Iraqi Journal of Science, 2023, Vol. 64, No. 3, pp: 1160-1170
DOI: 10.24996/ijs.2023.64.3.12

Iraqi Journal of Science

ISSN: 0067-2904

Studying some cytotoxic and cytogenetic potentials of Dandelion methanolic extract on MCF-7 cancer cell line: an *in vitro* study

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Received: 4/2/2022 Accepted: 2/7/2022 Published: 30/3/2023

Abstract

This study aimed to evaluate the anticancer activity and cell division arresting by dandelion methanolic extract on breast cancer cell line MCF-7 cancer cell line. For achieving this goal, cytotoxicity assay (MTT assay), multipara system assay: High Content Screening (HCS) which include (viable cell count VCC; membrane permeability MP; cellular mitochondrial permeability CMP; nuclear intensity NI and cytochrome C releasing), reactive oxygen species detection and cell cycle phases division were tested. The results of this study showed the ability of the plant to reduce cancer cell viability in a dose-dependant manner within IC50 (141.0) in comparison to IC50 ~~of~~ (334.4) on the human normal cell line (WRL-68). Furthermore, the results of HCS demonstrated the ability of plants to enhance apoptosis of cancer cells and increase the production of ROS from cancer cells treated with plant extract and doxorubicin drug in comparison with negative control (50.07±2.2, 53.03±2.6 *vs.* 44.77±1.4) respectively. Also, the results of the cell cycle for control, 400 µg\ml of dandelion extract and doxorubicin drug showed the ability to arrest the division of cancer cells at G1 phase by decreasing its transmission to S, G2, and M phases of the cell cycle (62%, 59% and 62.46% at G1) (27.46%,26.50% and 24.66% at S Phase) (16.50%, 20.50% and 18.46% at G2\M) respectively. All these effects are attributed to the active compounds (secondary metabolites) such as terpenes, polyphenols, and different minerals that possess anticancer activity against different cell lines like MCF7.

Keywords: breast cancer, MTT assay, cell cycle, apoptosis, Free radicals, antioxidant.

التأثير السمي والوراثي الخلوي للمستخلص الميثانولي لنبات الداندليون على المزرعة الخلوية لخط سرطان الثدي : دراسه خارج جسم الكائن الحي

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الخلاصة

صممت هذه الدراسة لتقييم الفعالية المضاده للسرطان للمستخلص الميثانولي لنبات الداندليون على مزرعة الخلايا السرطانية لسرطان الثدي. تمت هذه التجربة من خلال اختبارات :حساب التركيز القاتل لنصف

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الخلايا MTT , (High Content Screening (HCS, الكشف عن تولد الجذور الحرة و مراحل الانقسام الخلوي. اظهرت النتائج قابلية البات على خفض حيوية الخلية بالاعتماد على التراكيز المستخدمه حيث كان التركيز القاتل لنصف الخلايا (IC50: 141.0) بالمقارنة مع التركيز القاتل للخلايا الطبيعية (IC50: 334.4). كما اظهرت نتائج (HCS) قدرة النبات وعقار الدوكسروبسن على زيادة انتاج الجذور الحرة من الخلايا السرطانية بالمقارنة مع الخلايا الغير معاملة (44.77±1.4 *vs.* 50.07±2.2, 53.03±2.6) على التوالي . كما اظهرت نتائج الانقسام الخلوي للخلايا الغير معاملة, والمعاملة بالنبات بتركيز 400 ملغم\مل وعقار الدوكسروبسن على قابلية النبات في حصر انقسام الخلايا السرطانية في طور G1 عن طريقه تقليل انتقال الخلايا السرطانية الى مراحل الانقسام الخلوي الاخرى المتمثلة باطوار S, G2 and M, G1 حيث كانت النتائج

(62%, 59% and 62.46% at G1)

(27.46%,26.50% and 24.66% at S Phase) (16.50%, 20.50% and 18.46% at G2\M) على التوالي. تعزى كل هذه التاثيرات الى وجود مركبات الايض الثانوي (التربينات, متعدد الفينولات وغيرها من المركبات التي تمتلك الخهاصية المضادة للسرطان كسرطان الثدي

1. Introduction

For hundreds of years, plants well thought-out as the ideal innate supply of treatments and curing of different diseases. New developments in science like biotechnology were created to use plants in a variety of manufacturing works to produce different biological compounds such as; therapeutic proteins in addition to producing drugs, medicines, and therapies [1]. The production of different Pharmaceuticals from plants are the result of an advanced application of biotechnology to permit them to use by the medical community to combat life-threatening illnesses, such as heart disease, cancer, HIV, diabetes, and cystic fibrosis[2]. The *Taraxacum* genus belongs to the Asteraceae family is scattered in different sites of the world especially the extreme zone of the Northern hemisphere and variety of sites of the world. The using of this plant differs according to the country that originated from it [3]. Taraxacum spp called (Dandelions) exhibited different activities such as anti-inflammatory, anti-oxidative, and diuretic activities of various parts of this plant [4]. Recent studies from our lab display a strong anti-cancer activity of dandelion extract [5]. Different studies demonstrated the ability of the plant to induce apoptosis on various cancer cell lines such as breast cancer cell line (MCF7) by affecting extracellular signal-regulated kinase(ERK) and affect on apoptotic factors like Bcl-2, Bax, and PARP-1[6]. Dandelion offers a forceful outline of bioactive components with different biological activities [7]. These include chicoric acid, taraxasterol (TS), sesquiterpene lactones, polyphenolic in leaves (the leaf extracts effectively against obesity and cardiovascular disease) and stems while different types of carbohydrates, carotenoids, minerals, sugars (e.g. glucose, fructose, and sucrose), choline vitamins, mucilage, and pectin are present in root[8] in addition to inulin and fructo-oligosaccharides that comprise (45% of root consist) with beneficial biological effects such as pathogens removal in the gastrointestinal tract, and repression of obesity, cancer, and osteoporosis [9,10,11]. This study was designed to assess the cytotoxic and cytogenetic effects of the dandelion methanolic extract on breast cancer cell line (MCF7).

2. Materials and Methods

2.1 Plant collection and Methanolic Extraction

The plant was collected during Sep. 2021 from Baghdad local market which was previously identified by the National Herbarium of Iraq then after washing the plant with distilled water and dried, the aerial parts of plant were extracted, which (50 g) of plant was extracted with

about (250 ml) of 80% methanol at 65°C for 3 hours using soxhlet apparatus then dryness to yield dried crude extract for laboratory assessments [12].

2.2 Cytotoxic Effect of dandelion extract on MCF-7 cancer cell line

- **MTT assay**

Different concentrations of plant extract ranging from (6.2, 12.5, 25, 50, 100, 200, and 400 µg/mL) were used to determine the cytotoxic effects of dandelion *in vitro* on MCF-7 by using a kit of MTT (Bio-Rad, USA) by using micro-titer plates (96-well). After cell line maintenance according to Freshney, 2010 [13] (briefly, after the cells in the vessel made confluent monolayer and washed with PBS, 3 ml of Trypsin\versine solution were added then the cells were incubated at 37 °C for 1 to 2 minutes, then 15 ml of RPMI medium added and further incubated at 37 °C in 5 % CO_2 incubator then the full concentration of Cell was accomplished by counting the cells using the haemocytometer). MCF-7 cells (1×10^4–1×10^6 cells mL^{-1}) were seeded at final volume (200 µL) and cultured (37 °C for 24 h). Then, the prepared concentrations from plant (6.2- 400 µg/mL) were added to each well and incubated for 24 h, followed by the addition of 10 µL of MTT solution, and also incubated at 37 °C, 5% -CO_2 for 4 h. Hundred mL of solubilisation solution from the kit was added after removing the media and further incubated (5 min). An ELISA microplate reader (at 570nm) was used to determine the formation of formazan.

- **High-content screening assay**

The method of Palaniswamy *et al*., 2012 [14] was used, using a kit from Thermo Scientific Cellomics multi-parameter cytotoxicity 3 kit (Thermo Scientific, Japan). Different plant concentrations (100, 200 and 400 µm), doxorubicin (20 µm), solutions and buffers were used in this essay such as (Cytochrome c) acts as a primary antibody and DyLight™ 649 conjugated goat anti-mouse IgG, Hoechst stain, (10× Dulbecco's phosphate buffered saline [PBS]), permeabilization buffer (10× Dulbecco's PBS with 1% Triton® X-100), and a blocking buffer (10×) was used for the immediate recognition of HCS parameters in the MCF7 breast cancer cell line followed by HCS system that sensitively involved to a computerized imaging microscope (ArrayScan XTI, ThermoScientific) (with a Zeiss 40× (0.75 NA) in which intensity of fluorescence cells imaged. The mitochondrial membrane potential (MMP) and the cell permeability dyes were added to cells treated with plant extract at different concentrations for 24 h and incubated for 30 min at 37 °C.

- **MnSOD and phospho-H2AX Induction Assay**

Plant extract (400 µm) and doxorubicin drug (20 µm) were used in this essay in addition to the control group (cells without any treatment). The method performed according to Culotta, 2006, [15] in which both (Paraquate was diluted to 1mM and iron citrate to 400µg/ml by using a culture medium and incubated to16-20 h. at 37ºC in 5% CO2. Then 50 µl from 400 µm plant, doxorubicin drug were added to wells and incubated 24 h., then, fixation solution (100 µl) added for 25 minutes followed by washing plate twice with 100 µl wash buffer and permeabilization buffers and incubated for 15 minutes at room temperature. Primary antibody solution 50 µl was added and incubated for 1 hour then aspirated and washed with blocking buffer. After that, FBS 100 µl was added, and after 15min (50 µl) of secondary antibodies were added and incubated for 45 min. Finally all buffer aspirate and washed with washing buffer and measured on the Array Scan HCS Reader.

- **Cell Cycle Analysis**

MCF-7 cell cycle was analyzed using a commercial kit (BD Bioscience, SanJose, CA)(Cycle Test™ Plus DNA). Cells were seeded at a concentration of 5 x 10^5 cells per well in plates incubated at 37 °C, 5 % CO_2 for 24 hrs., after removing media, cells were treated with the plant extract at 400 µg/mL and doxorubicin for 24 hours then the growth media aspirated and cell washed with PBS. Three ml of trypsin/versine (solution A) was added and incubated for 2 minutes and RPMI-1640 medium (15-20 mL) was added followed by centrifuged for 5 minutes at 300rpm and 1mL of buffer and 250µL of trypsin buffer was added after removing the supernatant. Solution B 200µL was added and incubated for 10 minutes. Finally, 200µL of cold (2°C–8°C) propidium iodide (PI) stain solution was added and put in the refrigerator, and filtered for flow cytometer analysis at 488 nm providing a blue-to-green range, and histograms were examined with appropriate DNA analysis software.

2.3 Statistical analysis

Graph Pad Prism Software Inc., La Jolla version 6 program used to analyze data as mean± standard deviation

3. Results and discussion

3.1. Cytotoxic effects of the plant extract using MTT assay

The results of these parameters indicated that cell viability was decreased in MCF-7 cell viability percentage noted by 400µg/ml (49.15± 5.184) while 6.2 µg/ml represented the uppermost cell viability to be reached (96.06± 0.5306). The IC50 rate of 141.0 µg/ml of plant cytotoxicity effect on cancer cells while the IC50 of 344.4 µg/ml on normal cell WRI-68 as shown in (Table 1)(Figure 1).

Table 1: Cytotoxicity effect of plant extract on MCF-7 and WRI-68 cells.

DANDELION concentrations µg/ml	Viable cell count of MCF7 cell line Mean± S.D.	Viable cell count of WRL-68 cell line Mean± S.D.
400	49.15± 5.184	73.42±3.387
200	61.96± 0.8209	86.03±0.8535
100	76.35± 3.244	92.13±1.557
50	87.65± 1.044	96.18±1.252
25	96.95± 1.752	96.95±1.142
12.5	96.03± 3.076	94.91±2.199
6.25	96.06± 0.5306	96.10±0.4820

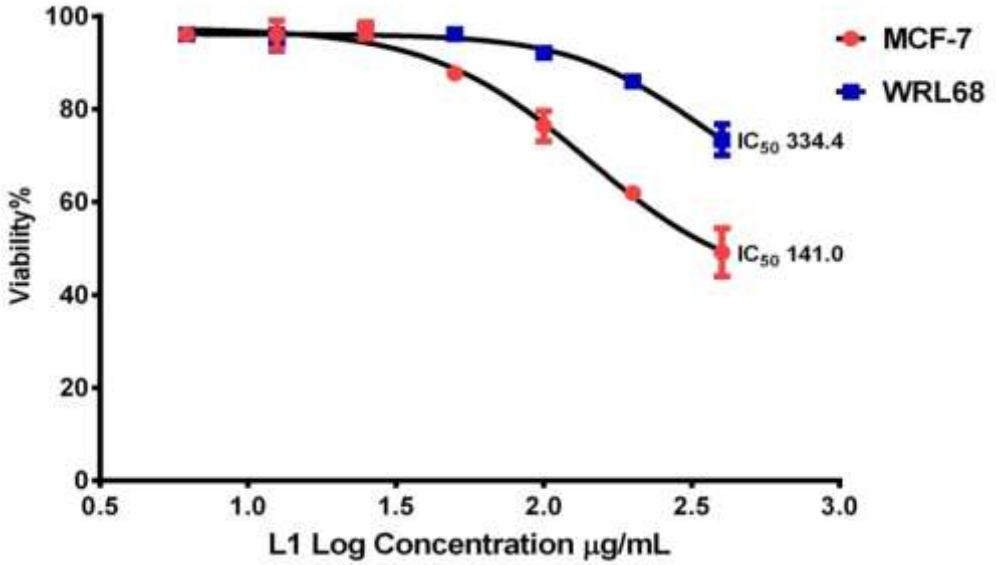


Figure 1: Cytotoxicity effect of plant extract on MCF-7 and WRI-68 cells using MMT assay.

3.2. Effects of plant extract on multipara system High Content Screening (HCS) :

The results of viable cell count ranged from 1008±1563, 1251±15.56, and 1505±26.16 for plant concentration (400, 200 and100 µg\ml) respectively in comparison with negative and positive controls (1790±14.14 and 975.5±6.36) as shown in (Figure 2)

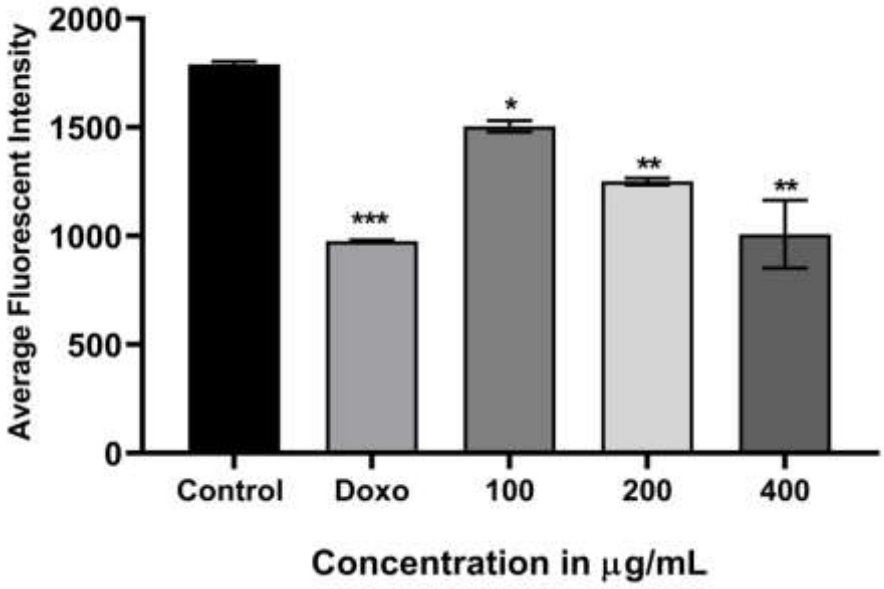


Figure 2: Cytotoxicity effect of plant extracts on viable cell count of MCF-7cell line on multipara system High Content Screening at 37 °C. Data expressed as (mean ± SD), *,**,***: $p \leq 0.01$, SD: Standard Deviation. (n = 3).

Membrane permeability results showed that 400 and 200 µg\ml of plant concentration increased the permeability significantly to (925.5±15.06 and 790.3±19.07) in comparison with negative control while 100 µg\ml showed non-significant differences to control group (Figure 3).

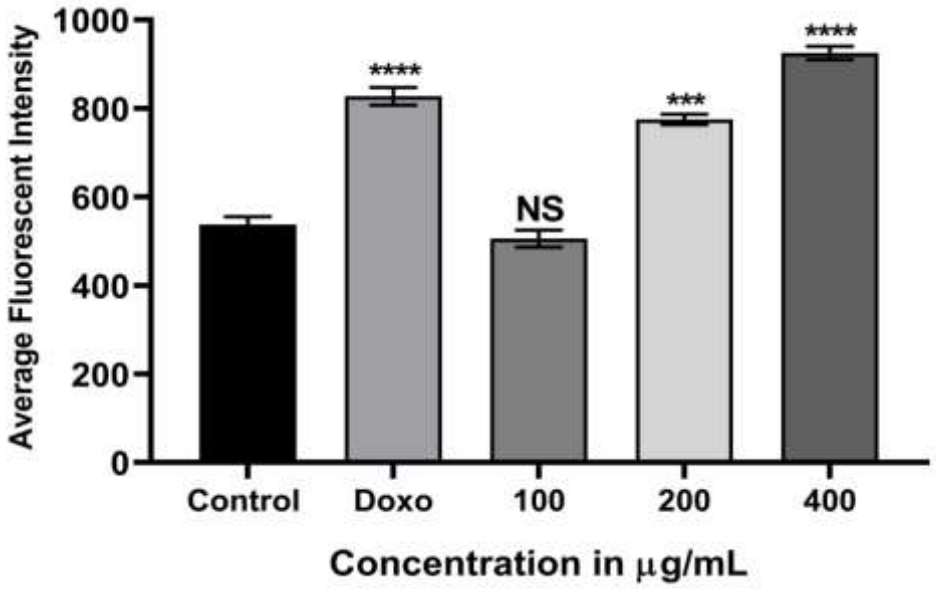


Figure 3- Cytotoxicity effect of dandelion extract on cell membrane permeability of MCF-7cell line on multipara system High Content Screening at 37 °C. Data expressed as (mean ± SD), *,**,***: $p \leq 0.01$, SD: Standard Deviation. (n = 3), NS: non-significant.

While the results of **MMP** indicated that both (400 and 200 µg\ml) caused significant differences in comparison with negative control (229.1±10.82 and 433.6±10.89 *vs.*433±10.86) (Figure 4).

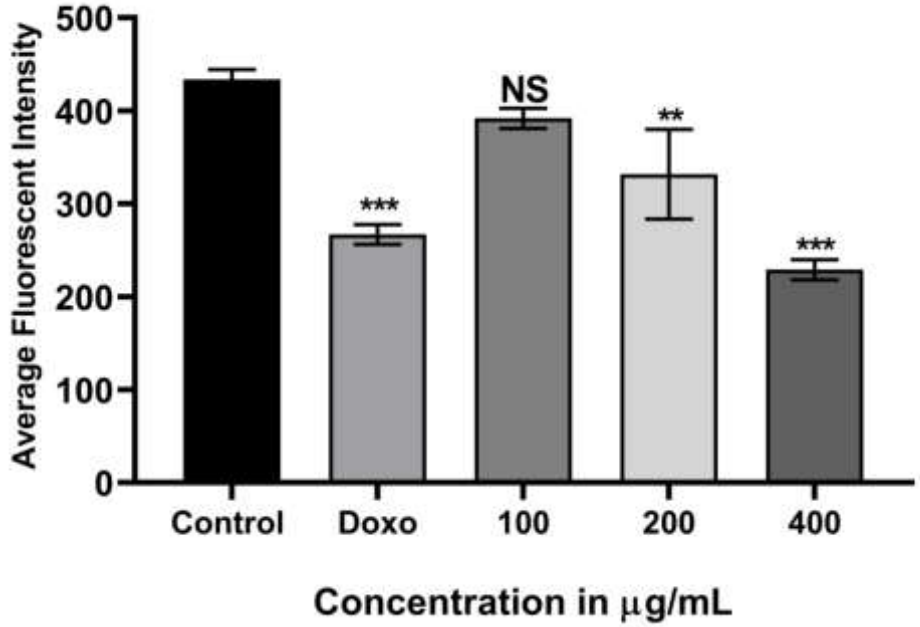


Figure 4: Cytotoxicity effect of dandelion extract on mitochondrial membrane permeability of MCF-7 cell line on multipara system High Content Screening at 37 °C. Data expressed as (mean ± SD), *,**,***: $p \leq 0.01$, SD: Standard Deviation. (n = 3), NS: non-significant.

The results of **nuclear intensity** declared that only 400 µg\ml abled to increase NI in comparison with negative control (525.3±88.95 and 272.9±15.49 respectively) (Figure 5)

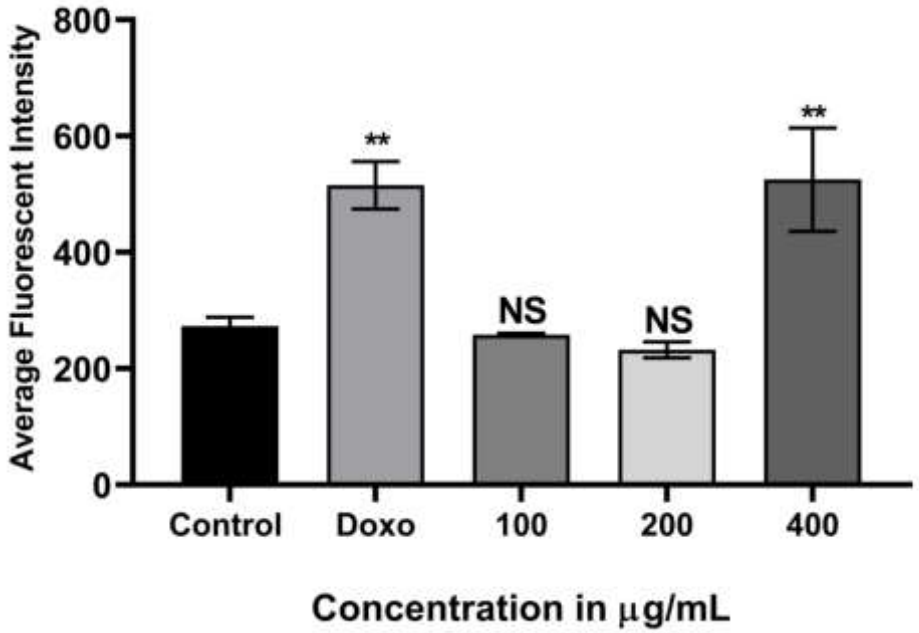


Figure 5: Cytotoxicity effect of dandelion extract on the nuclear intensity of MCF-7 cell line on multipara system High Content Screening at 37 °C. Data expressed as (mean ± SD), *,**,***: $p \leq 0.01$, SD: Standard Deviation. (n = 3), NS: non-significant.

Results of **cytochrome- C** releasing indicated that (400 and 200 µg\ml enhance the releasing to (270.4±1.3 and 232.1±6.6) in comparison to (161.4± 9.6) of control negative releasing (Figure 6)

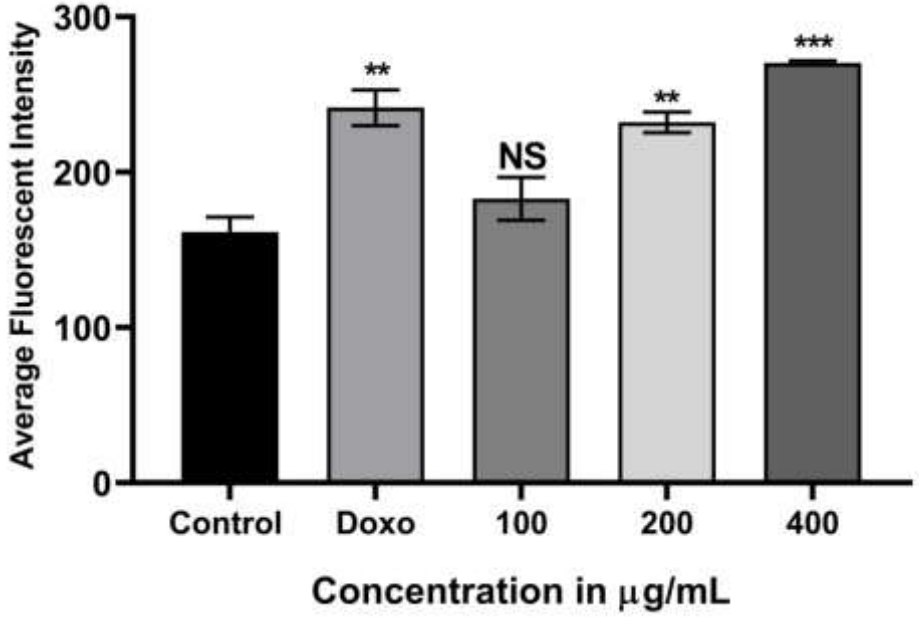


Figure 6: Cytotoxicity effect of dandelion extract on cytochrome C of MCF-7 cell line on multipara system High Content Screening at 37 °C. Data expressed as (mean ± SD), *,**,***: $p \leq 0.01$, SD: Standard Deviation. (n = 3), NS: non-significant.

3.3. Effects of dandelion extract on ROS generation

The results of ROS generation indicated the ability of plant extract (400 mg/ml) and doxorubicin drug to increase ROS scavenger in comparison with negative control (50.07±2.2, 53.03±2.6 *vs.* 44.77±1.4) respectively (Figure 7).

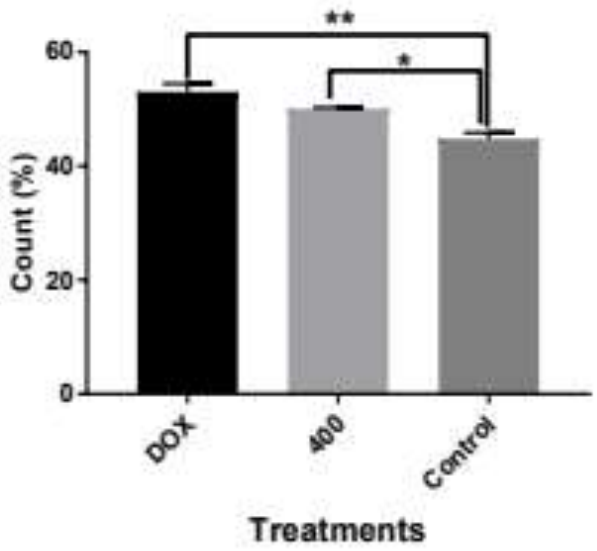
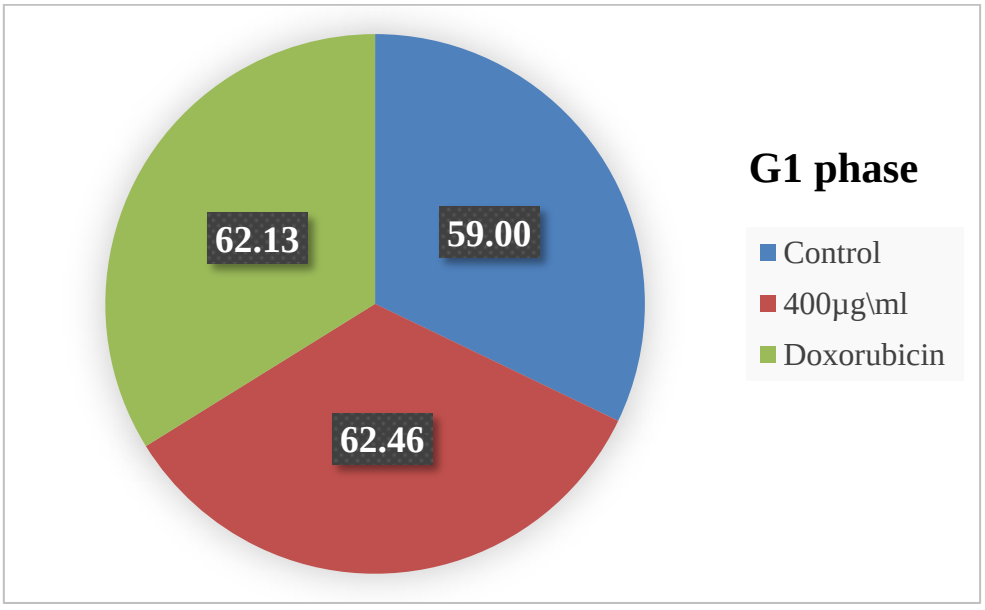


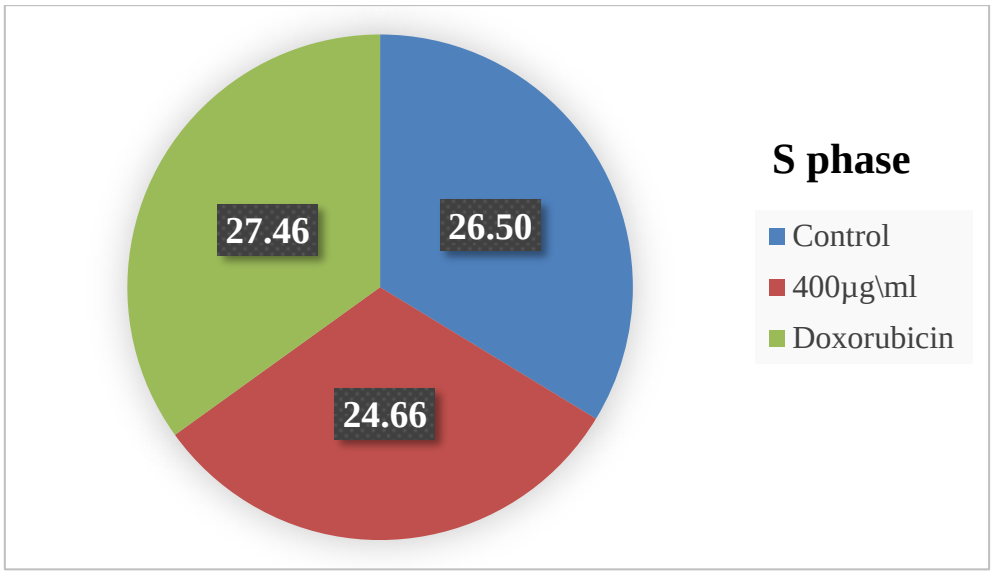
Figure 7: Effect of dandelion extract and doxorubicin drug on ROS generation in MCF-7 cells.

3.4. Effects of dandelion extract on MCF-7 cell cycle analysis

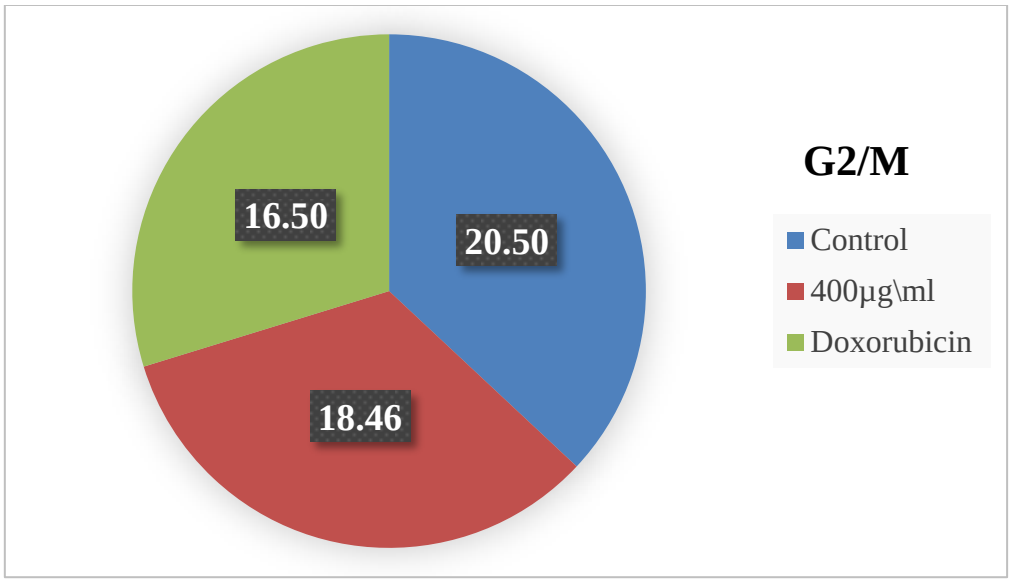
This assay assessed to evaluate the ability of plant extract to inhibit MCF7 cell viability related to or not a cell cycle. The resulted flow cytometric analysis revealed that plant extract possessed the ability to accumulate MCF-7 cells at G1 phase which suggests that the transition from G1 to S phase was blocked by plant methanolic extract at 400 µg/ml (62.46 cells at G1) while at S phase (24.66 cells), at G2\M minimum cells number (18.46 cells) in comparison to doxorubicin and control groups (Figure 8; A: G1, B:S phase. C: G2\M).



(A)



(B)



(C)

Figure 8; A, B, C- Effect of dandelion extract and doxorubicin drug on MCF-7 cell cycle phase distribution (A: G1 phase, B: S phase, and C: G2\M phase).

Natural plant extracts have been necessary for the development of many drugs. These components are used in different conventional medicines and the current studies on the use of natural plant extracts as therapeutics for specific diseases produce scientific support for their application [16]. According to the fact that there is a relationship between herb and drug so herbal drugs are used as chemotherapy such as dandelion methanolic extract [17]. In the recent study, the result shows the effectiveness of dandelion methanolic extract that contain lupeol, a lupane-type triterpene a significant line for anti-cancer chemotherapy that plays an important task to enhance the discrimination of tumor cells into benign or normal cells[18]. Dandelion may affect on human cancer cell line by inducing differentiation or repressed cell growth. A variety of mechanisms could enhance cell death .firstly the dandelion methanolic extract was able to enhance any oxidative tension and diminish mitochondrial membrane potential that related to apoptosis induction in cancer cells by which high reactive oxygen species levels that may prepare cancer cells and stimulate the cellular stress mechanisms also

foremost to apoptosis [19]. Dandelion extract causes levitation of the manufacture of tumor necrosis factor (TNF)-α and interleukin (IL)-1α and other cytokines types that may regulate cell death. Different studies indicated that TNF-α and IL-1α are two persuasive inducers of cancer cell apoptosis. Recent studies explained that both extrinsic (cell death receptor) and intrinsic (mitochondrial) pathways may stimulate apoptosis by different plant extracts. Dandelion possessed the ability to reduce the invasion of cancer cells via lowering the phosphorylation levels of focal adhesion kinase as well as dampening the activities of matrix metalloproteinases (MMPs) such as MMP-2 and MMP-986.our results showed higher activity of the dandelion extracts and the advantage of increasing antiproliferative effects [20].

4. CONCLUSION

From the results of the current study, it was concluded the potential and efficient use of natural bioactive compounds in our primary health care. The compounds from natural sources, such as dandelion can serve as a reservoir of potent bioactive compounds that might inhibit different forms of cancer without any side effects. Furthermore, the rigorous fractionation and isolation of the active principle(s) present in the various extracts of dandelion would play a crucial role in explaining the anti-proliferative effect of dandelion on the MCF7 cancer cell line.

5. **ETHICAL CLEARANCE** The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education and scientific research ministries in Iraq

6. **CONFLICT OF INTEREST**: The author declares that they are no conflicts of interest

7. **FUNDING**: College of Biotechnology\Al-Nahrain University

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