

Cytotoxic Activities of Extracts of Medicinal Plants of Euphorbiaceae Family Studied on Seven Human Cancer Cell lines

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Abstract Plant extracts of species of the family Euphorbiaceae used by traditional healers for the treatment of ulcers, cancers, tumors, warts and other diseases. were tested *in vitro* for their potential anti-proliferative activity. The objective of the present study was to evaluate the *in-vitro* anti-cancer effects of ethanolic extract of three plant species namely *Ricinus communis* Linn, *Euphorbia helioscopia*, *Jatropha curcas* of the family Euphorbiaceae by SRB assay against seven human cancer cell lines. Colon cancer cell line (Colon HT-29, SW-20, SiHa, Colon 502717), Liver cancer cell line (Hep-2), Breast cancer cell line (T-47D), Cervix cancer cell line OVCAR-5, Prostrate cancer cell line (PC-3) and Lungs (AF-49). The SRB assay was done in replicates to test cytotoxic activity of the three above mentioned plants against seven human cancer cell lines. The activity was evaluated at 100 µg/ml concentration of test material. *Jatropha curcas* showed 47% activity against SiHa. The ethanolic fraction of seed part of *Ricinus communis* showed 41% activity against Colon 502713 while stem part exhibited maximum activity against SiHa (47%). The ethanolic extract of *Euphorbia helioscopia* inhibited the growth of three cancer cell lines viz Hep-2, T-47D and PC-5. Hep-2 showed 27% activity.

Keywords Euphorbiaceae, Ricinus Communis, Jatropha, E.Helioscopia, Cytotoxic Activity

1. Introduction

Several plants of Euphorbiaceae family have been tested for their anticancer property, partly based on information concerning plants that have traditionally been used as medication to treat various human diseases (Bernal & Correa 1990, Unander et al.1995). Antitumor activity against sarcoma 180 ascites, leukemia in mice and cytotoxic activity against certain cancer cell lines has also been observed (Itokawa et al. 1989, Wu et al. 1991, Fatope et al.

1996). There seems to be increasing possibility of finding biological activity among plants with recorded medicinal uses rather than from plants randomly selected (Unander et al. 1995, Cordell 1995). Furthermore, selection of plants gives better criteria for screening programs especially in its initial phases, compared to the screening of compounds isolated or purified from natural products (Kusumoto et al. 1995, Cordell 1995, Baker et al. 1995). The objective of our work was to evaluate using SRB assay, the *in vitro* cytotoxic activity of some *Euphorbia* species that are known in India to have traditional medicinal uses against cancers.

2. Materials and Methods

2.1. General Method of Ethanolic Extract Preparation of Three Plants

The plant part was placed in glass percolator of appropriate size. Sufficient quantity of solvent was added to submerge the plant material. After standing for about 16 hours percolate was collected and filtered if required. The process was repeated four times for exhaustive extraction of the plant material. The ethanolic extract was evaporated under reduce pressure at 50°C using rotavapor and round bottom flask. The finally it was concentrated in a vacuum desiccators. The extract was transferred to glass container of appropriate size. This form the stock extract.

2.1.1. Source of Human Cancer Cell Line

Human cancer cell line were obtained from National Centre for cell science, Pune- 411007 (India) and National Cancer Institute, DTCD, Fredrick Cancer Research and Development Centre, Fairview centre, Suite 205, 1003, West -7th Street Frederick MD-21701- 8527 (USA)

2.1.2. Selection of Human Cancer Cell Line

The cell line were selected in such a way that almost all the cell line grow on a single growth medium (RPMI-1640) in

tissue culture flask (TCP) and the mass doubling time was such that enough cell were obtained for screening. Cell which were used were free from bacteria, yeast, mould, mycoplasma and in special cases from viruses at all the stages. If contamination appeared at any stage, the stock in which it occurred was discarded immediately. Cancer of central nervous system CNS, Lung cancer cell line A-549, Colon cancer cell lines, Colo-205, Colon 502713, Liver cancer cell line, Hep-2, Ovarian cancer cell line, OVCAR-5, Prostrate cancer cell line PC-5 were taken for the study.

2.2. Procedure for *In Vitro* Cytotoxicity Assay of Plants Extract

Cytotoxicity of test sample was performed against seven human cancer cell lines in replicates. 96 well flat bottom tissue culture plates were taken. There were four types of well in TCP, control blank (CB, without cells, complete growth medium only) and control growth (GC, with cell in absence of test material) to determine 100% growth. The growth in the presence of test material was determined from the difference of test growth (GT, cell with test material) and test control (CT, test material without cells). The desired human cancer cell lines were grown in tissue culture flask at 37°C in an atmosphere of 5% in CO₂ and 90% relative humidity in complete growth medium to obtain enough number of cells. The cells were harvested by the treatment of trypsin –EDTA and complete growth medium added. Viable cells were counted in haemocytometer by using trypan blue. Viable cell density was adjusted 5000- 40,000 cells/100 µl depending upon the cell line (Monks *et al* 1991). Cell suspension 100 µl was added. Complete growth medium was added and incubated at 37°C for 24 hours in an atmosphere of 5% CO₂ and 90% relative humidity in a CO₂ incubator. After 24 hours test material was added. Plates were incubated at 37°C for 48 hours in an atmosphere of 5% CO₂ and 90% relative humidity in a CO₂ incubator. The growth was determined after 48 hours by SRB assay.

2.3. SRB Assay

SRB assay was carried out as described by Skehan *et al.*, 1990, using SRB dye in replicates. After 48 hours incubation of cells with test material, the plates were taken out and 50 µl of chilled 50% TCA was gently layered on top of the medium in all the wells to produce a final concentration of 10%. After that Tissue culture plate were incubated at 4 °C in a refrigerator to fix the cells attached to the bottom of the wells. After one hour the plates were taken out from refrigerator and all the contents of all the wells were pipetted out and supernatant was discarded. The plates were washed five times with distilled water to remove TCA growth medium, low molecular metabolites, serum protein etc. For washing, the wells of Tissue culture plates were filled with distilled water and the liquid in the wells was discarded by sharply flicking plate over sink. Plates were air dried and can be stored until use. SRB solution (100 µl) was added to each well of the plates and the plates were incubated for 30 minutes at room temperature. The unbound SRB was removed quickly (to avoid desorption of protein bound dye) by washing the wells of the plates five times with 1 % acetic acid. Plates were than air dried. After that Tris buffer (100 µl /well) was added in the plates. The plates were gently stirred for 5 minutes on a mechanical shaker and optical density was recorded on ELISA reader at 540 nm.

3. Results and Discussion

The aim of this study was to evaluate the anti-cancer activity of three Euphorbiaceae plants namely *Ricinus communis*, *Euphorbia helioscopia* and *Jatropha curcas*. The cytotoxic activity of these plants was determined against seven human cancer cell lines. Following seven human cancer cell lines were taken Colon cancer cell line (Colon HT-29, SW-20, SiHa), Liver cancer cell line (Hep-2), Breast cancer cell line (T-47D), Cervix cancer cell line OVCAR-5, Prostrate cancer cell line (PC-3). SRB assay was done in replicates to determine the cytotoxic activity of these plants. The results summarized in Table1.shows anti-proliferative activity of three plants.,

Table1. *In- Vitro* Cytotoxicity of Plant Extract of *Euphorbia helioscopia*, *Ricinus communis* Linn and *Jatropha curcas* Against Human Cancer Cell Line

	Plant name	Plant part	Conc µg/ml	Hep-2	Breast T-47D	Colon HT-29	Prostrate PC-3	SW-620	Colon SiHa	Ovary OVCA R-5
			% Growth Inhibition							
1.	<i>Euphorbia helioscopia</i>	Whole Plant	100	27	7	0	11	--	-	-
2.	<i>Ricinus communis</i> Linn.	Stem	100	9	-	31	-	0	40	-
3.	<i>Jatropha curcas</i>	Leaves	100	-	0	-	-	-	47	30

(-) means that extract was not evaluated with particular human cancer cell line

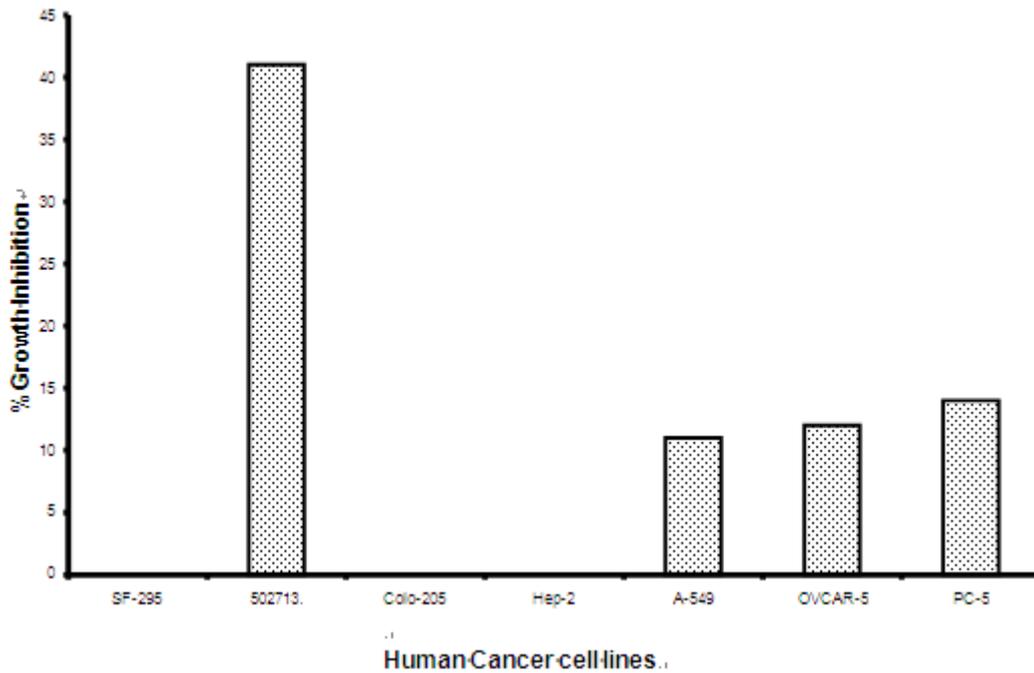


Figure 1. The ethanolic extract of *Ricinus communis* inhibited the growth of only four cancer cell lines viz colon 502713, A-549, OVCAR-5 and PC-5. The cytotoxic activity was observed in Colon 502713, A549, OVCAR-5, PC-5 are 41%, 11%, 12%, 14% respectively. Extract was observed to show no cytotoxic activity against these human cancer cell lines SF-295, Colo-205, Hep-2.

In vitro cytotoxicity of *Euphorbia helioscopia* (ethanolic extract) against human cancer cell lines

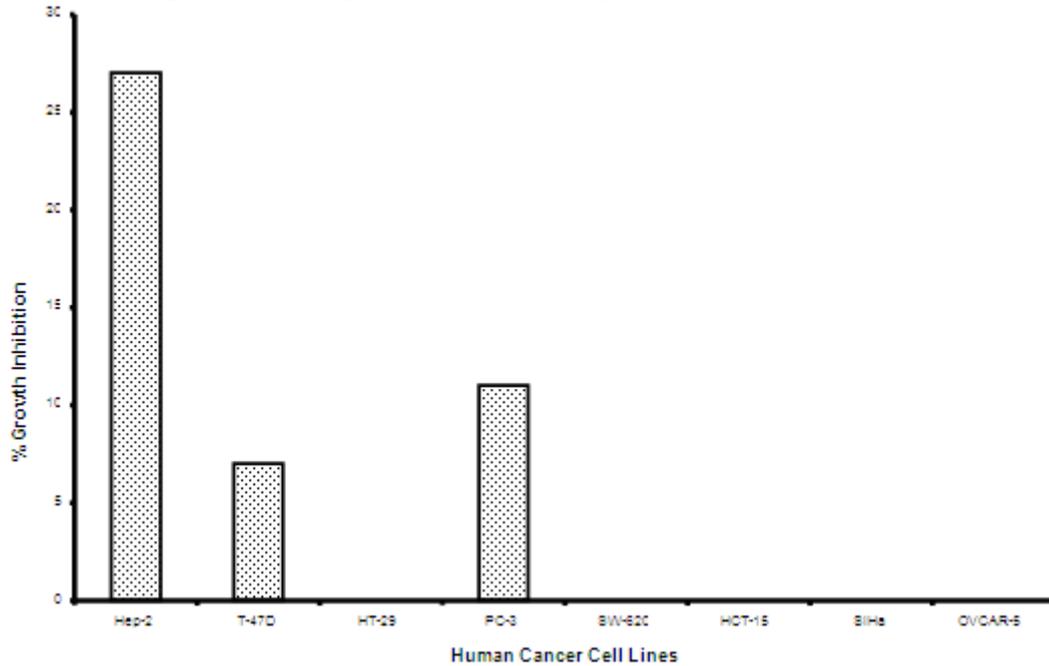


Figure 2. The ethanolic extract of *Euphorbia helioscopia* inhibited the growth of only three cancer cell lines viz Hep-2 (27%), T-47D (7%) and PC-5(11%). The cytotoxic activity was observed to be nil against following cancer cell lines SW-670, HCT-15, SiHa, OVCAR-5.

In vitro cytotoxicity of *Jatropha curcas* (ethanolic extract) against human cancer cell lines

Table 2. *In-Vitro* Cytotoxicity of Plant Extract of *Ricinus communis* Linn (seed part) Against Human Cancer Cell Lines

	Plant name	Plant part	Conc $\mu\text{g/ml}$	SF295	Colon 502713	Colon Colo205	Liver Hep-2	Lung A-549	Ovary Ovar-5	Prostrate PC-5
			% Growth Inhibition							
1.	<i>Ricinus communis</i> Linn.	Seed	100	-	41	-	-	11	12	14

(-) means that extract was not evaluated with particular human cancer cell line

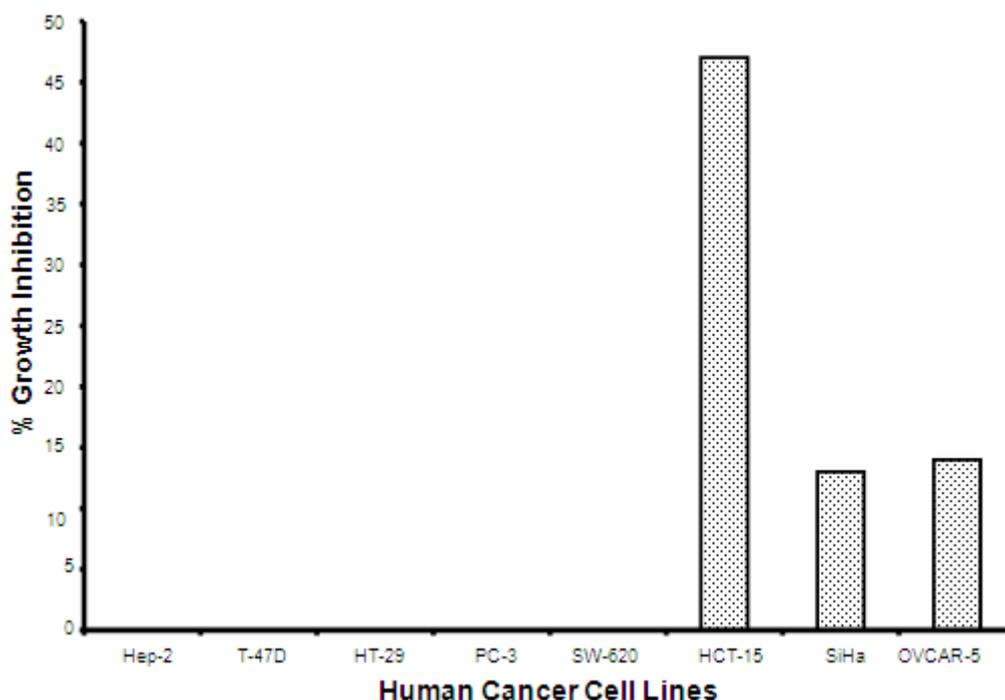


Figure 3. The ethanolic extract of *Jatropha curcas* showed nil activity against T-47D Human cancer cell line. HCT-15, SiHa, OVCAR-5 showed 47 %, 13 % and 14 % activity.

Total seven human cancer cell lines were taken to study the extract of seed and stem part of *Ricinus communis*. The ethanolic extract of seed part of *Ricinus communis* found active against Colon 502713, A-549, OVCAR-5 and PC-5 human cancer cell lines. The cytotoxic activity found to be 41%, 11%, 12% and 14% against Colon 502713, A549, OVCAR-5, PC-5 respectively. The ethanolic extract of stem part of *Ricinus communis* shows 9%, 31% and 40% activity against Hep-2, HT-29 and SiHa cell lines at 100 $\mu\text{g/ml}$ concentration. Fig1.shows cytotoxic activities of *Ricinus communis* against mentioned cell lines. The ethanolic extract of *Euphorbia helioscopia* inhibited the growth of three human cancer cell lines namely Hep-2, T-47D and PC-5. 27%, 7% and 11% activity was observed against these three cell lines respectively. Figs 2 explain the percentage activity against human cancer cell lines. The cytotoxic activity of ethanolic fraction of *Jatropha curcas* was found to be 47 % and 30% against SiHa and OVCAR-5 human cancer cell lines respectively as shown in Table 2.

In conclusion. the highly potent activities exhibited by *Jatropha curcas* and *Ricinus communis* and *Euphorbia helioscopia* even at low concentration (100 $\mu\text{g/ml}$) suggest that these compounds could be developed further as anticancer drugs.

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