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Study of Cytotoxic Activity of Two Species of Portulaca on Cancer Cell Lines

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Abstract:

Aim: *Portulaca oleracea* and *Portulaca quadrifida* are found growing as weed in Asia, Africa, and parts of Mediterranean region. These plants belonging to the family Portulacaceae have been used in traditional systems of medicine in Asia and Africa for their pharmacological activities. This study was conducted to study the *in-vitro* cytotoxic activities of extracts of these plants on established cancer cell lines.

Methods: Morphological observations, MTT assay and DNA fragmentation assay were employed to investigate their activity against HeLa and MCF-7 cancer cell lines.

Result: All the extracts exhibited cytotoxicity at higher concentrations and the *P. oleracea* seed extract was found to be most active amongst the extracts studied. Cytotoxicity of *P. quadrifida* harvested from western India has not been reported in detail in literature and this study helps to compare the cytotoxicity of these closely related plant species. The phytochemicals previously identified from these extracts have been reported to possess cytotoxic activity which may help explain the observed cytotoxic activity against cancer cell lines.

Conclusion: Although the crude extracts of the two plant species exhibit cytotoxicity to cancer cell lines *in-vitro*, further *in-vitro* and *in-vivo* studies using isolated phytochemicals from these extracts may provide a source of potential anti-cancer phytochemicals.

Keywords: *Portulaca oleracea*, *Portulaca quadrifida*, cytotoxicity

INTRODUCITON:

Portulaca oleracea, commonly known as purslane or chickenweed or 'ghol' in the local Marathi language, belongs to the family Portulacaceae. The plant has a characteristic sour taste due to which it is consumed in the form of salad, soup or pickle. The ancient folk medicine systems of Asia and Africa state that the plant has various pharmacological properties. The plant is classified taxonomically as Kingdom- Plantae; Order- Caryophyllales; Family- Portulacaceae; Genus – *Portulaca*; Species- *Portulaca oleracea Linn.*

P. oleracea grows in warm climate and is a green annual herb with branched and succulent stem which is horizontal near the base and ascending near the top and is 15- 30 centimeters (cm) tall. The plant water content is over 90 per cent (%). The plant bears small, yellow flowers which grow in clusters of 3-6 on the tips of branches and on forks. The fruit has a transverse aperture and is oblong in shape. The seeds are black in color with a diameter of 0.5 millimeter (mm) [1]. The plant may have originated in Asia, but is now also found in Africa, Middle East, Europe, North America and Australia. Traditional systems of medicine in Asia and Africa make use of the plant for its anticancer, antidiabetic, hypocholesteremic, neuroprotective, hepatoprotective, nephroprotective, anti-inflammatory, antiulcer, antimicrobial activities. In *P. oleracea*, different plant parts contain varying amounts of flavanoid compounds [2]. According to reports, the plant contains flavanoids such as kaempferol, myricetin, luteolin, apigenin, quercetin, genistein and display selective cytoxicity towards SF-268, NCI-H460, SGC-7901 human cancer cell lines [1-3]. The plant also contains alkaloids such as noradrenaline, dopa, cyclodopa, dopamine. *P. oleracea* contains monoterpenes such as aportulosides A and B and diterpenes suc as potulene, β- amyrin type triterpenoids. The plant extracts contain alanine, catechol, saponins, tannins, steroids, carbohydrates, urea, and minerals such as calcium, iron, copper, phosphorus, manganese and zinc [4-5]. Additionally, the plant has been investigated and proven to have neuroprotective activity, anti-cancer activity, anti- ulcer activity, hepatoprotective activity, immune-modulator, nephroprotective activity, anti-diabetic, anti- inflammatory, insecticide and wormicide activities, antimicrobial activity, and anti-asthmatic activity [5-7].

Portulaca quadrifida is another species of Portulaca, commonly known as chickenweed is smaller than P. oleracea with moist leaves and timbered stem with yellow flowers [8]. The taxonomical classification of is Kingdom- Plantae; Order- Caryophyllales; Family- Portulacaceae; Genus – *Portulaca*; Species- *Portulaca quadrifida Linn.* Just like *P. oleracea*, *P. quadrifida* has been indicated to

possess medicinal properties against asthma, cough, urinary discharges, inflammations and ulcers, haemorrhoids [8-9]. These two species of Portulaca are often found growing together as weed and have many active constituents. The present study was conducted to investigate their cytotoxic activity against established cancer cell lines. The ethanolic extracts were prepared using microwave extraction from different parts of plants. This study was beneficial from a pharmacological point of view since the plants were easily available as weed and after significant experimentation might lead to availability of phytochemicals with anti-cancer properties.

MATERIALS AND METHODS:

1. Collection of plant materials and authentication:

The whole plants of *Portualca oleracea L.* and *Portulaca quadrifida L.* were collected from agricultural fields in Walva taluka, District Sangli, Maharashtra State. The plants were found to be growing in the same area as neglected weed. The plants were not supplied with additional nutrients, pesticides, herbicide, etc. The whole plants were harvested in the months of September and October. The plants were separated and any foreign matter such as other plants, twigs, leaves, etc. were disposed off and gently washed under running water.

The two plant species were then authenticated by Dr. Dhanaji S. Pawar, Associate Professor, Department of Botany, M. H. Shinde Mahavidylaya, Tisangi, Maharashtra and the respective specimen were deposited with voucher number V01 and V04 for *Portulaca oleracea L.* and *Portulaca quadrifida L.* respectively.

After authentication, the *P. oleracea* plants were dried in shade. Some of the plants which were to be used as fresh samples, were collected at the time of use and processed further without drying. The seeds of some of the *P. oleracea* plants were separated and processed further. As a result the following four specimens were separated and are referred as samples 1,2,3,4 henceforth:

a) *P. oleracea* fresh whole plant (sample 1)
b) *P. oleracea* seeds (sample 2)
c) *P. oleracea* dry whole plant (sample 3)
d) *P. quadrifida* fresh whole plant (sample 4)

The fresh whole plant samples of *P. oleracea* and *P. quadrifida* were cut into small pieces separately and used for further processes. The dried whole plant samples of *P. oleracea* were ground using mortar- pestle to a uniform powder consistency.

2. Microwave-assisted extraction:

Microwave Assisted Extraction were carried out in a controlled Catalyst microwave system having maximum power output 800 Watt, 50 g sample and 120 ml ethanol for 20 minutes. The extracts were evaporated to dryness 50 – 60 °C in a thermostat water bath. The dry extract thus obtained was stored at -20°C in air tight container until required.

3. Study of cytotoxic activity:

3.1 Cell line maintenance:

The established cell lines used for this study were HeLa and MCF-7 which are derived from cervical and breast cancer, respectively. Both the cell lines were maintained in Minimum Essential Medium (MEM) supplemented with 10 % fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml Streptomycin in 25 cm^2 culture flasks in a humidified 37 °C 5% carbon dioxide (CO_2) incubator. The media in the flasks was replaced every 2-3 days and the cell lines were passaged at sub- confluency.

3.2 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay:

The MTT assay was performed using both HeLa and MCF-7 cell lines separately [10]. For the MTT assay, cell cultures at sub-confluency were dissociated from the culture flasks and cell count was measured using Trypan blue dye and hemocytometer. The cells were then seeded in 96-well culture plates at a density of 10,000 cells per well in the above mentioned media. The plates were then incubated in 37 °C 5% CO_2 incubator for 24 hours. The media was then replaced with fresh media and varying concentrations of extracts (0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml. 0.4 mg/ml, 0.7 mg/ml). No extract was added to the cell control wells. The plates were then incubated for 24 hours in 37 °C 5% CO_2 incubator. The media was then discarded and replaced with fresh media and 0.5 mg/ml MTT reagent and again incubated for 4 hours. After the incubation, the media was discarded and the formazan crystals were dissolved in 200 µl dimethyl sulfoxide (DMSO) and absorbance was measured at 540 nm. Each sample was tested in triplicate and mean value of absorbance was considered used to calculate the % inhibition of cell growth. The samples were tested in three independent trials and results were expressed as % inhibition of cell growth of mean ± standard error of mean (S.E.M.) of the three trials at each sample concentration.

$$\% \text{ Inhibition of cell growth} = \frac{[(Absorbance\ of\ cell\ control - Absorbance\ of\ sample)]}{(Absorbance\ of\ cell\ control)} * 100$$

3.3 De-oxyribose nucleic acid (DNA) fragmentation assay:

The DNA fragmentation assay was performed using both HeLa and MCF-7 cell lines [11]. The cells were harvested at sub-confluency and counted using hemocytometer. 5×10^4 cells per well were seeded in 12-well culture plate for 24 hours in 37 °C 5% CO_2 incubator. The media was then replaced with fresh medium and varying concentrations of extract (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml). No extract was added to the cell control well. The plate was then incubated for 48 hours. After the incubation, the cells were then harvested using Trypsin and washed twice with Hanks' balanced salt solution (HBSS). 300 µl of cell lysis buffer was added to the cell pellet containing 10 mM Tris-HCl, 1 mM EDTA, 0.2% triton X -100, 0.5% sodium dodecyl sulfate (SDS). 5 µl of 10 mg/ml RNase A was added and incubated for 1 hour at 37°C. DNA was then precipitated using $1/10^{th}$ volume 5 M sodium chloride (NaCl) and equal volume of isopropanaol at -80°C for 2 hours. The suspension was the centrifuged at 12,000 rpm for 20 minutes at 4 °C. The precipitated DNA pellet was washed with 400 µl 70% ice-cold ethanol, air- dried and re-suspended in 10 mM Tris 1mM EDTA buffer (pH 8.0). The samples were analyzed using 1.5% agarose gel electrophoresis with ethidium bromide and visualized using

ultra violet light. Along with the samples, 100 base pair (bp) DNA ladder and 1 kilo base pair DNA ladder were also run to estimate the length of DNA fragments of the samples.

RESULTS AND DISCUSSION:

The cytotoxic activity of plant extracts may be attributed to phenolic compounds such as flavanoids, tannins, alkaloids, etc. The study of cytotoxicity of crude plant extracts may lead to isolation of suspected active principles. The cytotoxicity activity of ethanolic extracts obtained using microwave assisted extraction (Samples 1, 2, 3, 4) was tested on established cancer cell lines derived from breast cancer (MCF-7) and cervical cancer (HeLa) using MTT assay. The assay is based on the reduction of MTT reagent to formazan crystals by mitochondrial enzymes of live cells. The absorbance of dissolved formazan may be considered proportional to the number of live cells. The cells were exposed to the extracts for 24 hours and the results obtained were as follows [Figure 1]:

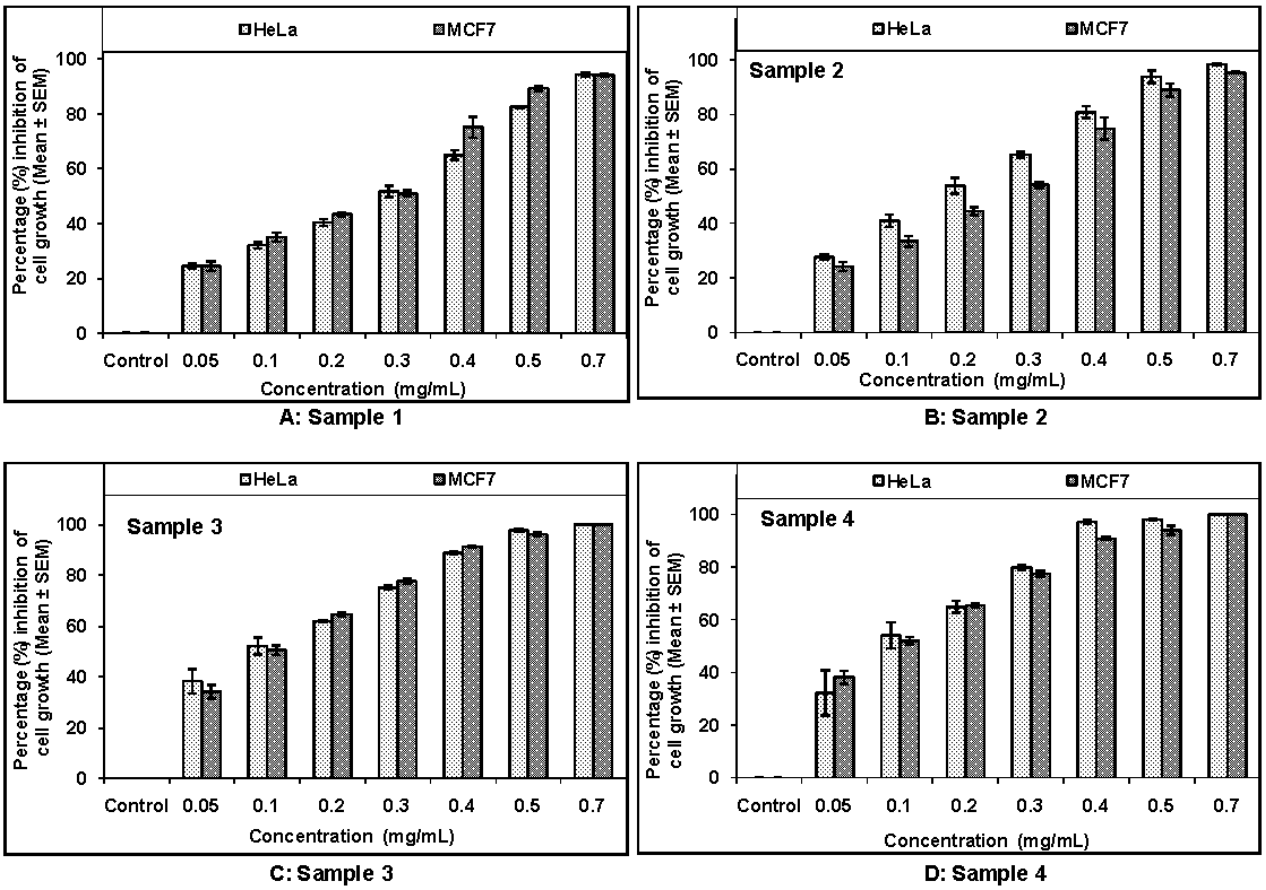
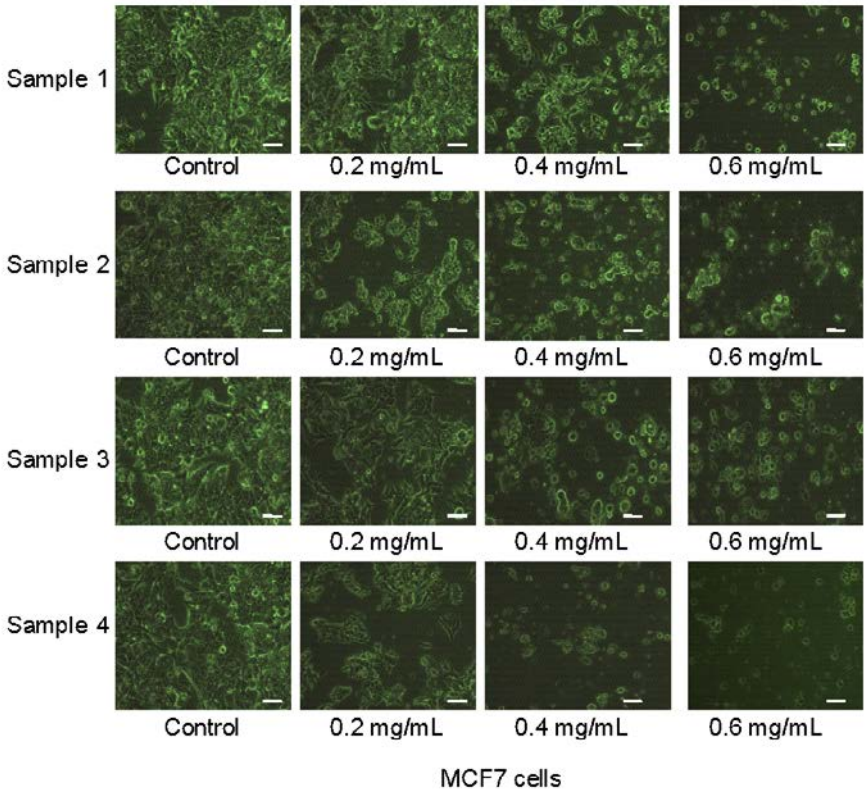


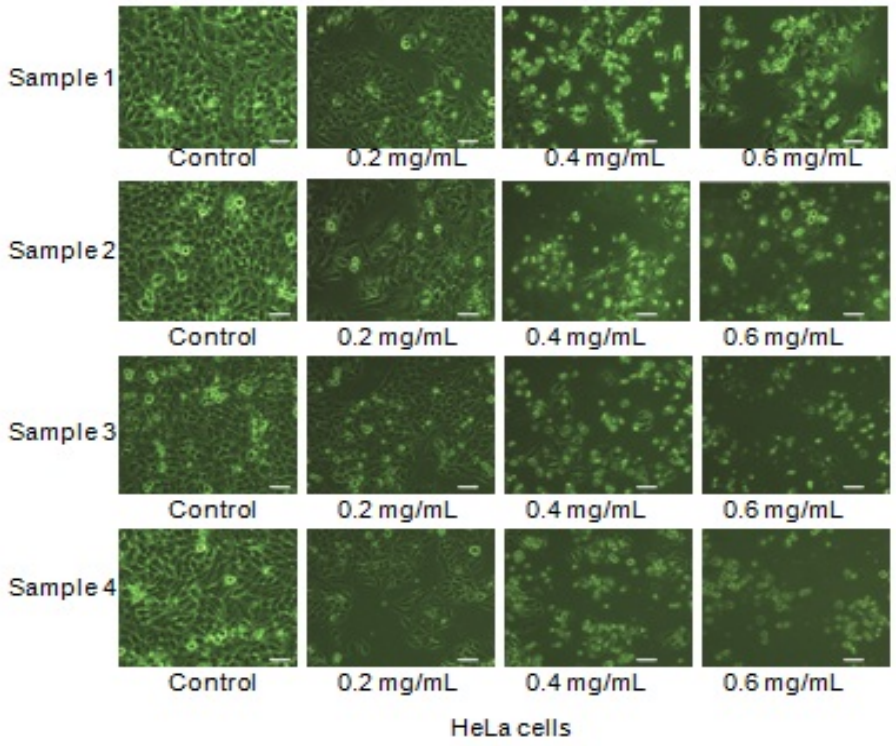
Figure 1: Viability of HeLa and MCF-7 cell lines calculated using MTT assay.

The results of MTT assay for HeLa cell line showed that sample 1, sample 2, sample 3 and sample 4 had significant cytotoxic activity over the concentration range tested ($F = 425.7$, $F = 264.18$, $F = 194.09$, $F = 96.11$, $p<0.05$ respectively). Sample 4 (*P. oleracea* seed extract) obtained using microwave extraction had significantly higher cytotoxicity ($F = 22.458$, $p<0.05$) as compared to other extracts at the lowest concentration of 0.05 mg/mL. Similarly, for MCF-7 cell line sample 1, sample 2, sample 3, and sample 4 had significant cytotoxic activity over the concentration range tested ($F = 374.45$, $F = 655.89$, $F = 273.76$, $F = 703.18$, $p<0.05$ respectively). Sample 4 (*P. oleracea* seed extract) obtained using microwave assisted extraction showed significantly higher cytotoxicity ($F = 58.609$, $p<0.05$) against MCF-7 cell line at the lowest concentration of 0.05 mg/mL.

The cell lines after exposure to the extracts showed dose dependant morphological changes. These morphological changes for MCF-7 cell line were observed using inverted microscope with phase contrast setting and captured as below [Figure 2a]. As can be seen from the images, at higher concentration of the extracts, the cells exhibit rounded borders and appear shrunken indicating dead cells. Similar morphological changes were observed in case of HeLa cells [Figure 2b].



(a)



(b)

Figure 2: Cytotoxic effects of extracts on morphology of (a) MCF-7 cells ; (b) HeLa cells.

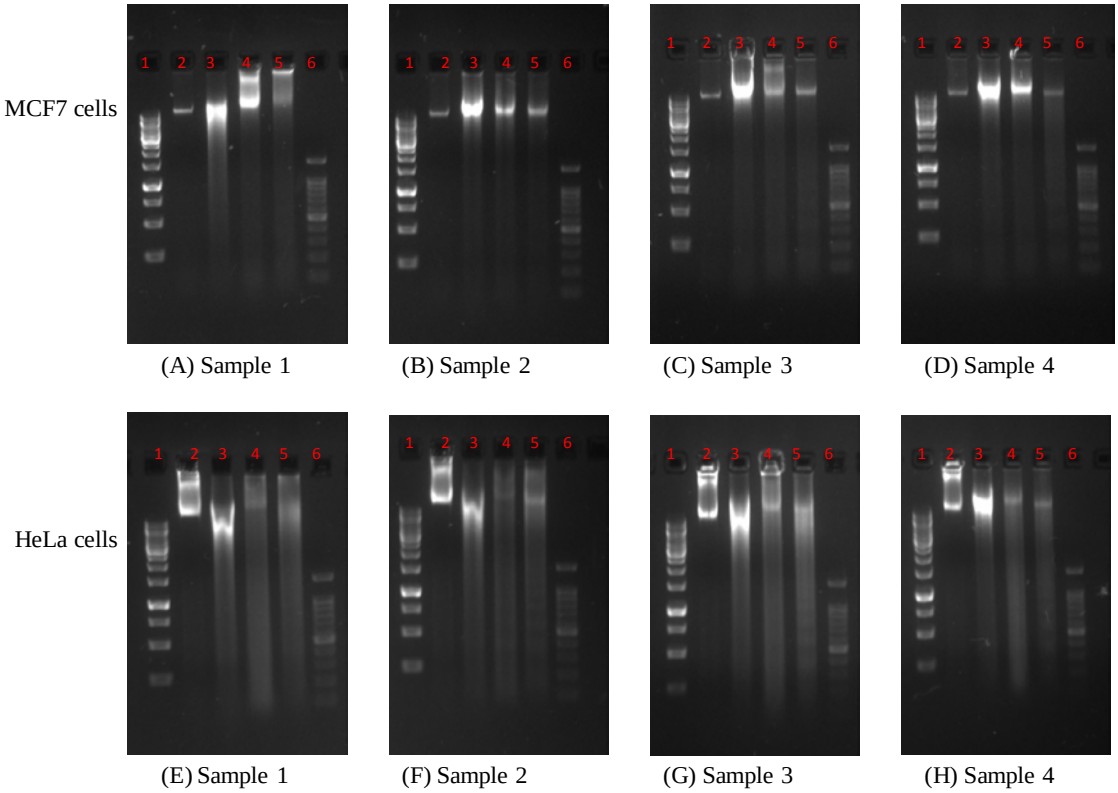


Figure 3: Representative agarose gel images showing DNA fragmentation in MCF7 cells treated with extracts samples 1, 2, 3, 4 (Upper panel) and HeLa cells treated with extract samples 1, 2, 3, 4 (Lower panel) at different solvent extract concentrations. In each representative gel, lane 1 is 1Kb DNA marker : Lane 2 is DNA from control cells followed by lane 3: DNA from 0.2mg/mL concentration of extracts treated cells, lane 4: DNA from 0.4mg/mL concentration of extract treated cells; lane 5: DNA from 0.6mg/mL concentration of extract treated cells and lane 6 is 100 bp DNA marker

Apoptosis or programmed cell death is induced by some cytotoxic agents and is characterized by fragmentation of cellular DNA. This DNA fragmentation assay was performed on MCF-7 and HeLa cell lines. Images of the cells were taken using phase contrast microscope and these images show change in morphology of cells after exposure to the extracts [Figure 3]. The DNA was isolated and analyzed on agarose gel electrophoresis showing DNA fragmentation or 'ladder-like' appearance characteristic of apoptosis. The MTT assay and DNA fragmentation assays showed that sample 4 (*P. oleracea* seed extract) obtained using microwave- assisted extraction showed the highest cytotoxic activity by inducing apoptosis.

Figure 3: Representative agarose gel images showing DNA fragmentation in MCF7 cells treated with extracts samples 1, 2, 3, 4 (Upper panel) and HeLa cells treated with extract samples 1, 2, 3, 4 (Lower panel) at different solvent extract concentrations. In each representative gel, lane 1 is 1Kb DNA marker : Lane 2 is DNA from control cells followed by lane 3: DNA from 0.2mg/mL concentration of extracts treated cells, lane 4: DNA from 0.4mg/mL concentration of extract treated cells; lane 5: DNA from 0.6mg/mL concentration of extract treated cells and lane 6 is 100 bp DNA marker

The extracts were observed to be cytotoxic to cancer cell lines HeLa and MCF-7 evaluated using MTT assay and apoptotic activity was studied using DNA fragmentation assay. Independent groups of researchers have attributed this anti-cancer property to polysaccharides, alkaloids, saponins present in the extracts. The anti-cancer activity of extracts and isolated compounds were demonstrated against HeLa, HepG2, K 562 cancer cell lines [12]. The methanolic extract of *P. oleracea* was reported to inhibit growth of HeLa and MCF-7 cell lines up to 20 % at maximum tested concentration of 100 µg/ ml [13]. The anti-cancer activity observed may be related to the presence of digitoxin, myristoleic acid, oleic acid, tributyl phosphate compounds which were identified in the extracts after GC-MS analysis [14]. The cardiac glycoside digitoxin is a known heart stimulant and cytotoxic agent with proven anticancer activity against a number of cancer cell lines was also found. The monounsaturated fatty acid Myristoleic Acid, also has been reported to be effective as a cytotoxic agent for the treatment of prostate cancer [15-17, https://pubchem.ncbi.nlm.nih.gov/compound/5281119,https

://pubchem.ncbi.nlm.nih.gov/compound/5838]. Oleic acid was reported to possess anti- inflammatory, anti-androgenic, cancer preventive, hypocholesterolemic activities [https://pubchem.ncbi.nlm.nih.gov/compound/445639, https://pubchem.ncbi.nlm.nih.gov/compound/94216, https://pubchem.ncbi.nlm.nih.gov/compound/12397]. Tributyl phosphate has been reported to possess cytotoxic activity [14].

CONCLUSION:

The ethanolic extract of seeds of *P. oleracea* was found to possess most potent cytotoxic activity amongst the extracts tested. Further purification of phytochemicals from this extract might point to potential lead compounds with anti-cancer activity which can be evaluated using *in-vivo* experiments.

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Conflict of interest: None to be declared.

REFERENCES

1. Masoodi MH, Ahmad B, Mir SR, Zargar BA, Tabasum N. *J of Pharm Res*. 2011 , *4(9)*, 3044-8.
2. Kamal-Uddin MD, Juraimi AS, Begum M, Ismail MR, Rahim AA, Othman R. *International Journal of Agriculture and Biology*. 2009, *11(1)*,13-20.
3. Uddin MK, Juraimi AS, Ismail MR, Brosnan JT. *Weed Technology*. 2010, *24(2)*,173-81.
4. Uddin M, Juraimi AS, Hossain MS, Nahar M, Un A, Ali M, Rahman MM. *The Scientific World Journal*. 2014, *2014*.
5. Syed S, Fatima N, Kabeer G. *International journal of Biology and Biotechnology*. 2016, *13(4)*, 637-41.
6. Lim YY, Quah EP. *Food chemistry*. 2007, *103(3)*, 734-40.
7. Uddin M, Juraimi AS, Ali M, Ismail MR. *International journal of molecular sciences*. 2012, *13(8)*,10257-67.
8. Kamil MS, Ahmed MD, Paramjyothi S. *International Journal of PharmTech Research*. 2010, *2(2)*,1386-90.
9. Mulla SK, Paramjyothi S. *International Journal of PharmTech Research*. 2010, *2(3)*,1699-702.
10. Fotakis G, Timbrell JA. *Toxicology letters*. 2006, *160(2)*,171-7.
11. Wiseman H, Halliwell B. *Biochemical Journal*. 1996, *313(1)*,17.
12. Zhou YX, Xin HL, Rahman K, Wang SJ, Peng C, Zhang H. *BioMed research international*, 2015, *2015*.
13. Payudara S, Dan Nasofarinks K, Tan G, Wong K, Pearle-wong GQ, Yeo SL, YEAP SK, YiAP BC, huEh ZA. *Sains Malays*, 2013, *42*, 927-35.
14. Durgawale TP, Khanwelkar CC, Durgawale PP. *Asian J Pharm Clin Res*, 2018, *11(9)*. 204-207.
15. Lunn J, Theobald HE. *Nutrition Bulletin*. 2006, *31(3)*, 178-224.
16. Elbaz HA, Stueckle TA, Tse W, Rojanasakul Y, Dinu CZ. *Experimental hematology & oncology*. 2012, *1(1)*, 4.
17. Inoue M, Craker LE. Medicinal and aromatic plants—Uses and functions. *InHorticulture: Plants for People and Places*, Dordrecht 2014.