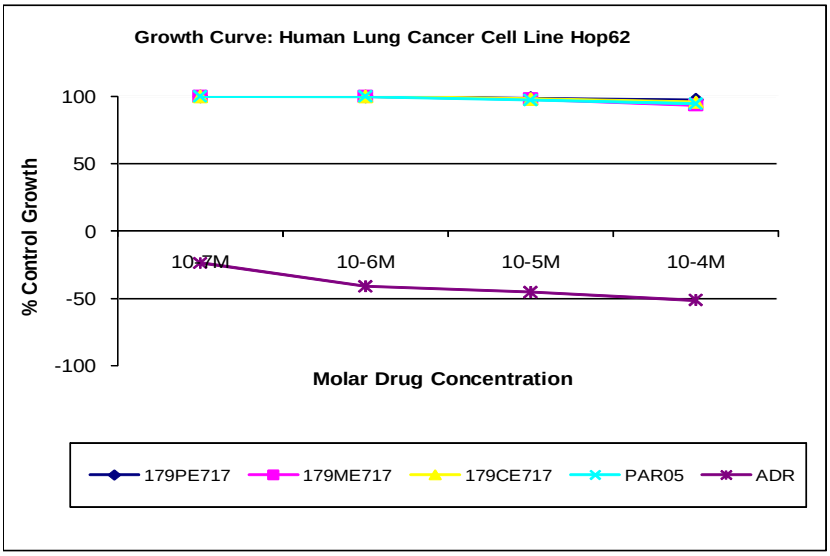


Table 6: % Control growth of various extracts of Lagenaria siceraria fruit on human lung cancer cell line Hop62.

	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
L.s. Petroleum extract	100±0	100±0	98.67±1.33	97.57±2.28
L.s. Methanol extract	100±0	100±0	97.8±2.20	93.47±3.03
L.s. Chloroform extract	100±0	100±0	97.97±2.03	95.73±3.14

Values are mean ± SEM

Conclusion:

Although steroid saponin were found to be essential for the antiapoptotic activity with regard to anticancer acivity, here results show that Petroleum ether, methanol and chloroform extract of *Lagenaria siceraria*, fruits in the concentration of 10^{-7} M, 10^{-6} M, 10^{-5}M and 10^{-4}M does not possess anticancer activity.

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