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Antitumor and antioxidant activity of *Aloe vera* leaf gel extract

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ABSTRACT

The purpose of the study was to determine the *in-vitro* antitumor and antioxidant activity of *Aloe vera* gel extract by evaluating the cytotoxic effect of extract and Zoladex (goserelin*)* on breast cancer cell line (MCF-7) and prostate cancer cell line (PC-3), in addition to measure the antioxidant activity to find out this kind of anticancer drug, which is a cheap, safe, less toxic, and more active drug contrasted to chemotherapy drug. The cytotoxic effect evaluated by cytotoxicity assay (MTT assay), while the activity of the *Aloe Vera* gel as antioxidant was tested using a scavenging test of free radical (2,2-Diphenyl-1-picrylhydrazyl) (DPPH), ferric reducing antioxidant potential (FRAP) and (Oxygen Radical Absorbance Capacity)(ORAC). Treatment of the extract against breast cancer cell line (MCF-7) and prostate cancer cell line (PC-3), in all concentrations, indicateda decrease in cell viability percentage with the concentration increasing. *Aloe vera* extract showed its best activity in the concentration of 400 ìg/ml. The same results observed with Zoladex treatment for breast cancer cell line (MCF-7) and prostate cancer cell line (PC-3) which represented a decrease in cell viability percentage with the concentration increasing. *Aloe vera* gel extract was more than to the activity of Zoladex drug and revealed that *Aloe vera* extract exhibits good cytotoxic activity. The antioxidant properties of the *Aloe vera* gel extract was effective. Antioxidant activities of *Aloe Vera* gel extract were estimated using different concentrations starting with 200 µg/ml, 100µg/ml, 50µg/ml, 25 µg/ml and, 12.5µg/ml. *Aloe Vera* gel extract have the maximum scavenging activity at 200µg/ml.

Key words : *antioxidant activity, Antitumor, Aloe vera*

Introduction

Plant-based systems persist to play an crucialrole in the essential health care of 80% of the world's population (Farnsworth *et al.*, 1985). Because of chemical studies directed toward the isolation of the active substances from plants used in traditional medicinean increasing number of chemotherapeutic agents are detected (Cragg *et al.*, 1997). Cell and tissue damages in the body induces by the presence of free radicals, this type of damage is known as oxidative damage (Halliwell and Gueridge, 1989). Cancer is a disease characterized by uncontrolled divisionand spread of abnormal arrangements of the body's own cells. It is one of the major reasons of death in the developed nations (Kumi Diaka *et al.*, 1999) in terms of incidence among women worldwide breast cancer is the leading tumor (Ferlay *et al.*, 2010). Among men prostate cancer is the second leading tumor in terms of world-wide incidence (Mistry *et al.*, 2011). As indicated by the World Health Organization expresses that 80% of the total populace utilizes therapeutic plants for the treatment of countless infections,the plants are presently utilized in drug arrangements by drug companies more than before. *Aloe vera* is adjusted to parched

and semiarid conditions and is notable for its potential wellbeing advancing properties, for example, immuno-incitement and cell regeneration (Mikolajczak, 2018). *Aloe Vera* is a short-stemmed bush also known as a wonder plant; it belongs to a family of many other plants of blooming, and most of this kind of plants is normally found in North Africa whichhave fleshy, erect and thick circular arrangement leaves. The gel acquired from the plant's leaves have got numerous utilization (Minjares-Fuentes *et al.*, 2017). *Aloe vera* has been the subject of attention throughout the most recent years to researchers, with respect to a few asserted remedial properties (Phumying *et al.*, 2013). As per England Kew garden research which is one of the royal botanical centers for excellence in the UK have named *Aloe Vera* which has been used many yearsas one of the current most popular medicinal plant (Quezada *et al.*, 2017). The human use for *Aloe vera* earliest researchers is dated back to sixteenth century and was initially from Egyptian clinical record known as Ebers Papyrus and is broadly utilized today in food, it is endorsed by the FDA as Herbal cures, food enhancements, seasoning and beautifiers (Nyerges, 2016). *Aloe vera* has various active components for instance, the minerals, salicylic, vitamins, amino acids, lignin, saponins, enzymes and the sugars (Femenia *et al.*, 1999).

Materials and Methods

Aloe Vera plant were collected from the plantation of college of Science for Women University of Baghdad and identified by professor Dr. Ali Almosawi at University of Baghdad, College of Science - Biology Department

Preparation of *Aloe vera* gel

Freshly expanded leaves of plant were selected, carefully washed from dust with water and cleaned by filter paper. After that, the leaves were longitudinally divided in two parts. The gel was obtained by scraping with a spoon followed by homogenized in a blender.

Aloe vera crude extract

The extract was prepared depending to Noor *et al*. After leaves were washed. The lower leaf base and the small spines are removed by blades. The epidermis of the leaves was removed to collect the parenchymatous tissue. The sold, colorless gel was cut into small parts. Finally lyophilized and ground the required gel of *Aloe vera* (Noor *et al.*, 2008).

Cytotoxicity assay (MTT assay)

The MTT assay was carried out in the MCF-7 cell lines and PC3 cell linesto determine the anticancer activity of *Aloe vera* gel and Zoladex (goserelin).

Depend on the manufacturer's directions for agreement (Rashid and Umamaheswari, 2017). The cells (1 x 10 4 to 1 x 10 6 cells/ ml) were cultured in 96-well plates to a final volume of 200 ml well. The plates were covered with gently shacked and incubated for 24 hours at 5% CO_2 with 37 °C. Then, the medium was discharged, and 2-fold serial dilution of the*Aloe vera* gel and Zoladex (6.2, 12.5, 25, 50, 100, 200, 400 mg ml) was added to eachwell. Triplicate was achievedfor all concentrations and control. For 48 hours the plates were incubated at 37 °C, 5%CO_2. After treatmentwith the compounds, 10 ml of MTT solution was added to the wells. Additional time of incubation for 4 hours at 37 °C, 5% CO_2. The medium was then aspirated, 100 ml of the dissolution solution was added to each well with incubation for 5 minutes. ELISA reader was used to examine the absorbance (Bio-rad, Germany) at 575 nm wavelength. Statistical analysis was done on the optical density to measure the IC50. Depending to the below equation:

Viability (%) = (optical density of sample/optical density of control) ×100

Free radical antioxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl)

The activity of the *Aloe vera* gel as antioxidant was tested using a scavenging test of free radical (2,2-Diphenyl-1-picrylhydrazyl)(DPPH) (Tohma, 2016). The radical scavenging activity of samples against DPPH free radicals was measured spectrophotometrically. Colorimetric differences from violet to lightyellow were measured at 517 nm. In this assay, a set of concentrations (12.5, 25, 50, 100 and 200 µg ml) was depended, while theascorbic acidbeas a control. The activity of free radical calculated as bellow:

$$\text{Inhibition\%} = \frac{\text{Abssorbance of} - \text{vecontrol} - \text{Absorbance of sample}}{\text{Absorbance of} - \text{vecontrol}}$$

Ferric reducing antioxidant potential (FRAP)

ab234626 Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Colorimetric)

1. Reaction mixand Background Mix was Prepared.
2. Into wells of standard, sample and positive control, 190 µl of Reaction Mix was added.
3. Into the wells of the background control sample, 190 µl of Background Reaction Mix was added.
4. Evaluate absorbance directly at 594 nm for 60 minutes at 37 °C.

ORAC (Oxygen Radical Absorbance Capacity) ab233473 ORAC Assay Kit

1. The diluted Antioxidant Standard or sampleswas added 25 µl to each 96-well Microtiter Plate.
2. To each well ,150 µl of the 1X Fluorescein Solution was Added. Mix gently then Incubated the plate for 30 minutes at 37 °C.
3. The Free Radical Initiat or Solution 25 µl was added to each well.
4. For homogeneity, reaction mixture mixed gently by pipetting.
5. Directly start the reading by a fluorescent microplate reader at 37 ºC.

Statistical analysis

In forms of mean ± standard deviation, the results of the tested parameters were given, and variation between them were calculated by (ANOVA) then the Duncan test.

Results and Discussion

Cytotoxicity assay

The cytotoxicity assay(MTT) was used to evaluate the cytotoxic effect of *Aloe vera gel* extract and Zoladex (goserelin*)* on breast cancer cell line (MCF-7) and prostate cancer cell line (PC-3). The viability of cell and inhibition percentage of tumor cell line was estimated by MTT Assay using various concentrations of *Aloe vera gel* extract and Zoladex. The concentrations ranged between 6.2-400 µg/ml. For *Aloe Vera gel* extract effect on breast cancer cell line (MCF-7), the results showed a decrease in cell viability in a dose-dependent manner, which decreased with increasing of concentrations. Cell viability of *Aloe vera* gel extracton MCF-7 reached the minimum in 400 µg/ml (52.66±2.47) while the maximum was at 6.2 µg/ml reached (95.67±0.29), as show in (Table 1). *Aloe vera* extract caused inhibitory effective with IC50 of 113.5 µg/mlon WRI-68 cell line while the IC50 of 453.9 µg/ml was obtained from the effect of extract on breast cancer cell line (MCF-7).

Cell viability of *Aloe vera* gel extract on prostate cancer cell line (PC-3) reached the minimum in 400 µg/ml (53.04±4.97) while the maximum was at 6.2 µg/ml reached (94.98±1.10), as show in (Table 2). *Aloe Vera* extract caused inhibitory effective with IC50 of 209.8 µg/ml on WRI-68 cell line while the IC50 of 207.0 µg/ml was obtained from the effect of extract on prostate cancer cell line (PC-3).

The anticancer drug Zoladex (goserelin) was evaluated for their cytotoxic activity on breast cancer cell line (MCF-7) and prostate cancer cell line (PC-3). For breast cancer cell line (MCF-7), signifi-

Table 2. Cytotoxicity effect of *Aloe vera* gel extract on PC-3 and WRI-68 cells

Aloe Concentrations (µg/ml)	Viable cell count of PC3cell line Mean± S.D.	Viable cell count of WRL-68 cell line Mean± S.D.
400	53.04±4.97	57.39±5.61
200	71.68±1.86	77.54±1.51
100	88.19±0.46	94.29±1.83
50	95.56±0.52	95.83±0.30
25	95.29±0.26	95.37±0.90
12.5	94.94±0.86	96.64±0.70
6.25	94.98±1.10	95.67±0.40

Table 1. Cytotoxicity effect of *Aloe vera* gel extract on MCF-7 and WRI-68 cells

Aloe Concentrations (ìg/ml)	Viable cell count of MCF-7 cell lineMean± S.D.	Viable cell count of WRL-68 cell lineMean± S.D.
400	52.66±2.47	75.84±4.78
200	63.00±3.90	87.92±3.57
100	74.34±3.32	92.20±2.77
50	87.19±5.69	96.48±1.29
25	95.98±0.98	96.95±1.14
12.5	95.98±0.94	94.29±2.97
6.25	95.67±0.29	96.06±0.11

cant reduction in viability was observed at 400 µg/ml of the drug (60.34±6.75), while it reached the maximum at 6.25 µg/ml (94.36±1.37), as show in (Table 3). Zoladex caused inhibitory effective with IC50 of 212.2 µg/mlon WRI-68 cell line while the IC50 of 120.1 µg/ml was obtained from the effect of drug on breast cancer cell line (MCF-7).

The anticancer drug Zoladex (goserelin) was evaluated for their cytotoxic activity onprostate cancer cell line (PC-3). Significant reduction in viability was observed at 400 µg/ml of the drug (76.00±2.32), while it reached the maximum at 6.25 µg/ml (95.94±0.20), as show in (Table 4). Zoladex caused inhibitory effective with IC50 of 224.5 µg/ml on WRI-68 cell line while the IC50 of 200.7 µg/ml was obtained from the effect of drug on prostate cancer cell line (PC-3).

Table 3. Cytotoxicity effect of Zoladex (goserelin) on MCF-7 and WRI-68 cells

Zoladex Concentrations (µg/ml)	Viable cell count of MCF-7 cell line Mean± S.D.	Viable cell count of WRL-68 cell line Mean± S.D.
400	60.34±6.75	75.73±2.73
200	69.59±1.21	84.49±2.53
100	78.85±3.01	92.13±1.55
50	93.71±0.77	95.02±0.87
25	95.06±0.06	95.71±1.00
12.5	94.52±0.85	94.90±2.19
6.25	94.36±1.37	95.33±0.87

Table 4. Cytotoxicity effect of Zoladex (goserelin) on PC-3 and WRI-68 cells

Zoladex Concentrations (µg/ml)	Viable cell count of PC3 cell line Mean± S.D.	Viable cell count of WRL-68 cell line Mean± S.D.
400	76.00±2.32	81.96±0.75
200	84.79±1.20	88.27±2.60
100	93.59±2.10	93.59±2.10
50	95.33±1.18	95.33±1.18
25	95.21±0.82	95.21±0.82
12.5	95.94±1.02	95.94±1.02
6.25	95.94±0.20	95.94±0.20

Antioxidant activities of *Aloe vera* gel extract

Antioxidant Activities of *Aloe vera* gel extract were estimated using different concentrations start with 200 µg/ml, 100 µg/ml, 50 µg/ml, 25µg/ml and, 12.5 µg/ml using three methods of scavenging activities. (1, 1-diphenyl 1-2-picryl-hydrazyl) DPPH assay, oxygen radical absorbance capacity (ORAC), andferric reducing antioxidant potential (FRAP).

Depending to the results in Table 5, 200µg/ml of *Aloe vera* gel extract have the maximum scavenging activity (76.00±2.64%) while the lowest scavenging activity of *Aloe vera* gel extract (17.63±7.19%) was at 12.5µg/ml. At the same time the Vitamin C showed differences in scavenging activity between the concentrations.

Aloe vera gel extract was more effective in FRAP scavenging activity than vitamin C at all concentrations (200 µg/ml, 100µg/ml, 50µg/ml, 25µg/ml and, 12.5µg/ml). According to the results in table (5), the concentration 200µg/ml of *Aloe vera* gel extract have the maximum scavenging activity (66.17±1.99%), while the lowest activity (16.74±3.91%) observed at 12.5µg/ml. At the same time the Vitamin C showed differences in scavenging activity between the concentrations.

The results of (Table 5) , showed that *Aloe vera* gel extract was more effective in ORAC scavenging activity than vitamin C at concentrations (200 µg/ml, 100 µg/ml, 50 µg/ml). At the same time the Vitamin C showed differences in scavenging activity between the concentrations.

Discussion

The outcomes demonstrated that *Aloe vera* extracts

Table 5. Antioxidant activity DPPH assay, ORAC, FRAP of *Aloe Vera* gel extract and vitamin C.

Concentrations (µg/mL)	DPPH assay Mean± S.D.		FRAPMean± S.D.		ORACMean± S.D.	
	Aloe Vera Extract	Vitamin C	*Aloe Vera* Extract	Vitamin C	*Aloe Vera* Extract	Vitamin C
200	*76.00±2.64	86.04±2.49	66.17±1.99	60.34±8.12	72.99±1.91	69.79±6.99
100	**64.97±1.74	76.21±1.00	55.36±1.63	46.53±7.92	60.22±2.60	55.71±8.43
50	55.63±0.85	60.70±1.63	36.77±8.23	33.14±8.80	50.00±3.98	39.04±5.48
25	39.85±5.35	38.62±2.90	28.97±4.26	24.61±1.38	28.70±7.33	29.24±5.20
12.5	17.63±7.19	23.64±3.29	16.74±3.91	15.78±0.935	14.54±1.34	17.40±4.20

has solid anticancer activity and repressed the development of MCF-7 and PC-3 cells through the induction of modified cell passing or apoptosis. The values of the IC50 showed that the *Aloe vera* extracts had a more powerful cytotoxic impact on MCF-7 cells contrasted with PC-3 cells. The distinction in affectability to the *Aloe vera* extracts between MCF-7 and PC-3 malignant growth cells and ordinary cells proposed that *Aloevera* extracts can be utilized as a chemotherapeutic for therapy of human intense myeloid leukemia and the breast cancer. Then again, in disease treatment killing dangerous cells through apoptosis is as ideal cell death. Thusly, current outcomes show the capability of the *Aloe vera* extracts as a fundamental enemy of cancer to repress tumor cell development and trigger apoptosis.

Majority of the studies have showed that plants are as a significant source of bioactive specialists that can instigate apoptosis in malignant growth cells (Wang *et al.*, 2012; Iqbal *et al.*, 2017). *Aloe vera* is one of the plants that have been utilized in customary medication for quite a long time to treat various illnesses, for example, diabetes, asthma, and herpes. Lately numerous confirmations have indicated that *Aloe vera* crude extract can go about as hostile to malignant growth alone or synergistically with chemotherapeutic medications (Shalabi *et al.*, 2015; Tomasin *et al.*, 2011). For moment, aloe-emodin, an anthraquinone compound present in the *Aloe vera* leaves, has shown anticancer movement against esophageal, colon, and pancreatic malignant growth cells and numerous different sorts of tumors. It appears to be that enemy of malignancy movement of the aloe-emodin is by down directing many key cancer advancing particles without cytotoxic impacts (Sanders *et al.*, 2017). As needs be, in the current examination, against malignant growth property, the cytotoxic and apoptotic action of *Aloe vera* separate on MCF-7 and PC-3 cancer cells were assessed.

Aloe vera gel composed mainly of (> 98%) water and PLS, involving pectins, cellulose, hemicellulose, glucomannan and acemannan, the latter considered as the main functional component of the gel. The mannose-6 p compound, which is a component of the gel sugar, possess ahealing activity of the wounds. In addition, glycoproteins found in the gel have antitumor and anti-ulcer activity and may increase the proliferation of normal human epithelial cells (Vijay and Swapna, 2018).

Emodin, anextract of *Aloe vera*, was appeared to restrain cell multiplication and prompt apoptosis in human liver disease cell lines through p53-and p21-subordinate pathways (Kuo *et al.*, 2002). Acemannan, a starch part got from *Aloe vera* leaf, has been found to induce cytokine creation in mouse macrophage cell line (Zhang and Tizard, 1996). It additionally showed immunomodulating activity by actuating development of dendritic cells (Lee *et al.*, 2001). Furthermore, aloe ride, a polysaccharide got from *Aloe vera* juice, was accounted for to be a powerful immunostimulatory that demonstrations by improving NF-kappa B exercises (Pugh *et al.*, 2001). What is more, di(2-ethylhexyl) phthalate (DEHP), obtain from *Aloe vera*, restrained leukemic cells, *in vitro* (Lee *et al.*, 2000).

Various investigations have been led to study the mechanisms of activity of aloe. Oligosaccharides from aloe extract were found to prevent bright radiation-instigated concealment of postponed type touchiness by lessening keratinocyte-determined immunosuppressive cytokines (Byeon and Pelley, 1998). Proposed instrument hidden enemy of psoriatic impact incorporates restraint of tumor necrosis factor (TNF) alpha initiated multiplication of keratinocytes and overactivation of the atomic factor (NF kappa B flagging pathway, by an aloe polysaccharide (Leng *et al.*, 2018). Furthermore, a polymer division of aloe was appeared to ensure the gastric mucosa against ethanol-instigated gastric harm by diminishing mRNA articulation levels of inducible nitric oxide synthase (iNOS), neuronal nitric oxide synthase (nNOS), and lattice metalloproteinase (MMP-9). The three catalysts are basic biomarkers in gastric ulceration (Park *et al.*, 2011). Different discoveries recommend that the radio-defensive impacts of aloe polysaccharides aredue toinhibition of apoptosis (Wang *et al.*, 2004).

Conclusion

This work is mechanized to ensure the ability of *Aloe vera* herbal extracts and Zoladex (goserelin) to inhibit the cancer cells growth and proliferation. The result indicted that *Aloe vera* possess a good antitumor propriety. so, this plant of *Aloe vera*, can be considered during cancer chemotherapy, In addition to antioxidant potential of *Aloe Vera* gel extract.

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