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Cytotoxicity Assessments of *Portulaca Oleracea* Plant Extracts on Some Cancer Cells

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Abstract

The present study was designed to investigated the chemical composition, aqueous extracts bioactive compounds and biological activities of *Portulaca oleracea* (PO) on human liver cancer (HepG2), human kidney cancer (Uo-31) cell lines and normal human lung cell lines (Wi-38) and compared with staurosparine (STA) as anticancer drug were evaluated. The obtained results the chemical composition of *Portulaca oleracea* powder was characterized with high protein, ash and total carbohydrate, which amounted in 15.07±0.01, 28.29±0.05 and 40.82±0.60% on D.W, respectively.

The anticancer activity of extracts of *Portulaca oleracea* on three cancer cells line were exposed to various concentrations (0.4- 1.6- 6.3- 25- 100 µg /mL) for 24h. Percentages of cells viability were analyzed by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)). From the obtained results showed that PO induced statistically significant ($p<0.001$) decrease in the cell viability of HepG2 cells in a concentration dependent manner. The HepG2 cells exposed to PO at 100 µg/mL and lower concentrations were found to be cytotoxic. The cells viability of (STA/HepG2) was recorded as 32.70, 42.94, 51.40, 60.44 and 66.67% at concentrations 100, 25, 6.3, 1.6 and 0.4 µg/mL of PO, respectively. The cell viability (S1/HepG2) was recorded as 22.98, 29.56, 38.71, 46.89 and 59.29% at the same concentrations above, respectively.

While, the decrease in the cell viability at 0.4, 1.6, 6.3, 25 and 100 µg/mL of PO extract was recorded to be 73.62, 65.27, 55.52,45.74 and 34.26% for (STA/UO-31) and 78.62, 70.51, 61.88, 52.49 and 38.24% for (S1/UO-31 cells), respectively. On the other hand, the normal cells line for (WI-38 cells) showed the heighest in the cell viability in a concentration dependent manner. The data from this study showed that exposure to extract of *Portulaca oleracea* resulted in significant cytotoxicity and inhibition of growth of the human liver cancer (HepG2) and human kidney cancer (UO-31) cells lines.

From the obtained results, it can be concluded that *Portulaca oleracea* extracts were a significantly reduced cell viability of HepG2 and Uo-31 cells in a concentration-dependent manner. HepG2 cells were also found to be more sensitive to PO extract, according to the findings.

Keywords: *Portulaca oleracea* – Cytotoxicity – Cancer cells – Cell viability – Anticancer agents – Staurosparine.

Introduction

For thousands of years, natural products have played a vital role in cure and prevention of diseases. Plants have grown in popularity as a result of their bioactive chemicals, which have a variety of medical properties and are vital to human health. In comparison to synthetic drugs, the use of plants as herbal therapy has recently been considered a good source of therapeutic compounds with low toxicity, natural origins, fewer side effects, and lower costs **(Morsy, 2015).**

Cancer is one of the major causes of death in developed countries, together with cardiac and cerebrovascular diseases **(WHO, 1998**). Hepatocellular carcinoma is the fourth most deadly cancer in the world **(Mohamad *et al.*, 2013 and Fazeli *et al.*, 2012)**.

Purslane (*Portulaca oleracea*) was an annual green herbaceous medicinal plant that grows in temperate and tropical climates **(Yang *et al.*, 2009).** The pharmacological potential of the *Portulaca oleracea*, such as anti-inflammatory **(Chan *et al.*, 2000),** antioxidant **(Dkhil *et al.*, 2011),** anti-bacterial **(Zhang *et al.*, 2002),** skeletal muscle relaxant **(Parry *et al.*, 1993),** woundhealing **(Rashed *et al.*, 2003),** and in vitro anti-tumor **(Yoon *et al.*, 1999).** The anticancer effect of extracts of *Portulaca oleracea* against hepatocarcinoma cells was tested and preventative features of these plant extracts the anti-hepatocellular carcinoma (HepG2) cell line is a well-established in vitro model **(Li *et al.*, 2014).**

Purslane's medicinal usefulness is mostly due to the presence of numerous biologically active substances, such as phenolic acids, flavonoids, alkaloids, saponins, vitamins, minerals, β-carotene, glutathione, coenzyme Q10 and a high concentration of Omega-3 fatty acids (**Okafor *et al.*, 2014)**.

Zhao *et al.* (2013*)* used *Portulaca oleracea* L. extracted as hypoglycemic and hypolipidemic activities, antioxidant and antitumor activities. They found that treatment of HeLa cell with POL-P3b inhibited cell proliferation, in addition, POL-P3b significantly inhibited tumor growth in U14-bearing mice.

Alam *et al.* (2014) analyzed the methanolic extracts of 13 accessions of purslane for their total phenol content (TPC), total flavonoid contents(TFC), and total carotenoid contents (TCC) and antioxidant activity of extracts was screened using FRAP assay and DPPH radical scavenging methods. They found that ornamental purslane was richer in antioxidant properties, whereas common purslane possesses more mineral contents than ornamental ones.

Farshori *et al.* (2014) studied the pharmacological potential, of *Portulaca oleracea* (PO) and *Petroselinum sativum* (PS) as preventive properties against the human hepatocellular carcinoma cells (HepG2). They found that demonstrated preliminary screening of anticancer activity of *Portulaca oleracea* and *Petroselinum sativum* extracts against HepG2 cells, which can be further used for the development of a potential therapeutic anticancer agent.

Hassan (2014) investigated that biological, histopathological and anticancer effect of *Portulaca oleracea* extracts. Infested rats by toxic hepatitis were feeding orally with aqueous extract compared with silymarin drug which led to prevent the increase of the serum hepatic enzyme level (ALT, AST and ALP), uric acid, nitric oxide, lipid profile and liver malanodialdehyde (MDA). They found that the highest HepG2 dead cell percentage by plant dried powder (0.547 liver cell of HepG2) at concentration of 12.50 µg/mL and the cytotoxic effect was determined with the Ic50 values of 17 µg/mL in HEPG2 cell line.

Al-Sheddi *et al.* (2015) evaluated the cytotoxic effects of *Portulaca oleracea* extract against human liver cancer (HepG2) and human lung cancer (A-549) cell lines. They found that *Portulaca oleracea* extracted showed in a significant cytotoxicity and inhibition of growth of the human liver cancer (HepG2) and human lung cancer (A-549) cell lines.

Habibian *et al.* (2017) studied the effects of dietary supplementation of purslane powder (PP) on performance, blood indices, and antioxidant status in broilers with triiodothyronine (T3)-induced ascites. They found that the supplementation of 3 gkg^{-1} of PP in diet improves oxidative status and reduces ascites incidence in broiler chickens without impairing their growth performance.

Mastud *et al.* (2018) utilized the purslane powder for the development of ice-cream and cookies. They found that purslane powder added ice-cream and cookies contains significant amount of protein and antioxidants with potential health benefits and nutritional characteristics.

Dabbou *et al.* (2020) determined the phytochemical composition and antioxidant potential of leaves and stems of spontaneous population of purslane. They found that stems showed higher content of moisture (89.9%) and anthocyanins (4.61 µg g^{-1} dry matter, DM), whereas leaves revealed higher chlorophyll concentrations (7.42 mg g^{-1} DM). Significantly higher levels of phenolic compounds and antioxidant capacities ($p<0.05$) were obtained in ethanolic extracts, compared with water extracts. Limonene (17.3–32.2%), carvone (38–46%), 2,6-dimethylcyclohexanol (2.2–6.4%), and nonanal (3.4–3.8%) were the most abundant volatiles which analysed by (GC-MS).

Ojah *et al.* (2021) studied the phytochemical and antibacterial properties of extracts from *Portulaca oleracea* Linn. (Purslane) utilized in the management of diseases. They found that phytochemical screening of P. oleracea L. showed the presence of carbohydrates, steroids, triterpenes, cardiac glycosides, and saponins. All extracts showed a high level of minimum inhibition concentration against the pathogens.

The aim of this study is to investigations the antioxidant activity and identify the main constituents presented in *Portulaca oleracea* extracts which are responsible for the bioactivity. Also, Carried out to screen the anticancer activities of *Portulaca oleracea* extracts against cancer cell lines i.e. human liver cancer cell line (HepG2), human kidney cancer cell line (UO-31) and human lung normal cell line (Wi-38).

Materials and Methods

Fresh specimens of *Portulaca oleracea* were collected in (2017) from the Botanical Garden, Agriculture Research Centre (ARC), Faculty of Agriculture at Moshtohor, Benha university and EL-Dair felids, Qalubia Government.

All chemical used in these experiments were provided of high quality and purity and obtained from El-Gomheria Co. Experimental cells were human liver cancer cell line (HepG2), human kidney cancer cell line (UO-31) and human lung normal cell (Wi-38) were obtained from American Type Culture Collection.

Preparation aqueous and methanolic extracts of *Portulaca oleracea* (AEPO and MEPO):

Samples of these materials were prepared by using fresh *Portulaca oleracea* specimens (2kg), then washed and the roots were separated from the leaves and stems. The leaves and stems were air-dried for six weeks before being pulverized in a laboratory mortar and pestle **(Oyedeji and Bolarinwa, 2012)**. The later sample was divided into two samples (A) and (B) for analyzed**.**

-Weighted portions (470 g) of sample (A) were macerated and extracted for 72 h at room temperature with distilled water (1:2 w/v). Also, weighted portions (500 g) of sample (B) were macerated and extracted for 72 h at room temperature with 70 % methanol (1:2 w/v) .Then evaporated in a steam bath, yielding a 12 % and 11%, respectively of the starting material. To make test solutions of known concentrations, the dried

material was reconstituted in distilled water **(Oyedeji and Bolarinwa, 2012).**

Analytical methods:

Chemical composition of *Portulaca oleracea* (Moisture, fat, ash, proteins, and carbohydrates) were determined using procedures according to **AOAC (2016)**.

Preliminary phytochemical screening was carried out by using standard procedure described by **Harborne (1998).** The phenolic acids contents were extracted and measured according to a modified procedure described by **Dragovic-Uzelac *et al.* (2005)**. Also, total flavonoids contents were extracted and determined according to a modification of the procedure described by **Han *et al.* (2007)**.

HPLC-DAD system for analysis of phenolic acids and flavonoids were used and determined according to a modification of the procedure described by **Dragovic-Uzelac *et al.* (2005)**. The phenolic acids and flavonoids were identified by comparing them to standard mixtures, and the results were calculated using response factors derived from known identify standards **(Siriamornpun and Suttajit, 2010).**

Free radical scavenging activity by DPPH method of different extracts of leaves and stems of *portulaca oleracea* plant were measured and the method described by **Braca *et al.* (2001).**

In vitro study:

Cytotoxicity assay protocol:

Plate cells (cells density 1.2–1.8×10^4 cells/well) in a volume of 100 µL complete growth medium + 100 uL of the tested compound per well in a 96-well plate for 24 h. After treatment of cells with the serial concentrations of the compound to be tested incubation is carried out for 48 h at 37ºC, then the plates were to be examined under the inverted microscope and the 3-(4,5-dimethylthiazol-2yl)-2,5-biphenyl tetrazolium bromide (MTT) method of monitoring in vitro cytotoxicity for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 106 cells/cm². For the MTT assay, data were expressed as mean optical density with standard deviations, and the IC_{50} was calculated using the dose-response inhibition equation (Log inhibition versus variable slope). Each test should include a blank containing complete medium without cells according to the method described by **Farshori *et al.* (2014)**

Experimental design:

HepG2, UO-31 and Wi-38 cells were exposed to various concentrations (0.4, 1.6, 6.3, 25.0 and 100.0 µg/mL) of *Portulaca oleracea* extracts for 24 h following the exposures, treated cells were tested for cytotoxicity using (MTT) method in HepG2, UO-31, and WI-38 cells according to the method described by **Farshori *et al.* (2014).**

Statistical analysis:

The statistical analysis was carried out using one-way ANOVA using SPSS, ver. 25 **(IBM Corp. Released 2013)**. Data were treated as a complete randomization design according to **Steel *et al.* (1997)**. Multiple comparisons were carried out applying **Duncun test.** The significance level was set at < 0.05.

Results and Discussion

Chemical composition of *Portulaca oleracea* plant powder was analysed to find the properties of this plant and the obtained results are recorded in the Table (1). From these data, the chemical composition of purslane *(P. oleracea*) powder revealed that moisture content, total protein, crude fat, crude ash and total carbohydrates were found to be 6.07±0.00, 15.07±0.01, 9.75±0.02, 28.29±0.05 and 40.82±0.60%, respectively at dried weight (D.W). *Portulaca oleracea* was characterized with high protein, ash and total carbohydrates**.** These results are in agreement with those reported by **Hassan (2014).**

Table 1. Chemical composition of dried powder *P. oleracea* (mean±SE).

Components	Percentage (%) by dried weight (D.W)
Moisture	**6.07±0.00**
Total Protein	**15.07±0.01**
Crude Fat	**9.75±0.02**
Crude Ash	**28.29±0.05**
Total Carbohydrate	**40.82±0.60**

Fractionation flavonoids and phenolics compounds of *portulaca oleracea* extracts by HPLC:-

The flavonoids and phenolics contents of *P. oleracea* were extracted by methanolic extracts (70%) and measurements of components by HPLC and the obtained results are presented in Tables (2 and 3).

Table 2. Fractionation of purslane (*P. oleracea*) methanolic extracts of flavonoids components by HPLC

Rate time (Rt)	Compontents	Concentrations (µg/mL)
4.6	**Rutin**	6.22
5.2	**Quersestin**	5.02
6.9	**Naringin**	8.11
8.1	**Kampferol**	5.33
9.0	**Apegenin**	6.14
10.0	**Luteolin**	13.45
12.01	**Catechin**	5.14
15.0	Chrysoeriol	20.08

Fractionation of purslane (*P. oleracea*) methanolic extracts showed a contains eight different flavonoids, including rutin, quersestin, naringin, kampferol, apegenin, luteolin, catechin and chrysoeriol as shown in Table (2). From the obtained results it can be seen that chrysoeriol (20.08 µg/mL) was found to be the maximum flavonoids in extract of *P. oleracea* plant, which followed by luteolin (13.45 µg/mL), naringin (8.11 µg/mL), rutin (6.22 µg/mL) and apegenin (6.14 µg/mL). While, the other contains of flavonoids were found to be 5.33,5.14 and 5.02 µg/mL for kampferol, catechin and quersestin, respectively. From the abovementioned results,it could be concluded that *portulaca oleracea* were characterized by highest amount of flavonoids componds. These results are in agreement to these data obtained by **Alam *et al.* (2014), Hassan (2014) and Dabbou *et al.* (2020).**

On the other hand, fractionation of (PO) methanolic extracts of phenolics were estimated and the results are tabulated in Table (3).

From these data, it can be concluded that syringenic acid was a maximum concentration (9.50 µg/mL) followed by caffeic (7.55 µg/mL) and ellagic (7.18 µg/mL). While, the following components of vanillic, gallic, isoferulic and p-coumaric contents were found to be 6.79, 6.42, 6.37 and 5.02 µg/mL, respectively. The other phenolic compounds were found in low concentrations (less than5.0 µg/mL). From the abovementioned data, it could be concluded that *P. oleracea* were characterized by highest amount of phenolics compounds. These results are increments with those reported by **Alam *et al.* (2014)**.

Table (3): Fractionation of purslane (*P. oleracea*) methanolic extracts of phenolics components by HPLC

Rate time (Rt)	Compontents	Concentration(µg/ml)
2.0	**Caffeic**	7.55
3.0	**Syringenic acid**	9.50
4.0	**p-coumaric**	5.02
4.7	**Gentisic**	4.32
6.8	**Gallic**	6.42
7.8	**Protocatechulic**	3.31
9.0	**Ferulic**	4.20
11.0	**Ellagic**	7.18
13.8	**Iso ferulic**	6.37
14.6	Vanillic	6.79

Antioxidant activity of purslane (*P. oleracea*) methanolic extracts by 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The antioxidant activity of plants is the result of the active compounds present in them. In this study the antioxidant activity of purslane fractions were evaluated with the use of in vitro assays, specifically by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity .

Table 4. Determination of free radical-scavenging activity of purslane methanolic extract.

Samples (Conc. µg /mL)	DPPH scavenging%
1000.00	**98.3**
500.00	**96.4**
250.00	**93.8**
125.00	**91.8**
62.50	**84.7**
31.25	**75.1**
15.63	**68.4**
7.81	**58.7**
3.90	**36.6**
1.95	**30.4**

IC50 = 5.5 µg /Ml

The scavenging activity of purslane methanolic extracted at different concentrations (1.95, 3.90, 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500 and 1000 µg /mL) were estimated by using DPPH and the obtained resulted are presented at Table 4). From the obtained data, it can seen that at the concentration of purslane methanolic extracted (PME) increased, a regular increase was observed in DPPH of PME. The highest value as DPPH scavenging (%) were found to be 98.3, 96.4, 93.8 and 91.8 % at concentrations 1000, 500, 250 and 125 µg/mL of PME, respectively. This results indicated that the purslane methanolic extracts had a higher antioxidant activity at high concentrations. While, the overall values of DPPH scavenging (%) were found to be 84.7, 75.1, 68.4, 58.7, 36.6 and 30.4 at concentrations 62.5, 31.25, 15.63, 7.81, 3.90 and 1.95 µg/mL, respectively. From the abovementioned results, it can concluded that as the concentration of phenolic compounds or the degree of hydroxylation of phenolic compounds increases, so does their DPPH radical scavenging activity also increases, which can be defined as antioxidant activity **(Mohdaly *et al.*, 2011).** The IC_{50} value was calculated, which is defined as the concentration of extract that causes a 50% reduction in absorbance. The inhibition concentration IC_{50} value was found to be 5.5 µg /mL for PME.

These results are partially similar to the findings of **Uddin *et al.* (2012)** and **Alam *et al.* (2014)** they demonstrated that purslane fractions contained substantial amounts of important phytochemicals, such as phenolics and flavonoids compounds, and other bioactive compounds. Which showed highest antioxidant activities.

-Cell lines studies for anticancer activity of *Portulaca oleracea* aqueous extracts:

To date, natural materials have been used to extract approximately 60% of anticancer medicines.

The current study was designed to provide comparative data on the in vitro anticancer activity of *Portulaca oleracea* (PO) extracts on human hepatocellular carcinoma cell lines (HepG2), human kidney cancer cell lines (Uo-31) and human lung normal (Wi-38) cell lines therefore, comparing with staurosporine (anticancer drug, STA).

Tetrazolium salt (3-(4,5-dimethylthiazol-2yl)-2,5-biphenyl tetrazolium bromide, (MTT)) assays were used to assess cytotoxicity. For 24 h all cells were exposed to (0.4-100 µg /mL) *Portulaca oleracea* extract. The obtained results are recorded in the Tables (5, 6 and 7).

Human liver cancer (HepG2) cell lines:

Hepatocellular carcinoma (HepG2) cells are endothelial cells derived from the liver. As a result, HepG2 cells are regarded as an excellent cell model system for research on drug design and development, mechanisms underlying hepatocarcinogenesis, xenobiotic metabolism, cytotoxicity, and genotoxicity **(Soldatow *et al.*, 2013).**

The effect of different concentrations of aqueous extracts of *P. oleracea* (PO) on human liver cancer (HepG2) cell lines and compared with anticancer drug (staurosporine, STA) were evaluated.

Cytotoxicity assessments by MTT assay, in HepG2 cells and anticancer drug were exposed to aqueous *portulaca oleracea* (PO) extract are summarized and data are recorded in Table (5). HepG2 cells were exposed to various concentrations (0.4-100µg/mL) of *P. oleracea* (PO) for 24 h. From the obtained results showed that (PO) induced statistically significant ($p<0.001$) decrease in the cell viability of HepG2 cells in a concentration dependent manner. The HepG2 cells exposed to PO at 25 µg/mL and higher concentrations were found to be cytotoxic. The cell viability (STA/HepG2) was recorded as 32.7%, 42.94%, 51.4%, 60.44% and 66.67% at 100, 25, 6.3, 1.6 and 0.4 µg/mL of (PO), respectively. While, the percentage of toxicity at the same concentration (0.4-100 µg/mL) of anticancer drug (STA) with HepG2 cell lines were found to be 67.30, 57.06, 48.60, 39.56 and 33.33%, respectively.

But, the IC_{50} value was defined as sample concentration inhibiting 50% of cell growth, and they were obtained from the lines regression of calibration cure, from these data the value of IC_{50} was found to be 7.15±0.40 µg/mL for anticancer drug under investigation.

On the other hand, the cell viability of the effect different concentrations of aqueous extracts of *P. oleracea* (PO) on human liver cancer cell lines (HepG2) were recorded as 22.98, 29.56, 38.71, 46.89 and 59.29% at 100, 25, 6.3, 1.6 and 0.4 µg/mL of PO, respectively. While, the toxicity percentage were found to be 77.02, 70.44, 61.29, 53.11 and 40.71%, respectively at the same above concentration atims. Therefore, the IC_{50} was calculated and the amounted was 1.23±0.07 µg/mL for PO aqueous extracts.

These results are in accordance with the previous studies showing anticancer activity of PO at 50-500 µg/mL concentration against the human cervical cancer (Hela) and the mouse cervical carcinoma (U14) cells **(Zhao *et al.*, 2013).**

From the obtained results present in this study suggest that the cytotoxicity of the PO might be due to the presence of antioxidant phytochemicals and the presence of bioactive compounds such as, polysaccharides, coumarins, monoterpene glycoside, rich source of omega-3-polyunsaturated fatty acids and flavonoids compounds, particularly kampferol, apegenin, myricetin, quercetin, luteolin, carotene and alkaloids **(Huang and Zou, 2011; Handique *et al.*, 2012; Uddin *et al.*, 2012; Kma, 2013 and Tan *et al.*, 2013)**, which is showing anticancer actively on HepG2 cells.

On the other hand, **Hassan (2014)** found that aqueous extract of *P. oleracea* antiproliferatiue efficacy against HepG2 cell lines. Moreover, it has been confirmed that after increasing the concentration of *P. oleracea* to 100 mg/mL, more HepG2 cells faced to cell death and showed the cytotoxic effect of extracts. Also, **Chen *et al.* (2010)** suggested that water soluble polysaccharides of *P. oleracea* possessed mild cytotoxicity activity against cervical cancer Hela cell line and the sulphated form of these polysaccharides enhanced the anti-tumor effect. *Portulaca oleracea* has contain large amount of dopamine and may possibly play a role as anti-tumor.

Dopamine may inhibit the production or relase of endogenous factor required for cell viability and proliferation **(Mohammad *et al.*, 2011)**.

Table 5. Effect of different concentrations of staurosporine (anti cancer drug STA) and aqueous crude extracts of *P. oleracea* on human cellular cancer (HepG2) cell lines.

Concent-ration (µg/mL)	Anti cancer drug (staurosporine STA)				Human cellular cancer (HepG2)			
	O.D.	**Viability (%)**	**Toxicity (%)**	**IC_{50}**	**O.D.**	**Viability (%)**	**Toxicity (%)**	IC_{50}
0	**0.583**	**100**	**0.00**	**7.15±0.40**	**0.583**	**100**	**0.00**	1.23±0.07
0.4	**0389**	**66.67**	**33.33**		**0.346**	**59.29**	**40.71**	
1.6	**0.352**	**60.44**	**39.56**		**0.273**	**46.89**	**53.11**	
6.3	**0.300**	**51.40**	**48.60**		**0.225**	**38.71**	**61.29**	
25	**0.260**	**42.94**	**57.06**		**0.172**	**29.56**	**70.44**	
100	0.191	32.70	67.30		0.134	22.98	77.02	

Human kidney cancer (UO-31) cell line

Human kidney cancer (UO-31) cells were tested at different concentration of staurosporine (anticancer drug, STA), 0.4-100 µg/mL for cytotoxicity using MTT assays and the obtained results are tabulated in Table (6). The decrease in the cell viability at 0.4, 1.6, 6.3, 25 and 100 µg /mL of anticancer drug staurosporine (STA) were found to be 73.62, 65.27, 55.52, 45.74 and 34.26%, respectively. But, the toxicity percentage were recorded to be 26.38, 34.73, 44.47, 54.26 and 65.74%, respectively for STA, anticancer drug. The value of IC_{50} was found to 12.40±0.70 µg /mL for STA.

Also, Table (6) show that the effect of different concentrations of aqueous extracts of *P. oleracea* (0.4 to 100 µg/mL) on human kidney cancer (UO-31) cell lines and the obtained data are recorded in them. From these results the cell viability were found to be 78.62, 70.51, 61.88, 52.49 and 38.24%, respectively after the UO-31 cells were exposed for 24 h.

Recently, plant extracts have also been shown to reduce the cell viability of various cancer cells, including human epidermoid cancer cells (Hep2), human breast adenocarcinoma cells (MCF-7), human amniotic epithelial cells (WISH), human lung cancer cells and UO-31 cells **(Al-Oqail *et al.*, 2013; Farshori *et al.*, 2013 and Al-Sheddi *et al.*, 2014)**.

Table 6. Effect of different concentrations of staurosporine (anti cancer drug STA) and aqueous crude extracts of *P. oleracea* on human kidney cancer (UO-31) cell lines.

Concent-ration (µg/mL)	Anti cancer drug (staurosporine STA)				Human kidney cancer (UO-31)			
	O.D.	Viability (%)	Toxicity (%)	IC_{50}	O.D.	Viability (%)	Toxicity (%)	IC_{50}
0	**0.575**	**100**	**0.00**	**12.40±0.70**	**0.575**	**100**	**0.00**	26.70±1.50
0.4	**0.423**	**73.62**	**26.38**		**0.452**	**78.62**	**21.38**	
1.6	**0.375**	**65.27**	**34.73**		**0.406**	**70.51**	**29.49**	
6.3	**0.319**	**55.52**	**44.48**		**0.356**	**61.88**	**38.12**	
25	**0.263**	**45.74**	**54.26**		**0.302**	**52.49**	**47.51**	
100	0.197	34.26	65.74		0.220	38.24	61.76	

-Human lung normal (Wi-38) cells line

The effect of different concentrations (0.4 to 100 mg/ml) of aqueous extracts from PO on human lung normal (Wi-38) cell lines, then evaluated by MTT assays and the obtained results are presented in Table (7). From these data, the values of the cell viability at 0.4, 1.6, 6.3, 25 and 100 µg/mL of PO STA were recorded to be 93.08, 80.40, 71.13, 56.74 and 43.83%, respectively. While, the percentage of toxicity under the same concentrations were found to be 6.92, 19.60, 28.87, 43.26 and 56.17%, respectively for STA anticancer drug. The IC_{50} was found to 54.2±3.05 µg/mL. On the other hand, when Wi-38 cell lines were exposed to PO extracts at different concentrations (0.4-100 µg/mL) show the highest of cell viability percentage and these values were 95.51, 86.46, 73.27, 60.16 and 49.68%, respectively at the above concentrations.

But, the percentage toxicity were recorded to be 4.49, 13.54, 26.73, 39.84 and 50.32%, respectively with aqueous extracted of *P. oleracea* at the same concentrations. While, the IC_{50} was 93.5±5.25 µg/mL for P.O.

From these data, it can be concluded that the *Portulaca oleracea* extract was a significantly reduced cell viability and altered cellular morphology in a dose-dependent manner. Among the cell lines, HepG2 cells demonstrated the greatest decrease in cell viability, followed by UO-31 cells in MTT assays. While, a normal cell line (Wi-38 cells) demonstrated the greatest increase in cell viability in a concentration dependent manner.

Table 7. Effect of different concentrations of staurosporine (anti cancer drug STA) and aqueous crude extracts of *P. oleracea* on humn lung normal (Wi-38) cell lines.

Concent-ration (µg/ml)	Anti cancer drug (staurosporine STA)				Human lung cancer (Wi-38)			
	O.D.	Viability (%)	Toxicity (%)	IC_{50}	O.D.	Viability (%)	Toxicity (%)	IC_{50}
0	**0.468**	**100**	**0.00**	**54.20±3.05**	**0.468**	**100**	**0.00**	93.50±5.25
0.4	**0.435**	**93.08**	**6.92**		**0.447**	**95.51**	**4.49**	
1.6	**0.376**	**80.40**	**19.60**		**0.404**	**86.46**	**13.54**	
6.3	**0.333**	**71.13**	**28.87**		**0.343**	**73.27**	**26.73**	
25	**0.265**	**56.74**	**43.26**		**0.281**	**60.16**	**39.84**	
100	0.205	43.83	56.17		0.232	49.68	50.32	

From the abovementioned results, it can be concluded that *Portulaca oleracea* (PO) extracts were significantly reduced cell viability of HepG2 and Uo-31 cells in a concentration-dependent manner. HepG2 cells were found to be more sensitive to PO extract, according to the findings. Also, *P. oleracea* aqueous extracts can act as an anticancer herbal drug.

References

AOAC (2016). Official Methods of Analysis of AOAC International; Horwitz, W., Latimer, G., Eds.; AOAC Int: Gaithersburg, MD, USA, 2016.

Alam, M.; Juraimi, A.S.; Rafii, M.Y.; Abdul Hamid, A.; Aslani, F.; Hasan, M.M. and Uddin, M. (2014). Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulaca oleracea* L.) accessions. BioMed. Res. Int. vol 2014, 10 pages.

Al-Oqail, M.M.; Farshori, N.N.; Al-Sheddi, E.S.; Musarrat, J.; Al-Khedhairy, A.A. and Siddiqui, M.A. (2013). *In vitro* cytotoxic activity of seed oil of fenugreek against various cancer cell lines. Asian Pac. J. Cancer Prev., 14(3): 1829-1832.

Al-Sheddi, E.S.; Farshori, N.N.; Al-Oqail, M.M.; Musarrat, J.; Al-Khedhairy, A.A. and Siddiqui, M.A. (2014). Cytotoxicity of Nigella sativa seed oil and extract against human lung cancer cell line. Asian Pac. J. Cancer Prev., 15(2): 983-987.

Al-Sheddi, E.S.; Farshori, N.N.; Al-Oqail, M.M.; Musarrat, J.; Al-Khedhairy, A.A. and Siddiqui, M.A. (2015). *Portulaca oleracea* seed

oil extracts cytotoxic effects on human liver cancer (HepG2) and human lung cancer (A- 549) cell lines. Asian Pac. J. Cancer Prev., 16(8): 3383-3387.

Braca, A.; Tommasi, N.D.; Bari, L.D.; Pizza, C.; Politi, M. and Morelli, I. (2001). Antioxidant principles from *Bauhinia terapotensis*. J. Nat Prod., 64: 892–895.

Chan, K.; Islam, M.W.; Kamil, M.A.; Radhakrishnan, R.; Zakaria, M.N.M.; Habibullah, M. and Attas, A. (2000). The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. Sativa (Haw.) Celak. J. ethnopharma, 73(3): 445-451.

Chen, T.; Wang, J.; Li, Y.; Shen, J.; Zhao, T. and Zhang, H. (2010). Sulfated modification and cytotoxicity of *Portulaca oleracea* L. polysaccharides. Glycoconjugate J, 27(6): 635-642.

Dabbou, S.; Lahbib, K.; Pandino, G.; Dabbou, S. and Lombardo, S. (2020). Evaluation of pigments, phenolic and volatile compounds, and antioxidant activity of a spontaneous population of *Portulaca oleracea* L. grown in tunisia. Agr., 10(8): 353.

Dkhil, M.A.; Moniem, A.E.A.; Al-Quraishy, S. and Saleh, R.A. (2011). Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. J. Med. Plants Res., *5*(9): 1589-1593.

Dragovic-Uzelac, V.; Pospišil, J.; Levaj, B. and Delonga, K. (2005). The study of phenolic profiles of raw apricots and apples and their purees by HPLC for the evaluation of apricot nectars and jams authenticity. Food Chem., 91: 373–383.

Farshori, N.N., Al-Sheddi, E.S.; Al-Oqail, M.M.; Musarrat, J.; Al-Khedhairy, A.A. and Siddiqui, M.A. (2013). Anticancer activity of Petroselinum sativum seed extracts on MCF-7 human breast cancer cells. Asian Pac. J. Cancer Prev., 14(10): 5719-5723.

Farshori, N.N.; Al-Sheddi, E.S.; Al-Oqail, M.M.; Musarrat, J.; Al-Khedhairy, A.A. and Siddiqui, M.A. (2014). Cytotoxicity assessments of *Portulaca oleracea* and Petroselinum sativum seed extracts on human hepatocellular carcinoma cells (HepG2). Asian Pac. J . Cancer Prev., 15(16): 6633-6638.

Fazeli, Z.; Pourhoseingholi, M.A.; Vahedi, M. and Zali, M.R. (2012). Burden of hepatocellular carcinoma in Asia. Asian Pac. J. Cancer Prev. 13: 5955-8.

Han, J.; Ye, M.; Xu, M.; Sun, J.; Wang, B. and Guo. D. (2007). Characterization of flavonoids in the traditional Chinese herbal medicine-Huangqin by liquid chromatography coupled with electrospray ionization mass spectrometry. J. Chromatogr. B, 848: 355–362.

Handique JG, Boruah MP and Kalita D (2012). Antioxidant activities and total phenolic and flavonoid contents in three indigenous medicinal vegetables of north-east India. Nat. Prod. Commun., 7, 1021-3.

Harborne, J.B. (1998). Phytochemical methods: A guide to modern technique of plant analysis, 3rd Ed, Champman and Hall, London, pp. 302.

Hassan, A. (2014). Chemical and remedial effects of purslane (*Portulaca oleracea*) plant. Life Sci. J., 11: 31-42.

Habibian, M.; Sadeghi, G. and Karimi, A. (2017). Effects of purslane (*Portulaca oleracea* L.) powder on growth performance, blood indices, and antioxidant status in broiler chickens with triiodothyronine-induced ascites. Archives Animal Breeding, 60(3): 315-325.

Huang, W. and Zou, K. (2011). Cytotoxicity of a plant steroidal saponinon human lung cancer cells. Asian Pac. J. Cancer Prev., 12: 513-517.

Kma, L. (2013). Roles of plant extracts and constituents in cervical cancer therapy. Asian Pac. J. Cancer Prev., 14: 3429-36.

Li, J.P.; Cao, N.X.; Jiang, R.T.; He, S.J.; Huang, T.M.; Wu, B. and Luo, G.R. (2014). Knockdown of GCF2/LRRFIP1 by RNAi causes cell growth inhibition and increased apoptosis in human hepatoma HepG2 cells. Asian Pac. J. Cancer Prev., 15: 2753-8

Mastud, S.K.; Mote, G.V. and Sahoo, A.K. (2018). Development of value-added products by using purslane (*Portulaca oleracea*). Int. J. Pharma. Phytochem., 7: 1761-1766.

Mohammad, M. H., Abdul-Gany, Z. S., & Hassan, A. A. (2011). Cytotoxic effect of the aqueous extract of Portulaca oleracea L. on some cell lines. *J. Biotechnology Res. Center*, *5*(1), 5-10.

Mohamad, A.P.; Fazeli, Z.; Ashtari, S. and Fatemeh, S.F.B. (2013). Mortality trends of gastrointestinal cancers in Iranian population. Gastroenterol Hepatol Bed to Bench, 6.

Mohdaly, A., Smetanska, I., Ramadana, M., Sarhan, M. andMahmoud, A. 2011. Antioxidant Potential of Sesame (Sesamum indicum) Cake Extract in Stabilization of Sunflower and Soybean Oils. Ind. Crops Prod., 34: 952-959.

Morsy, S.A.M. (2015). Biochemical studies on jatropha plant. MSC, Biochemistry Del, Fac of agri, ben.

Ojah, E.O.; Oladele, E.O. and Chukwuemeka, P. (2021). Phytochemical and antibacterial properties of root extracts from *Portulaca oleracea* Linn. (Purslane) utilised in the management of diseases in Nigeria. J. Med. Plants for Economic Development, 5(1): 103.

Okafor, I.A.; Ayalokunrin, M.B. and Orachu, L.A.(2014): A review on *Portulaca oleracea* (purslane) plant – its nature and biomedical benefits, Int. J. Biomed. Res., 5: 75–80.

Oyedeji K.O. and Bolarinwa A.F. (2012). Effects of crude extracts of *Portulaca oleracea* on

haematological and biochemical parameters in albino rats, Afr. J. Biomed. Res., 15: 41 – 47.

Parkin, D.M.; Bray, F.; Ferlay, J. and Pisani, P. (2005). Global cancer statistics, 2002. CA. Cancer. J. Clin., 55: 74-108.

Parry, O.; Marks, J.A. and Okwuasaba, F.K. (1993). The skeletal muscle relaxant action of *Protulaca oleracea*. J. Ethnopharma 40: 187-194.

Rashed, A.N.; Afifi, F.U. and Disi, A.M. (2003). Simple evaluation of the wound healing activity of a crude extract of *Portulacaoleracea L.* (growing in Jordan)in usmusculus JVI- 1. J. Ethnopharmacol, 88(2-3): 131- 136.

Siriamornpun, S. and Suttajit, M. (2010). Microchemical components and antioxidant activity of different morphological parts of Thai wild purslane (*Portulaca oleracea*). Weed Sci., 58(3): 182-188.

Soldatow, V.Y.; LeCluyse, E.L.; Griffith, L.G. and Rusyn, I. (2013). In vitro models for liver toxicity testing. Toxicology Res., 2(1): 23-39.

Sparg, S.G.; Light, M.E. and Van Staden, J. (2004). Biological activities and distribution of plant saponins. J. Ethnopharma, 94: 219-243.

Steel, R.; Torrie, J. and Dickey, D. (1997): Principles and procedures of Statistics: A Biometrical Approach, 3rd Ed., McGraw-Hill, New York, NY.

Tan, G.C.S.; Wong, K.M.; Pearle-Wong, G.Q.; Yeo, S.L.; Yeap, S.K.; Yiap, B.C. and Chong, H.Z. (2013). In vitro Cytotoxic and antiproliferative effects of *Portulaca oleracea* methanol extract on breast, cervical, colon and nasopharyngeal cancerous cell lines. Sains Malays, 42: 927-35**.**

Uddin, M.; Juraimi, A.S.; Ali, M. and Ismail, M.R. (2012). Evaluation of antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. Int. J. Molecular. Sci., 13(8): 10257-10267.

World Health Organization (WHO) (1998). World Health Statistics Annual 1996, World Health Organization, Geneva.

Yang, Z., Liu, C., Xiang, L., and Zheng, Y. (2009). Phenolic alkaloids as a new class of antioxidants in *Portulaca oleracea*. Phytotherapy Research: An Int. J. Devoted to Pharma and Toxicological Evaluation of Nat. Product Derivatives, 23(7): 1032-1035.

Yoon, J.W.; Ham, S.S. and Jun, H.S. (1999). *Portulaca oleracea* and tumor cell growth. US Patent. 5869060. Washington DC: Patent and Trademark Office.

Zhang, X.J.; Ji, Y.B.; Qu, Z.; Xia, J. and Wang, L. (2002). Experimental studies on antibiotic functions of *Portulaca oleracea* L in vitro. Chin. J. Microbiol., 14(35): 277-80.

Zhao, R.; Gao, X.; Cai, Y.; Shao, X.; Jia, G.; Huang, Y. and Zheng, X. (2013). Antitumor activity of *Portulaca oleracea L.* polysaccharides against cervical carcinoma *In vitro* and *In vivo*. Carbohydrate Polymers, 96(2): 376-383.

تقييم السمية الخلوية لمستخلصات نبات الرجلة علي بعض الخلايا السرطانية

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*** قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة بنها – مصر**

تهدف هذه الدراسة إلي إلقاء الضوء على المستخلصات المائية والكحولية لنبته الرجلة (البقلة) وكذلك فؤائدها العلاجية للتصدى لخلايا الكبد ,الكلي (السرطانية) والرئه (الطبيعيه) وتأثيرها كمضادات للأكسدة ولذا إشتملت الدراسة على ما يلى:

إعداد وتجهيز المستخلصات المائية والكحولية من نبته الرجلة بغرض تقدير المكونات الكيميائية الحيوية ذات النشاط الحيوى وذلك بإستخدام أحدث الأجهزة بجهاز HPLC– DAD – system لتقدير الفينولات والفلافونيدات و تقييم النشاط المضاد للأكسدة لمستخلصات الرجلة. وكذلك تم إجراء دراسات لفحص الأنشطة المضادة للسرطان لمستخلص الرجلة على كل من خلايا الكبد السرطانية من نوع ($HepG_2$) وخلايا الكلي السرطانية من نوع (UO–31) وكذلك تم إستخدام خلايا الرئة الطبيعية (كحالة كنترول) من نوع (WI–38). وكذلك استخدم مادة علاجية مضادة للسرطان (أستيرو إسبرين STA).

أظهرت النتائج التى تم التحصل عليها أن المكونات الرئيسية الكيميائية للرجلة فكانت نسبة الرطوبة (6.07±صفر)، البروتينات الكلية 0.10±15.07 والدهون الخام تمثل نسبة 0.2±9.75 وبينما الرماد الخام فكان 0.05±28.29 وأخيراً كانت نسبة الكربوهيدرات الكلية تمثل أعلى نسبة فكانت 0.016±40.82% على أساس الوزن الجاف.

– تم تقييم السمية الخلوية بإستخدام مقايسات إم تى تى MTT يشير إلي أن النتائج التي تم الحصول عليها أظهرت أن مستخلص الرجلة *Portulaca oleracea* ذات دلالة إحصائية ($P<0.001$) يسبب انخفاض في حيوية الخلية لخلايا (HepG2) بطريقة تعتمد على التركيز وخلايا (HepG2) المعرضة لمستخلص الرجلة عند 100 ميكروجرام/ملليلتر وتركيزات اقل كانت سامه للخلايا. تم تسجيل حيويه الخلية لخلايا الكبد السرطانية والمقارنة مع الدواء المضاد للسرطان (STA/HepG2) على أنها 32.70 و 42.94 و 51.40 و 60.44 و 66.67٪ عند التركيزات 100 ، 25 ، 6.3 ، 1.6 و 0.4 ميكروجرام/ملليلتر من الدواء المضاد للسرطان، على التوالي. تم تسجيل حيويه الخلية لخلايا (S1/HepG2) على أنها 22.98 ، 29.56 ، 38.71 ، 46.89 و 59.29٪ بنفس التركيزات أعلاه. بينما تم تسجيل الانخفاض في حيويه الخلية عند 0.4, 1.6,6.3, 25 و 100 ميكروجرام/مل من الدواء المضاد للسرطان بنسبة 73.62, 65.27, 55.52, 45.74 و 34.26٪ لخلايا الكبد السرطانية (STA/UO–31) و 78.62, 70.51, 61.88, 52.49 و 38.24٪ (لخلايا S1/UO–31) على التوالي.

من ناحية أخرى ، أظهر خط الخلايا الطبيعي لخلايا الرئة الطبيعية (WI–38) الارتفاع في حيويه الخلية اعتمادا على التركيز.

من النتائج التي تم الحصول عليها يمكن استنتاج أن مستخلصات الرجله غيرت الشكل الخلوي لخلايا الكبد السرطانية (HepG2) وخلايا الكلى السرطانية (Uo–31) اعتمادا على التركيز. كذلك كانت خلايا الكبد السرطانية (HepG2) كانت أكثر حساسية لمستخلص الرجله.