



## Pharmacological Study

# Assessment of cytotoxicity of *Portulaca oleracea* Linn. against human colon adenocarcinoma and vero cell line

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### Abstract

**Background:** *Portulaca oleracea* Linn. (*Portulacaceae*) is commonly known as purslane in English. In traditional system it is used to cure diarrhea, dysentery, leprosy, ulcers, asthma, and piles, reduce small tumors and inflammations. **Aim:** To assess cytotoxic potential of chloroform extract of *P. oleracea* whole plant against human colon adenocarcinoma (HCT-15) and normal (Vero) cell line. **Materials and Methods:** Characterization of chloroform extract of *P. oleracea* by Fourier transform infrared (FTIR) spectroscopy was performed. Cytotoxicity (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay was used for assessment of cytotoxic potential of chloroform extract of *P. oleracea*. The concentrations of 1000–0.05 µg/ml were used in the experiment. Doxorubicin was considered as standard reference drug. **Results:** FTIR spectrum showed the peak at 1019.52 and 1396.21 center. The 50% cell growth inhibition (IC<sub>50</sub>) of chloroform extract of *P. oleracea* and doxorubicin was 1132.02 µg/ml and 460.13 µg/ml against human colon adenocarcinoma and 767.60 µg/ml and 2392.71 µg/ml against Vero cell line, respectively. **Conclusion:** Chloroform extract of *P. oleracea* whole plant was less efficient or does not have cytotoxic activity against human colon adenocarcinoma cell line. It was not safe to normal Vero cell line. But, there is a need to isolate, identify, and confirm the phytoconstituents present in extract by sophisticated analytical techniques.

**Key words:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, Fourier transform infrared, human colon adenocarcinoma, *Portulaca oleracea*, purslane

## Introduction

*Portulaca oleracea* Linn. is a warm-climate, fleshy herbaceous succulent annual plant belonging to the *Portulacaceae* family.<sup>[1]</sup> It has a cosmopolitan distribution in Africa, China, India, Australia, Middle East, Europe, and the United States.<sup>[2-4]</sup> *P. oleracea* plant and its seeds are used in treating diseases of kidney and bladder, as strangury, dysuria, hematuria, gonorrhoea, and lungs.

It is beneficial to the intestinal mucous membrane, relieves tormina, dysentery, and mucous diarrhea. Sour leaves are used as a vegetable. The seeds are said to be used as a vermifuge and to treat to dyspnea.<sup>[5,6]</sup> *P. oleracea* is commonly known as *Brihat Lonika*, *Lona*, *Loni*, *Ghoddhika*, *Ghotika*, *Upodika*,

*Khursa* in Ayurveda. It has properties and actions such as, *Rasa* (taste): *Amla* (sour); *Guna* (properties): *Guru* (heavy), *Ruksha* (dry), *Sara*; *Virya* (potency): *Ushna* (hot); *Vipaka*: *Amla*; *Karma*: *Kaphahara*, *Pittakara*, *Vatahara*, and *Vanidosahara*.<sup>[7,8]</sup> *P. oleracea* in ancient times was looked upon as one of the anti-magic herbs and strewn around a bed was said to afford protection against evil spirits and nightmares.<sup>[9]</sup> *P. oleracea* has reported to have more omega-3-fatty acids, alpha linoleic acid in particular than any other leafy vegetable plant.<sup>[10]</sup> It also contains Vitamins A, C, and E as well as dietary minerals such

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as calcium, potassium, magnesium, and iron, pigments, and betacyanins with potent antioxidants properties.<sup>[11,12]</sup>

*In vitro* cytotoxicity testing has become an integral aspect of drug discovery because it is a convenient, cost-effective, and predictive means of characterizing the toxic potential of new chemical entities. The early and routine implementation of this testing is testament to its prognostic importance for humans.<sup>[13]</sup> Medicinal plants constitute a common alternative for cancer prevention and treatment worldwide.<sup>[14-17]</sup> Approximately 60% of the anticancer drugs currently have been isolated from natural products. At this time, more than 3000 plants worldwide have been reported to possessed anticancer properties.<sup>[18]</sup> Therefore, based on the above considerations and to explore traditional use of this plant scientifically, author has assessed cytotoxicity of *P. oleracea* against human colon adenocarcinoma and normal cell line.

## Materials and Methods

### Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye powder (Serva Electrophoresis), dimethyl sulfoxide cell culture grade (BioWorld), amphotericin B (HiMedia), penicillin and streptomycin solution stabilized (Sigma), ethylene di-amine tetra-acetic acid (EDTA; MP Biomedicals), DPBS/modified IX (Dulbecco's phosphate buffer saline without Ca<sup>+</sup> and Mg<sup>+</sup>) (HiMedia), fetal bovine serum (Quaditive), chloroform (SD Fine Chem). All other chemicals used for this experimental work were of analytical grade.

### Instruments

Fourier transform infrared (FTIR) spectroscopy (Agilent Cary 630), biosafety cabinet Class II and cytotoxic safety cabinet (Esco), CO<sub>2</sub> incubator (RS Biotech, mini galaxy A), deep freezer (Dairei), ELISA plate reader (Thermo), micropipettes (Eppendorf), RO water system (Millipore), chloroform (SD Fine Chem) 96-well microtiter plate (flat bottom, U bottom, V bottom), tissue culture flasks (75 cm<sup>2</sup> T Flask vented and 150 cm<sup>2</sup> T Flask vented), falcon tubes (15 ml, 50 ml), cryotubes (2 ml), etc.

### Preparation of extract

The whole plant of *P. oleracea* was collected from the Khote Nagar of Jalgaon city, Maharashtra [Figures 1 and 2], was authenticated by taxonomist, Department of Botany, Dr. AGD Bendale Mahila Mahavidyalaya, Jalgaon, Maharashtra, India and herbarium specimen (No. Bot/14/129) was preserved. The plant was dried, powdered, and extracted with chloroform to get nonpolar cytotoxic phytoconstituents present in it using percolation method. The excess solvent was completely removed, get concentrated, and preserved in an airtight container under refrigeration.

### Characterization of extract by Fourier transform infrared

The characterization of chloroform extract of *P. oleracea* was performed by FTIR spectrophotometer.

### Procurement and maintenance of cell lines

Human colon adenocarcinoma (HCT-15) and normal (Vero) cell line were procured from National Centre for Cell Science,



Figure 1: Photograph of *Portulaca oleracea* Linn.

Pune. Stock cells of these cell lines were cultured in Dulbecco's modified eagles media, supplemented with 10% FBS. Along with media, cells were also supplemented with 5% Hanks' balanced salt solution, penicillin, streptomycin, and amphotericin B, in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C until confluence reached. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock cultures were grown initially in 25 cm<sup>2</sup> tissue culture flasks, then in 75 cm<sup>2</sup>, and finally in 150 cm<sup>2</sup> tissue culture flask and cytotoxicity experiment was carried out in 96-well microtiter plates.

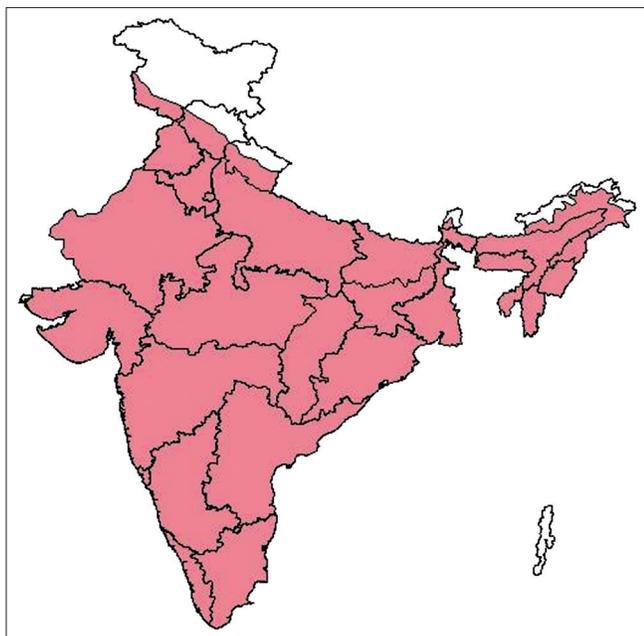
### Cytotoxicity (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay

MTT assay is a sensitive, quantitative, and reliable colorimetric assay that measures viability, proliferation, and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate MTT into a dark blue formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in range of cell lines.<sup>[19]</sup> The protocol described by Mosmann with some modifications was adopted.<sup>[20-26]</sup> Briefly, cell lines in exponential growth phase were washed, trypsinized, and resuspended in complete culture media. Cells were seeded at a concentration of  $2 \times 10^4$  cells/well in 96-well microtiter plate and incubated for 24 h during which a partial monolayer forms. The cells were then exposed to various concentrations of the test compounds and standard drug doxorubicin, (i.e., Conc. 1000–0.05 µg/ml). Control wells were received only maintenance medium. The plate was incubated at 37°C in humidified incubator with 5% CO<sub>2</sub> and 75% relative humidity for 24 h. At the end of 24 h, 10 µl MTT labeling mixture was added and incubates for 4 h. The absorbance was measured using microplate ELISA reader at wavelength 590 nm. The percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

$$\% \text{ Viability} = (A_T - A_B)/(A_C - A_B) \times 100.$$

Where,  $A_T$  = Absorbance of treated cells (drug),  
 $A_B$  = Absorbance of blank (only media), and  
 $A_C$  = Absorbance of control (untreated).

$$\% \text{ Cell growth inhibition/\% Cytotoxicity} = 100\% \text{ cell survival.}$$



**Figure 2: Geographical distribution map of *Portulaca oleracea* in India (ENVIS database)**

### Statistical interpretation

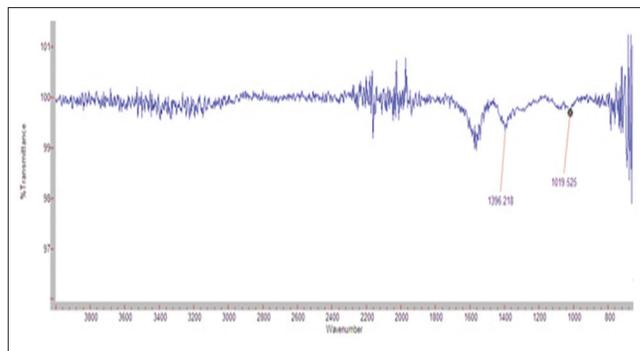
All the values of percent cell growth inhibition was means of three independent observations ( $n = 3$ ), and 50% cell growth inhibition ( $IC_{50}$ ) was determined by interpolating concentrations (X-axis) versus % cell inhibition (Y-axis) by linear regression method or equation using Microsoft Excel, 2007, Microsoft Corporation, USA application.

### Results

FTIR spectrum of chloroform extract of *P. oleracea* showed the peak at 1019.52 and 1396.21 center at the wavelength region of 4000.00–650.00/cm and resolution 4/cm [Figure 3]. The peaks/centers corresponds to 1300–1000 (C–O stretching) alcohol and phenols, 1220–1020 (aliphatic C–N stretching) amines, 1300–1420 (C–H stretching in combination) organic compounds, 1430–1360 (oxygen-bonded complex) nitrogen or oxygen atom-containing compounds might be present in the extract. The 50% cell growth inhibition ( $IC_{50}$ ) of chloroform extract of *P. oleracea* and doxorubicin was 1132.02  $\mu\text{g/ml}$  and 460.13  $\mu\text{g/ml}$  against human colon adenocarcinoma cell line [Figures 4 and 5] and 767.60  $\mu\text{g/ml}$  and 2392.71  $\mu\text{g/ml}$  against normal cell line, respectively [Figures 6 and 7].

### Discussion

Numerous cancer research for chemotherapeutic potential of medicinal plants has been carried out in an effort to discover new therapeutic agents that lack the toxic effects associated with current therapeutic agents.<sup>[27]</sup> Herbal medicines have been proven to be a major source of novel agents with various pharmaceutical applications.<sup>[28]</sup> The result of characterization of chloroform extract of *P. oleracea* by FTIR technique



**Figure 3: Fourier transform infrared spectrum of chloroform extract of *Portulaca oleracea***

was in line with the above findings. Cytotoxicity assays are performed to predict potential toxicity, using cultured cells which may be normal or transformed cells. These tests normally involved short-term exposure of cultured cells to test substances, to detect how basal or specialized cell functions may be affected by the substance, before performing safety studies in whole organisms. It can also provide insight toward the carcinogenic and genotoxic dispositions of herb-derived compounds and extracts. The ability of a plant extract to inhibit cellular growth and viability can also be ascertained as an indication of its toxicity. Assessment parameters for cytotoxic effects include inhibition of cell proliferation, cell viability markers (metabolic and membrane), and morphologic and intracellular differentiation markers.<sup>[29]</sup> The results of our studies revealed that the 50% cell growth inhibition ( $IC_{50}$ ) of chloroform extract of *P. oleracea* and doxorubicin was 1132.02  $\mu\text{g/ml}$  and 460.13  $\mu\text{g/ml}$ , respectively, against human colon adenocarcinoma cell line. As per the United State National Cancer Institute Plant Screening Programme, a crude extract is generally considered to have *in vitro* cytotoxic activity if the  $IC_{50}$  value in carcinoma cells, following incubation between 48 and 72 h, is <20  $\mu\text{g/ml}$  while it is <04  $\mu\text{g/ml}$  for pure compounds.<sup>[30]</sup> The extract was less efficient or does not have cytotoxic activity as  $IC_{50}$  is more than the concentrations used in the study due to the nonextraction of nonpolar cytotoxic bioactive constituents into chloroform solvent or either not cytotoxic to HCT-15 cell line. The last main consideration in cytotoxicity testing is the manner in which cells die. The mechanism of death can be quite important if cytotoxicity testing is initiated for ancillary safety concerns (e.g. off-target cytotoxicity from pharmaceuticals, cosmetics, and nutritional supplements), or specifically, as for identifying new chemical entities for cancer therapy. Simply stated, compounds that cause primary necrosis in cell culture may carry unacceptable cytotoxic liabilities whereas compounds that cause apoptosis can be preferable.<sup>[31-33]</sup> Therefore, cytotoxic potential of chloroform extract of *P. oleracea* was also checked against normal cell line for its the safety.

### Conclusion

It can be concluded that the chloroform extract was found to be toxic, and doxorubicin was safe with respect to the concentrations used against normal cell line. Further studies

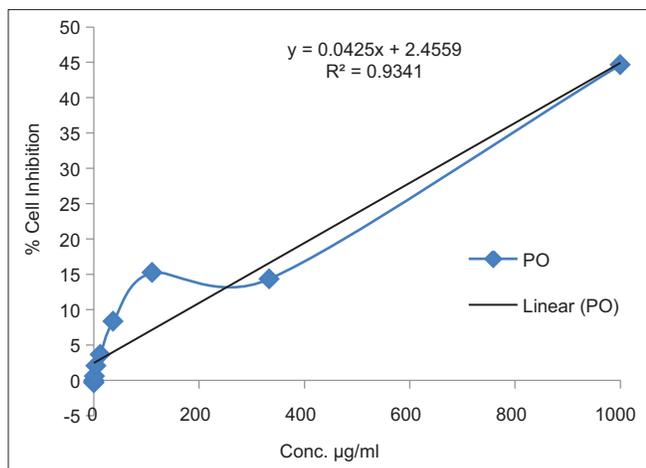


Figure 4: % cell inhibition of chloroform extract of *Portulaca oleracea* against human colon adenocarcinoma cell line

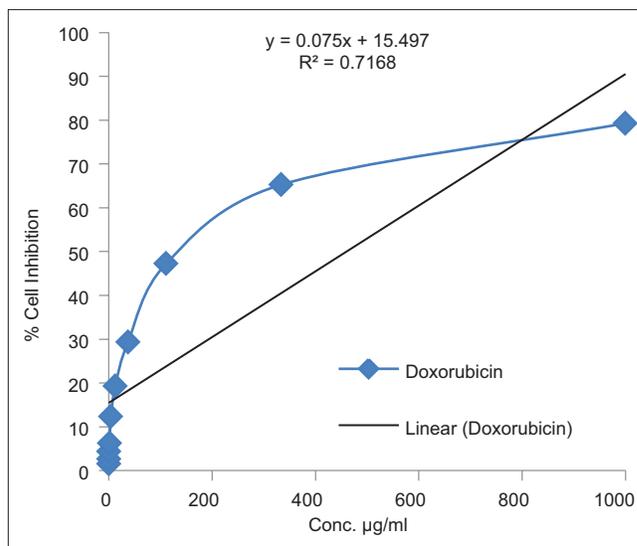


Figure 5: % cell inhibition of doxorubicin against human colon adenocarcinoma cell line

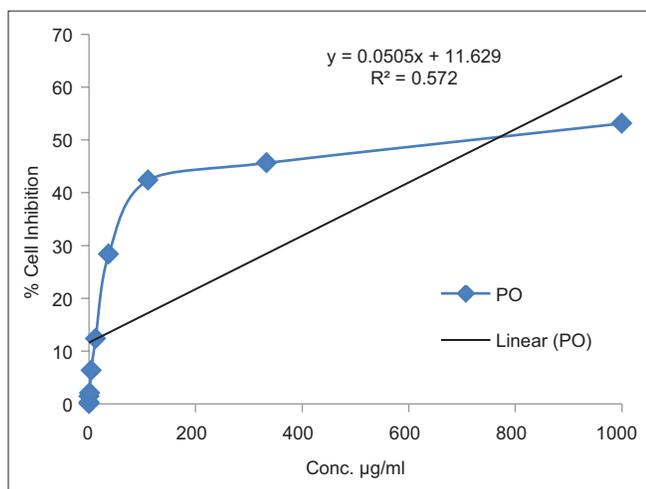


Figure 6: % cell inhibition of chloroform extract of *Portulaca oleracea* against Vero cell line

will not required on chloroform extract of *P. oleracea* whole plant using human colon adenocarcinoma and normal cell line. However, there is a need to isolate, identify, and confirm the phytoconstituents present in the extract using sophisticated analytical techniques which were found in the FTIR peak centers.

### Acknowledgment

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### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

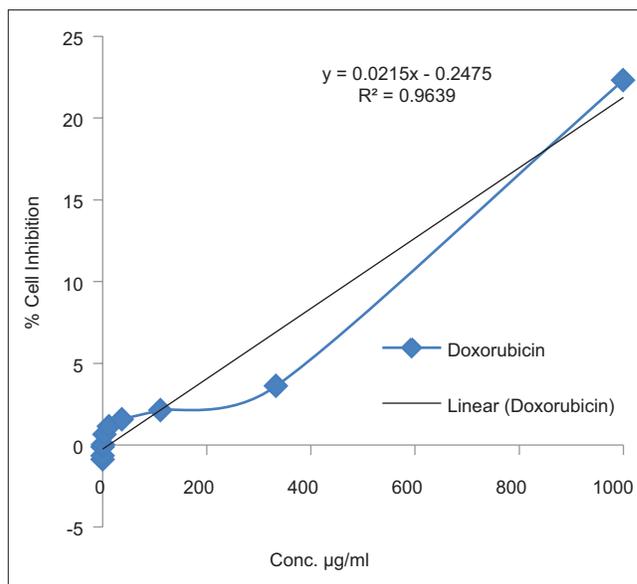


Figure 7: % cell inhibition of doxorubicin against Vero cell line

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## हिन्दी सारांश

### मानव कोलन के ग्रंथिकर्कटल और सामान्य कोशिका पर पोर्च्युलेका ओलेरेसिया की साइटोटॉक्सिसिटिका आंकलन

प्रशांत वाई माली

पोर्च्युलेका ओलेरेसिया आमतौर पर अंग्रेजी में पर्सलेन के रूप में जाना जाता है। पारंपरिक प्रणाली में, यह दस्त, पेचिश, कुष्ठ, अल्सर, दमा, बवासीर का इलाज, छोटे ट्यूमर और सूजन को कम करने के लिए प्रयोग किया जाता है। अध्ययन का उद्देश्य मानव कोलन के ग्रंथिकर्कटता (एच.सी.टी.-१५) और सामान्य (वेरो) सेल लाइन के विरुद्ध पी.ओलेरेसिया के क्लोरोफार्म सत्व निकालने की साइटोटॉक्सिक क्षमता का आंकलन करने के लिए किया गया था। इसके लिए साइटोटॉक्सिसिटी (एम.टी.टी.) एसे का उपयोग किया गया था। १०००-०.०५ माइक्रोग्राम/एम.एल. की सांद्रता प्रयोग में इस्तेमाल किया गया। डोक्सोरोबिसिन मानक संदर्भ दवा के रूप में माना जाता था। एफ.टी.आई.आर.स्पेक्ट्रम १०१९.५२ और १३९६.२१ केन्द्र में शिखर पर पी.ओलेरेसिया और डॉक्सोरोबिसिन का क्लोरोफार्म सत्व निकालने का ५०% सेल के विकास में निषेध हुआ (आयसी ५०) मानव कोलन के ग्रंथिकर्कटता के विरुद्ध और क्रमशः वेरो सेल लाइन के खिलाफ ७६७.६० और २३९२.७१ माइक्रोग्राम/एम.एल. के पी. ओलेरेसिया क्लोरोफार्म सत्व निकालने में पूरे सयंत्र की कार्य क्षमता कम थी। इसमें मानव कोलन के ग्रंथिकर्कटता सेल लाइन के विरुद्ध साइटोटॉक्सिक गतिविधि नहीं थी। यह सामान्य वेरो सेल लाइन के लिए सुरक्षित नहीं था। लेकिन इसमें अलग करने, पहचान करने और फाइटोकेमिस्ट्रिब्यूट्स की वर्तमान पुष्टि करने के लिए परिष्कृत विश्लेषणात्मक तकनीकों की आवश्यकता है।