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Exploring Possible Anticancer Potential Of Some Plants Of Cucurbitaceae Family

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Research Article

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ABSTRACT

Today's major concern among the public is cancer treatment. Herbal medicines play vital role in this context. *Cucurbita pepo* (CP, Pumpkin), *Cucumis melo* (CM, Muskmelon) and *Cucumis sativus* (CS, Cucumber) seeds are consumed as nutritious snacks. In the present studies, crude *n*-hexane and petroleum ether seed extracts of CP, CM and CS were screened for *in vitro* cytotoxicity activity against human prostate cancer DU-145 and human breast cancer MCF7 cell lines by Sulforhodamine B (SRB) assay method. Growth inhibition of 50% (GI_{50}) was analyzed by comparing it with standard drug Adriamycin. The extracts did not show any activity when compared to Adriamycin at different concentrations (µg/ml) on both the cell lines. Thus, authors have attempted to provide importance to this family of the plants by subjecting them to anticancer studies. In future, new cell lines may provide relevance to these species and they can be actual put in therapeutic role

INTRODUCTION

Treatment against cancer has become of the one of the major concern, worldwide. The common types prevalent in India are lung, stomach, colorectal, liver, breast types of cancers. Lung and breast cancer are the most common cancers diagnosed in men and women respectively. Many countries may witness, different cancers alamaring to full strength by 2020 affecting maximum generations. Even though maximum research and efforts various countries has taken, still cancer is regarded to be one of the major killer disease. During the last decades, novel synthetic chemotherapeutic agents currently in usage have found not to be satisfactory clinically [1-3]. Plant derived herbal products have received increasing attention due to their multiple therapeutic roles even those related to many lifesaving diseases. It has been stated that plant-derived compounds are potential inhibitors of various stages of tumour genesis and associated inflammatory processes. More than 50% of plants derived are reported to be used for cancer treatment. The frequency of use of such products is reaching 50% in Asian population [4-10].

Plant species in this work belongs to Cucurbitaceae family. Phytochemistry has helped us to identify different phytoconstituents belonging to families of flavonoids, triterpenes, sterols, carotenoids and fatty acids from the plants. They have been reported to play vital role in varied areas like inflammation, ulcer, helminthiasis, fungal, bacterial, viral, cancer and diabetes [11-15]. SRB assay is considered to be one of the quick, accurate, reliable and cheap ideal methods for cancer screening. It gives colorimetric end point which is stable and visible under normal eye. It works on the principle of SRB dye that stains total cellular protein and thereby estimates the cell number. It provides a sensitive measure of drug-induced cytotoxicity useful in quantitating clonogenicity and suited for high volume, automated drug screening techniques [16, 17].

Statistics has relieved that 6.9% forms the new cases of lung cancer and 9.3% are related to death in both male and females every year [18]. The day is not far that we will witness more lung cancers patients in metropolitan cities in the upcoming years [19]. Similarly, we are facing breast cancer as major threat in India. It has started affecting population in the age of thirties to forties in most cities and second most common occurrence in rural areas [20]. Thus, population needs to be screened for the cancer which is need of hour so that the accurate treatment at right time can be initiated. Hence, taking the visionary of such clinical situations in progressing Indian nation, we have attempted to screen the plants for these two cell lines.

In this paper, studies are focused on screening *n*-hexane and petroleum ether seed extracts of CP, CM and CS on human

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prostate cancer DU-145 and human breast cancer MCF7 cell lines using SRB assay protocol.

MATERIALS AND METHODS

Collection, authentication and extraction

CP, CM and CS seeds were collected, authenticated and extracted as reported previously [21].

After extraction they were filtered using Whatman Filter No.1 and concentrated by evaporating the solvents on evaporating dish. The extracts obtained were stored in amber colored air tight containers in the refrigerator at 4°C to prevent any kind of decay.

SRB assay method

Both extracts were screened against specifically selected cell lines *viz*; human prostate cancer DU-145 and human breast cancer MCF7. Cell cultures, media and standard drug Adriamycin (ADR) were procured and maintained at ACTREC, Kharghar, Navi Mumbai.

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of CP, CS and CM extracts and standard drug.

CP, CS and CM seed extracts and standard drug were initially solubilized in dimethyl sulfoxide (DMSO) at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

CP, CS and CM seed extracts and standard drug addiction plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. SRB solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength [16,17].

Percent growth calculation was done on a plate-by-plate basis for test wells relative to control wells. Percent Growth has been expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels.

Percentage growth inhibition was calculated using formula,

[Ti/C] x 100 %

RESULTS AND DISCUSSION

The extracts did not produce significant effect on the human prostate cancer DU-145 and human breast cancer MCF7 cell lines used in these studies as depicted in **Tables 1- 4** and **Figures 1-4**. Plants can be screened for their potential with various extraction techniques.

Researchers citing the paper should try to explore different cell lines at different concentrations.

Table 1. Human Breast cancer cell line MCF7 of *n*-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin). The table gives results in triplicate with average for n-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin)

Human Breast Cancer Cell Line MCF7								
% Control Growth and Drug Concentrations (µg/ml)								
Details	Experiment 1				Experiment 2			
Conc.	10	20	40	80	10	20	40	80
Hexane CM	108.1	105.5	102.7	102.6	105.1	99.8	98.0	101.7
Hexane CP	100.7	102.2	100.3	99.9	102.7	96.3	90.5	96.2
Hexane CS	98.1	103.9	99.2	101.3	102.8	96.4	93.8	97.1
Pet ether CS	101.0	99.9	96.1	103.1	102.0	98.5	93.8	98.4

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Pet ether CP	103.0	104.4	98.3	102.8	97.8	97.1	90.3	100.7
ADR	-64.8	-71.3	-75.6	-64.4	-70.1	-70.6	-75.9	-69.1
Details	**Experiment 3**				**Average Values**			
Conc.	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
Hexane CM	103.4	109.2	106.5	105.8	105.5	104.8	102.4	103.4
Hexane CP	100.9	107.4	110.6	104.6	101.4	102.0	100.5	100.2
Hexane CS	106.5	110.7	109.4	102.4	102.5	103.6	100.8	100.3
Pet ether CS	109.4	111.4	110.6	111.2	104.1	103.2	100.2	104.2
Pet ether CP	109.6	109.1	102.6	104.1	103.5	103.5	97.1	102.5
ADR	-66.3	-73.4	-71.2	-55.7	-67.1	-71.8	-74.3	-63.1

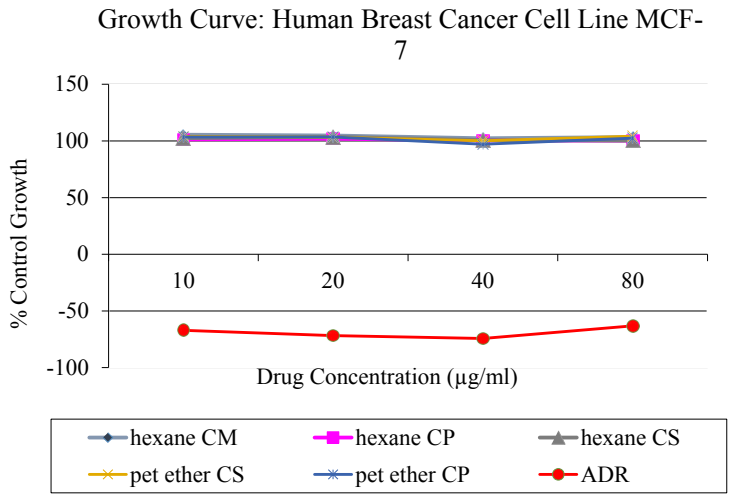

Figure 1. Growth Curve: Human Breast cancer cell line MCF7 of *n*-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin).

Table 2. Drug concentrations (µg/ml) calculated from graph of Human Breast cancer cell line MCF7 of *n*-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin). GI50 value of ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds whereas GI50 value ≤ 20µg/ml in extracts, Total growth inhibition (TGI).

Human Breast Cancer Cell Line MCF7 Drug concentrations (µg/ml) calculated from graph			
MCF7	**LC50**	**TGI**	**GI50**
n-Hexane CM	NE	NE	>80
n-Hexane CP	NE	NE	>80
n-Hexane CS	NE	NE	>80
Pet ether CS	NE	NE	>80
Pet ether CP	NE	NE	>80
ADR			<10

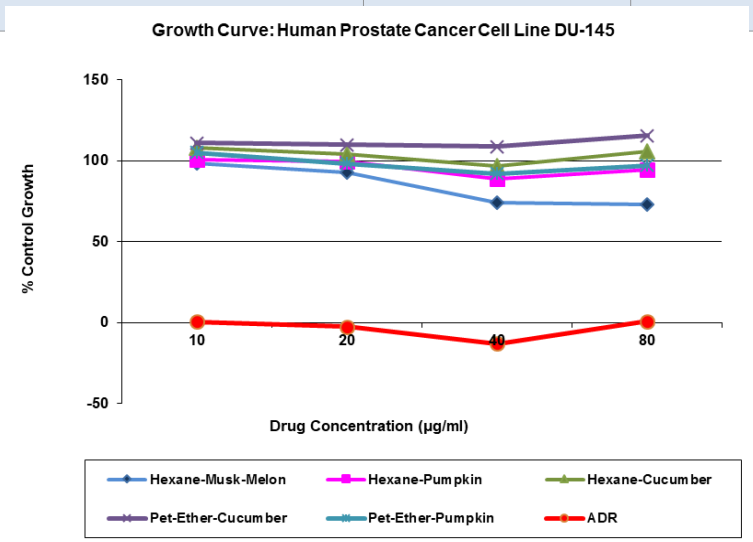

Figure 2. Growth Curve: Human Prostate Cancer Cell Line DU-145 of *n*-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin).

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Table 3. Human Prostate Cancer Cell Line DU-145 of *n*-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin). The table gives results in triplicate with average for n-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin)

HE : *n*-Hexane extract; PE : Petroleum ether extract

Human Prostate Cancer Cell Line DU-145								
% Control Growth and Drug Concentrations (µg/ml)								
Details	**Experiment 1**				**Experiment 2**			
Conc.	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
Hexane CM	96.3	98.4	72.5	65.6	99.5	84.4	65.8	68.3
Hexane CP	101.0	100.8	87.1	87.1	99.5	93.5	82.6	98.1
Hexane CS	110.7	106.3	97.9	100.3	108.9	102.5	93.0	110.3
Pet ether CS	113.7	117.8	112.8	117.6	109.2	102.6	105.7	115.2
Pet ether CP	108.4	107.7	100.8	103.7	103.0	88.3	89.0	96.3
ADR	4.3	2.8	-6.0	7.1	1.2	-9.0	-16.4	2.6
Details	**Experiment 3**				**Average Values**			
Conc.	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
Hexane CM	98.8	95.2	84.3	84.6	98.2	92.6	74.2	72.9
Hexane CP	101.9	103.5	96.8	98.8	100.8	99.3	88.8	94.7
Hexane CS	105.1	103.8	98.9	105.7	108.2	104.2	96.6	105.5
Pet ether CS	109.7	109.1	107.2	113.8	110.9	109.8	108.6	115.5
Pet ether CP	103.4	98.6	86.5	91.2	104.9	98.2	92.1	97.1
ADR	-4.0	-1.7	-16.6	-7.6	0.5	-2.6	-13.0	0.7

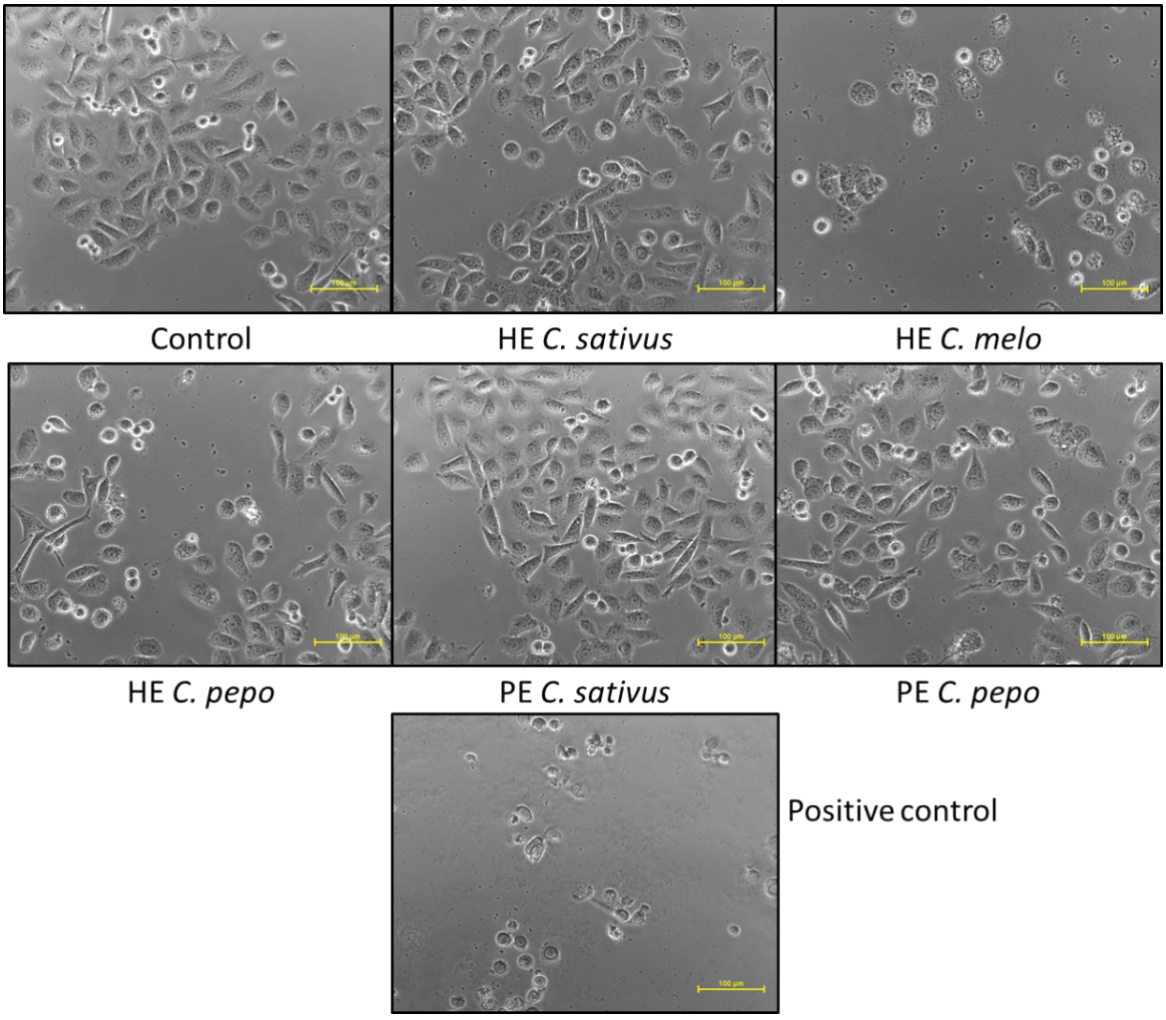

Figure 3. Images of Human Prostate Cancer Cell Line DU-145.

Table 4. Drug concentrations (µg/ml) calculated from graph of Human Prostate Cancer Cell Line DU-145 of *n*-hexane seed extracts of CP, CM, CS and petroleum ether seed extracts of CP and CS and ADR (Adriamycin). GI50 value of ≤ 10µg/ml is considered to demonstrate activity in case

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of pure compounds whereas GI50 value ≤ 20µg/ml in extracts, Total growth inhibition (TGI).

Hexane extract; PE: Petroleum ether extract

Human Prostate Cancer Cell Line DU-145 Drug concentrations (µg/ml) calculated from graph			
MCF7	**LC50**	**TGI**	**GI50**
Hexane CM	NE	NE	>80
Hexane CP	NE	NE	>80
Hexane CS	NE	NE	>80
Pet ether CS	NE	NE	>80
Pet ether CP	NE	NE	>80
ADR	NE	<10	<10

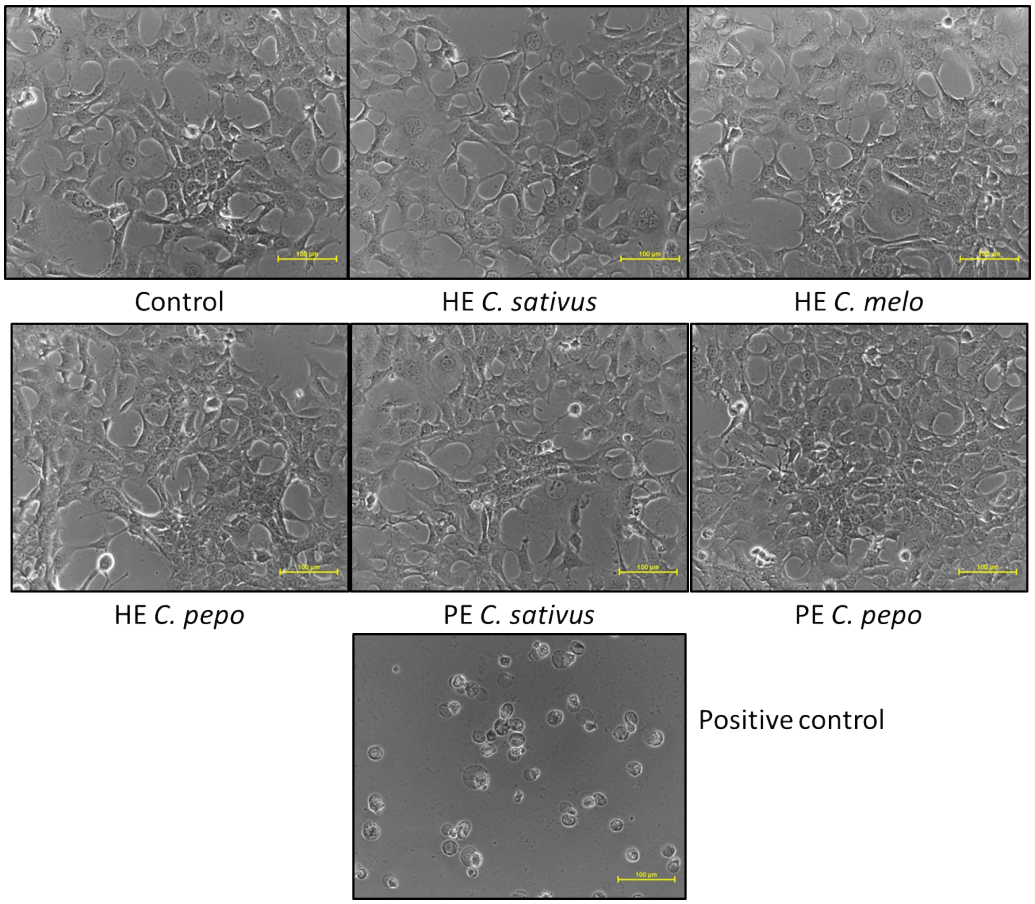

Figure 4. Images of Human Breast Cancer Cell Line MCF7.

CONCLUSION

The present studies are an attempt to explore the potential of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* for cancer remedy.

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