Exploring the Anticancer Potential of crude extract Containing Bioactive Secondary Metabolites isolated from Endophytic Fungi of the Host plant *Physalis angulata* L"

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Abstract:

The aim of this research is to evaluate anticancer potential of extract containing secondary metabolites obtained from culturing plant endophytic fungi isolated from*Physalis angulata* L. commonly Known as angular winter cherry belonging to Solanaceae family. *Physalis angulata* containing alkaloids, flavonoids and Vitamin C as phytoconstituents induced anticancer, anti-inflammatory, antimicrobial and antibacterial activities. The crude extract isolated from endophytic fungi of *Physalis angulata* L. Evaluated for Cytotoxic potential by Sulforhodamine B (SRB) Assay. We have used three cell line MFC-7 ADR, Lungs cancer Hop-62S, Colon cancer COLO-205. EN-PAR-CET showed 50% Growth inhibition.

Key words: Secondary metabolites, endophytes, cytotoxicity, cancer, lungs, colon, ADR.

Introduction:

Chemical compounds and various secondary metabolites derived from medicinal plants are utilised for the treatment of diseases and infections. Plant derived secondary metabolites are always favoured because these phytoconstituents shows very less side effect and have improved

therapeutic efficiency and also reduce the drug resistance [1]. Utilization of plant derived foods and compound have a low risk of cancer. Foods obtained from herb exhibit Antioxidant, free radical scavenging effect and also having potential not only to attenuate physiological side effect, but also to abate genotoxicity [2]. Modern Research studies have proven that herbal extracts and phytoconstituents shows killing or inhibiting properties to cancer cell and possess antitumor activity [3]. It has been observed that death occurs due to liver cancer. Worldwide, liver cancer is on sixth number and the fourth dominant leading cause of liver cancer. Superior incidence of liver cancer commonly seen in South Asia, East Asia, North and South Africa.

Diagnosis, annual testing, early meditation for cancer are recently deficient on various accounts, which include the increase in human population, deficient supply of medicine, cost drug treatments, side effect of synthetic drugs, increasing resistance to the drug substances are used [4].The definition of cancer, cancer is genomic disease caused by the alteration in the genomic DNA of an organism. Now a day, herbal medicine is choicely selected for the treatment of cancer. Recent studies revealed that plants have a potential as anti-tumour (Vinod kumar Nelson1*)(Alioune Dior Falla)(Archana Bhumireddya)(Ting Feng)(Ashwini S. Shinde)effect [5].

Physalis angulata Linn. is a wild species commonly known as Cut-leaved, Ground cherry, cape gooseberryPlants is blogs to Solanaceae family and distributed in different part of country. Physalis angulata is a medicinal plant used for the treatment of various infectious diseases such as Anticancer, anti-inflammatory, Asthma, Diuretic, Hepatitis, malaria, antibacterial, Antimicrobials and skin diseases. Plant contain alkaloids, flavonoid, Glucocorticoids, sterols, carbohydrates, and vitamins, minerals. Various phytochemicals were isolate from the plant such as, Physalin A, Physalin B, Physalin C, Physalin D, Physalin E, Physalin E, Physalin F, Withanolide [6,7].

Material and methods:

1. **Collection of plant:** Physalis angulata Linn plant Root were collected from Rural area Near kazipura Tal-barshitakli Dist-Akola Maharashtra India. Plant shaded dried sent for authentication. Plant authentication done by Dr. Benniamin Scientist F Botanical Survey of India (BSI) Pune Maharashtra India.
2. **Isolation of Plant Endophytic fungi:** Fresh root of Plant Physalis angulata Linn. Were collected in a polythene bag with protection to avoid any physical damage. Root were washed with running tap water to remove unwanted matter. The surface of root was sterilized with 4% sodium hypochlorite, 70% ethanol and Distilled water. Small pieces sizes 3-5 cm of root were

cut with the help of sterilized blade and transferred in petri plates containing Potato dextrose Agar Media for the growth of Endophytic fungi. To prevent the growth of bacterial endophytes, antibacterial drug is supplemented to culture media. The petri plates were kept for incubation for 5-6 days at a temperature 25-26°C. the growth of endophytic fungi was observed and picked up endophytic fungi for subculturing. One pure culture of endophytic fungi was sent to Biokart Pvt Ltd Bangalore Karnataka India for the identification and PCR analysis.

3. Potato dextrose broth were prepared and transferred in three litter flasks. Plates of pure endophytic culture were mixed with the broth and kept in incubator at 25-27°C for three weeks. Broth were filtered with the help of Muslin cloth to separate mycelia and broth were centrifuged at 400 rpm to get the supernatant.
4. The supernatant was collected and subjected to liquid -liquid extraction chloroform, methanol, n-butanol. The organic phase was separated and collected for evaporation. After evaporation we get the crude extract from endophytic fungi.
5. The crude extract were sent for screening of Cytotoxicity effect on different cell line.

Cytotoxic Potential using Sulforhodamine B assay:

In the present study, Three Human cell line were used namely human breast cell lines MCF-7, Human Lungs cancer cell line Hop-62 and Human Colon cancer cell line COLO-205 by using sulforhodamine B (SRB) assay[20] at Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer Sector-22, Kharghar, Navi Mumbai, Maharashtra.

In vitro evaluation of cytotoxic potential extract: [8,9,10,11]

The cell line was grown by using a medium containing 10% bovin serum and 2 M L-glutamine. In this evaluation we have used 5000 cell/ well were inoculated into 96 well microtiter plates in 100 microliters. After completion of inoculation of cell, the microtiter plates were incubated for complete one day (24 hrs) at a temperature 37° C, 5 % CO2, 95 % air and 100 % relative humidity pre-existing to addition of experimental drug.

Experimental drug are solubilized in a solvent at 100mg/mi and diluted to 1mg/ml using H_2O and stored frozen before use. During the time of addition, an aliquote of frozen concentration of experimental drug was warmed and diluted to 100, 200, 400 and 800 Microgram/ml with complete medium containing test article.Aliquotes of 10 µl of these variance drug dilutions were added to the microliter wells previously containing 90 µl of medium, resulting in the final drug concentration i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After addition of compound, plates were incubated for 48 hrs (2 days) and assay was terminated by the addition of cold TCA. The cells were fixed in situ by addition of 50 µl of cold TCA (10% TCA) and incubated for one minute at a temperature 4°C. the supernatant abdicated, the plates washed with running tape water five times and dried by air. Sulforhodamine B solution at 0.4 % in 1% acetic acid was added to each well, and plate incubated at room temperature for 20 minutes. After the staining, unbounded dyes were recovered and the residual dyes was separate by five time washing with 1 % acetic acid. The plate was air dried. Bound stain was afterward washed with 10 mM trizma base, and the absorbance was read on plates reader at wavelength of 540 nm with 690 nm reference wavelength.

Percentage growth was deliberate or calculated on the basis of plate-by-plate for test well relative to control wells. Growth percentage expressed as ratio of average absorbance of test well to the average absorbance of control wells.

Using the 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. Growth inhibition of 50 % (GI50) drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Result and discussion:

The aim of this study was to Evaluate in vitro Cytotoxic potential of *Physalis angulata* Root Extract against cancer cell lines. The treatment of EN-PAR-CET Extract + ADR, ADR was administered on breast cancer cell line (MCF-7), lung cancer cell line (HOP-62) and Colon cancer cell line (Colo-205) by using Sulforhodamine B (SRB) assay. Adriamycin (ADR) drug was used as positive control OR Reference compound. The susceptibility of cells to the drug employed was recognized by Growth inhibition 50% (GI_{50}), Concentration of drug causes 50% inhibition of cell growth (IC_{50}), Concentration of drug causing total inhibition of cell (TGI). And concentration of drug causing 50% cell kill (LC_{50}) as given in tables.

Sample drug with different concentrations were used for treating Breast cancer (MCF-7) for 24 hours. anticancer activity was found in EN-PAR-CET when compared with Adriamycin LC_{50}Is >80µg/ml, TGI is >80 µg/ml and GI_{50}µg/ml is 61. Cancer cells treated exhibit morphological changes characterized by irregular shapes of cells, cells shrinkage and showed cytotoxicity.

Cytotoxic potential of EN-PAR-CET on Breast cancer cell line MCF-7:

observation table and graph demonstrated that Sample drug concentratio LC50 >80 µg/ml, TGI >80 µg/ml and GI50 61 µg/ml were used and showed moderate cytotoxic activity, with a value indicating 50% growth inhibition at 61 µg/ml.

Table 1: Human Breast Cancer Cell Line MCF-7

Human Breast Cancer Cell Line MCF-7																
% control Growth																
EN-PAR-CET	Drug Concentrations (µg/ml)															
	Experiment No-1				**Experiment No-2**				**Experiment No-3**				**Experiment No-4**			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
	92.6	85.4	72.3	33.8	94.3	82.6	67.7	32.9	95.3	79.6	70.2	35	94.1	82.5	70.1	33.9
ADR	-70.7	-76.9	-83.8	-73.8	-71.0	-76.6	-81.2	-76.0	-78.9	-76.0	-83.7	-67.8	-73.5	-76.5	-82.9	-72.5

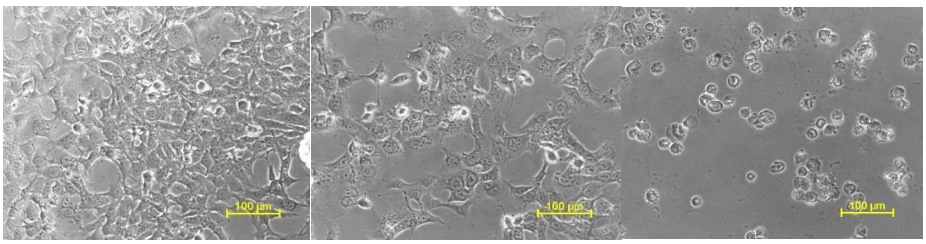

Figure 1: MCF-7 ControlMCF-7 EN-PAR-CETMCF-7 ADR

Table 2: Drug Concentrations (ug/ml) calculated from graph (MCF-7)

MCF-7	**LC50**	**TGI**	**GI50**
EN-PAR-CET	>80	>80	61
ADR	<10	<10	<10

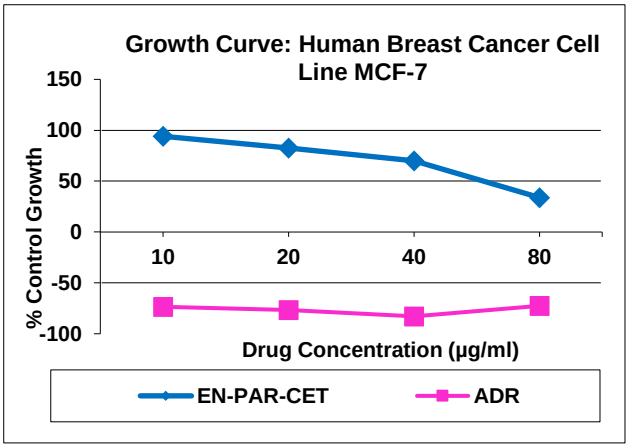

Figure 2: Growth Curve: Human Breast Cancer Cell Line MCF-7

Cytotoxic potential of EN-PAR-CET on Lungs cancer cell line Hop-62:

EN-PAR-CET Sample drug at a concentration LC50 Not Evaluated, TGI Not Evaluated and GI50 >80 µg/ml were used and exhibited negligible cytotoxic activity on Hop-62 with no significant inhibition observed up to the tested drug concentrations.

Table 3: Human Lungs Cancer Cell Line Hop-62

Human Lungs Cancer Cell Line Hop-62																
% control Growth																
EN-PAR-CET	Drug Concentrations (ug/ml)															
	Experiment No-1				**Experiment No-2**				**Experiment No-3**				**Experiment No-4**			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
	112.6	99.9	106.9	108.8	108.6	108.1	104.3	97.3	116.7	107.8	98.9	112.3	112.7	105.3	103.4	106.2
ADR	7.9	9.6	-3.6	-29.1	9.3	4.8	-2.4	24.5	4.6	3.8	-9.4	-31.8	7.3	6.1	-5.1	-28.5

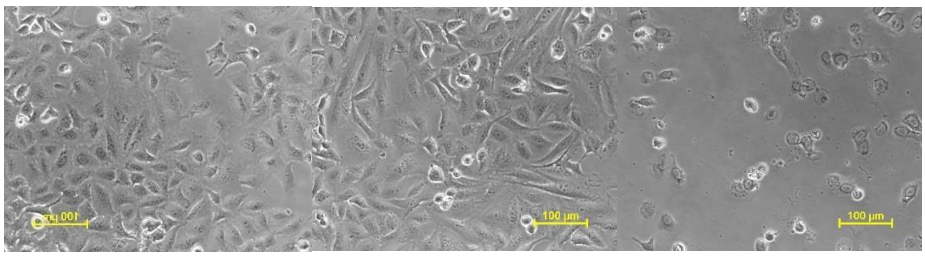

Figure 3: HOP-62 ControlHOP-62 EN-PAR-CETHOP-62 ADR

Table 4: Drug Concentrations (ug/ml) calculated from graph (Hop-62)

Hop-62	LC50	TGI	GI50
EN-PAR-CET	NE	NE	>80
ADR	NE	28	<10

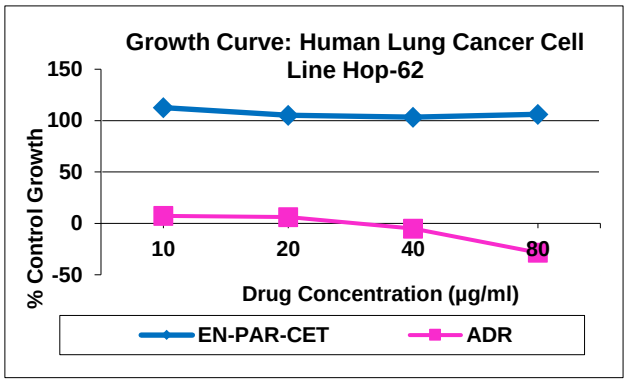

Figure 4: Growth Curve: Human Lung Cancer Cell Line Hop-62

Cytotoxic potential of EN-PAR-CET on Colon cancer cell line COLO-205:

EN-PAR-CET Sample drug at a concentration LC50 Not Evaluated, TGI >80 µg/ml and GI50 µg/ml were used and

COLO-205	LC50	TGI	GI50

exhibited negligible cytotoxic activity on COLO-205with no significant inhibition observed up to the tested drug concentrations.

Table 5: Human Colon Cancer Cell Line COLO-205

Human Colon Cancer Cell Line COLO-205																
% control Growth																
EN-PAR-CET	Drug Concentrations (ug/ml)															
	Experiment No-1				Experiment No-2				Experiment No-3				Experiment No-4			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
	101.2	91.5	72.5	62.4	94.0	95.1	74.8	50.4	100.3	100.1	69.7	48.8	98.5	95.6	72.3	53.9
ADR	-28.8	-25.4	-30.3	2.3	-34.1	-29.4	-41.5	-24.3	-35.8	-26.6	-27.4	-18.5	-32.9	-27.1	-33.0	-13.5

Table 6: Drug Concentrations (ug/ml) calculated from graph (COLO-205)

EN-PAR-CET	NE	>80	>80
ADR	NE	<10	<10

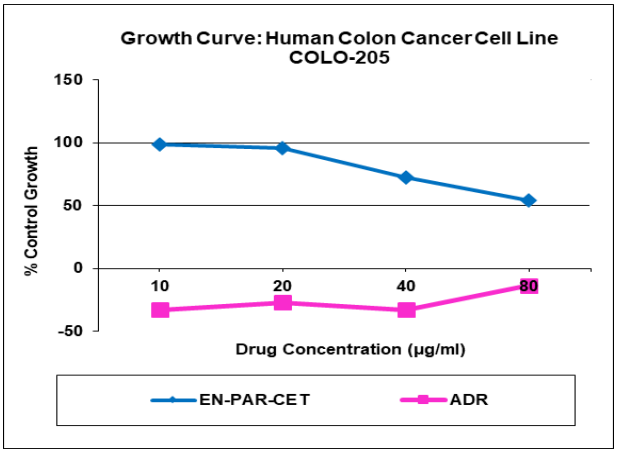

Figure 5: Growth Curve: Human Colon Cancer Cell Line (COLO-205)

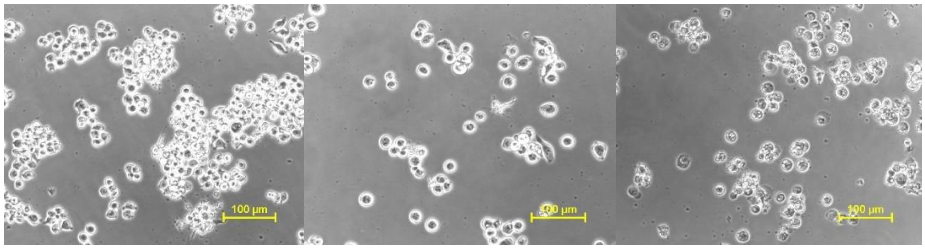

Figure 6: Colo-205 ControlColo-205 EN-PAR-CETColo-205 ADR

Conclusion

There are various researchers carried cytotoxic activities by using different medicinal plants. We have carried out isolation of plant endophytic fungi and culturing of isolated endophytic fungi to obtained the pure culture. We have obtained the extract of secondary metabolites which is produced by the plant endophytes. We have evaluated the extract on three different cell line as to evaluated the cytotoxic potential of Physalis angulata endophytic extract on breast, lungs and colon cancers cell line. the study showed that EN-PAR-CET Exhibited Growth inhibition (GI50) of cancer cells.

Conflict of Interest Declared None

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