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DETERMINING THE ANTI-TUMOR EFFECTS OF DIFFERNT EXTRACTING METHODS OF ARUM PALAESTINUM ON DIFFERENT CANCER CELL LINES BY IN VITRO ASSAY

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Abstract

Cancer chemotherapy has faced remarkable problems, which attracted the scientists to search about alternative medicine. Several studies have revealed that natural products exhibit an extensive spectrum of biological activities, one of medicinal plant were selected to investigate in this study is Arum palaestinum which belongs to Araceae family, and commonly is ingested as an herbal remedy to fight cancer. The aim of this study is to investigate the anti-cancer effect of different extracts from Arum palaestinum: ethyl acetate, methanol, chloroform and water extracts on the growth of different cancer cell lines. Plant sample was extracted in different solvents to prepare ethyl acetate, methanol, chloroform and water extracts, these extracts were then tested for the ability to inhibit the proliferation of chronic myelogenous leukemia (K562), colorectal cancer (HCT116), prostate cancer (PC3), and breast cancer cells (MCF-7). Cell viabilities were evaluated using MTT assay to identify which of the cancer cell lines are most affected by Arum palaestinum. The results showed that Arum palaestinum extracts were found to be most effective against leukemia (k562) and colon cancer (HCT-116), and ethyl acetate extracts showed the best effect. No significant anti-proliferative effects of the extracts were observed on human normal skin fibroblast. In summary, the results suggest that Arum palaestinum has shown anti-tumor activity which were strongly correlated with their bioactive contents. This plant may be used as functional food for prevention of cancer.

Keywords: Arum palaestinum , MTT assay, anti-tumor, extract

Introduction

Cancer is a major health problem in the world and one of the leading causes of death worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012 [1]. More than 20% of all deaths among the world's population are due to cancer [2,3]. An increase in life expectancy and prolonged exposure to risk factors such as: chemicals, radiation, tobacco smoking, viral infection, some food, and environmental factors may increase the risk of getting cancer [4]. Types of cancer are usually named for the organs or tissue where the cancers originate from [5], for example, the carcinomas appear in 90% of all cancers, such as lung, breast, and colon cancer; whereas the sarcomas are present in the cells that support connective tissue: bone and cartilage; Finally, the leukemia arise from the cells of lymph nodes and blood. [6].

Chemotherapy works by killing the cancer cells, stopping them from spreading, or slowing their growth by shrink tumors that are causing pain and other problems. However, drug chemotherapy travel throughout the body, thus it can also harm the growth of healthy cells, which causes side effects such as, nausea, vomiting, mouth sores, hair loss, greater chance of infections, tiredness and pain from nerve damage. Cancer chemotherapy has faced remarkable problems, poor selectivity of conventional anticancer agents, which might cause damage not only for malignant cells but also for normal cells [7]. Drug resistance is another major reason for the fail of most cancer treatments [8], this motivated the scientists to increase interest in search for alternative anticancer agents from the flora of different countries [9]. Plants have potent biological active chemicals with various promoting effects against many diseases e.g. cancer [10]. The use of an alternative medicine has been rapidly increasing among patients with cancer throughout the world [11]. Natural products have become an increasingly important option for inducing apoptosis. Apoptosis is an active, programmed cell death mechanism and plays a balancing role in the regulation of animal cell populations in both normal tissues and malignant neoplasms [12].

Herbal medicine, also called botanical medicine or phytomedicine, refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes 36-13]. Thousands of years ago, herbs, plant products and animals species were used in folk medicine in treating a wide spectrum of diseases [13]. Medicinal plants serve as sources of direct therapeutic agents and raw materials for the manufacture of more complex compounds [14]. The extraction of these natural products has low cost, safe for use and it is available [11]. The use of plants in modern medicine and demands for herbal medicine are accelerating [15]. The interest in studying the biological effects of traditional medicinal plants or isolating their phytochemicals for treatment of illness has been increasing all over the world due to the fact that plant remedies have less serious side effects [16]. The term phytochemical is defined as any chemical compound that naturally exists in plants which has biological effects in the treatment of disease. Important phytochemical constituents derived from traditional medicinal plants can inhibit or decrease some type of cancers [17]. Approximately 80% of the population in this world according to (WHO) depend on complementary and traditional medicine for their primary health needs [18]. Among cancer populations, studies about using natural product among cancer patients in the Western region of Turkey showed that nearly half of the patients (42.3%) of 220 cancer patients were using at least one form of natural product [19]. A study about using natural product among cancer patients attending the out patients departments of King Hussein Cancer Centre (KHCC) a specialist cancer center in Jordan showed that out of 1138 cancer patients 404 (35.5%) were using at least one form of natural product and most of them were above 48 years of age [20]. People who use a high level of natural herbal product have a low incidence of gastric cancer [21], for example, a high consumption of soybean products in Asian countries reduces the incidence of colon cancer [22]. In addition high consumption of vegetables reduces the risk of colon cancer mortality [23], and recently, medicinal plant extracts have the ability to control the proliferation of prostate cancer cells [24,25].

The bioactive herbal compounds are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many others [26]. All of these natural compounds enter in the foundations of new prescription of drugs as we know today. Studies have revealed that some compounds such as isoflavones in medicinal plants enhance immunity in cancer patients and inhibit angiogenesis and prevent the development of cancer [27]. Phytochemicals are divided into two parts, primary and secondary constituents, primary constituents are common in amino acids, sugars, proteins and chlorophyll; but whole secondary constituents contain alkaloids, terpenoids, and phenolic compounds [28]. Flavonoids, the main important part of plant extract, have long been recognized to have antiviral, anti-inflammatory, antiallergenic, anti-proliferative, antioxidative activities [29], and lower the risk of lung cancer [30], stomach cancer [31], coronary heart disease [32] and stroke [33]. Moreover, Flavonoids and phenolics and their derivatives are present in most plants, they

work as a cell-wall support materials [34], colorful attracters for insects, birds aiding in seed dispersal and pollination and resistance to pathogen [35]. Additionally flavonoids, carotenoids and terpenoids that are found in some plants have been reported for their antioxidant chemical activities play various roles in blocking various hormone actions and metabolic pathways that are associated with cancer progress [36]. Studies carried out by Ahmad et al (2000) indicated the presence of flavonids and catechins in medicinal plants were responsible for inducing apoptosis in human carcinoma cells, thus any agent that has the ability to stimulate programmed cell death can be used as antitumor treatment [37].

With 109 genera and over 3700 species were described for Araceae family, and it has a worldwide distribution and is found in a wide range of environments [38], One of the few Palaearctic representatives of Araceae is the herbaceous genus Arum, which comprises 26 described species [39, 40]. It is characterized by its flowers, showing adaptations for trapping pollinators [41], and its ability to produce odors (Figure 1).

Arum palaestinum has the following taxonomy:
Kingdom: plantae
Subkingdom: Viridaeplantae
Phylum: Tracheophyta
Subphylum: Euphyllophytina
Class: Spermatophyta
Subclass: Arecidae
Order: Alismatales
Family: Araceae
Genus: Arum
Species: Arum palaestinum[43] .

The leaves of A. palaestinum are considered edible only after cooking with oil or roasting or after sun drying or steeping them in salty water. Often it is boiled and then the leaves are fried in olive oil and eaten, because Arum is considered a poisonous plant, the leaves and other plants parts can cause vomiting and swelling in the mouth and throat mucous membranes [42]. This inconvenience can be stopped by using olive oil as reported in the Palestinian folklore [44,45].

Arum palaestinum is used by herbal practitioners and local rural healers in the treatment of several diseases such as: cough, constipation, heart burn, urinary tract infections, cancer, diabetes, hemorrhoids, toxicity, worms in the GIT, stomach acidity, atherosclerosis, urinary retention, and kidney infections [46-48]. Also, A. palaestinum was reported to be used for internal bacterial infections and disturbances of the circulatory system [49], and it is believed to protect from colon cancer. In fact, A. palaestinum Boiss is one of the most commonly utilized plants for cancer patients [50]. It can be regarded as a promising functional food for human consumption and as a potential plant for drug discovery research. The phytochemical screening of Arum plants showed that these plants characterized by presence of alkaloids, proanthocyanidins, polyhydroxy alkaloids, flavones, polyphenols, and their C-glycosides (flavonoids, saponin and cyanogenic groups), and flavonols [40, 51], 2-heptanone, indoles, p-cresol, (E)-caryophyllene, monoterpenes, and two unidentified sesquiterpenes [52,53]. Isoprenoids or terpenoids consist mainly from isoprene units and have antibacterial, antifungal, antiviral and antiprotozoal activities [54]. One of the main constituents of Arum tubers is lectin, which has insecticidal activity that tested against Lipaphis erysimi and Aphis craccivor and these two are economically important.

The extraction of these compounds in different solvents depends on the polarity and the molecular weight of the compounds. Table 1 shows some of extracted bioactive compounds by different solvents [55-57].

El-Desouky et al. (2007a, 2007b) demonstrated that the polyhydroxyl alkaloid and Piperazirum -a bioactive alkaloid isolated from A. palaestinum- have a significant in vitro cytotoxic activity against some tumor cell lines [40,58]. Recently, El-Desouky et al (2014) reported another new diketopiperazine derivative was shown to possess a mild cytotoxic activity against cultured multidrug-resistant human cells [51]. Moreover, Afifi et al. (1999) reported the flavonoid isoorientin (6-C glucoside of luteolin), that was isolated from A. palaestinum possess myolytic activity on animals smooth muscle. However, care should be taken when using A. palaestinum, since it may cause negative side effects [59]. Aboul-Enein et al., (2012) and Diab-Assaf et al., (2012) have studied the phenolic contents, antioxidant and anti-cancer activities of different organic solvents extracts of A. palaestinum, It demonstrated that methanol extract showed the highest total phenolic and flavonoid contents compared to chloroform and ethyl acetate extracts. On the other hand, the reduction in cell proliferation was shown to be dose dependent [60,61].

Cole et al. (2015) have studied the anti-cancer activity of A. palaestinum against aggressive androgen independent prostate cancer models in vivo and in vitro, clearly demonstrate an effect of fortified A. palaestinum Bioss on suppressing prostate cancer cells with activation of caspase 6 and prostate tumor in mice [62].

Abu-Reidah et al. (2015) have studied the comprehensive phytochemical analysis of A. palaestinum, a total of 180 metabolites were tentatively identified in A. palaestinum by using the correct and acceptable data of liquid chromatography–tandem mass spectrometry together, main phytochemical metabolites detected and characterized in A. palaestinum leaves (Fructosyl-valine, Fructosyl-leucine, Phenylalanin, 4-Caffeoyl-5-feruloylquinic acid, Lucenin-2, R1: glucose, isoorientin, Diosmetin-7-neohesperidoside, Chrysoeriol-7-D-glucoside, Verproside, Linoleic acid 13-hydroperoxide, Triglochinin, [6]-Shogaol, Dihydrocapsiate, Isovitexin, Vitexin, Orientin) [63].

This plant is also known to contain antioxidant activity [48,58], Al- Mustafa et al. (2008) have studied the antioxidant capacity of A. palaestinum extracts and showed that the potential role in radical scavenging of these plants extracts and their antioxidant activities agreed with their uses as traditional anti-diabetic agents by the Jordanian population [64].

The need for an extensive identification of the most phytochemical components of this important plant seems imperative. Undoubtedly, the medicinal use of this plant as anti-cancerous food has robustly prompted us to carry out this anti-cancer investigation on this promising plant.

Methods

Plant Material

The plant used for this study was obtained and authenticated by Dr. Salim Abderrahman, Department of Biology and Biotechnology, The Hashemite University.

Washed leaves of plant were dried at room temperature and finely ground to a moderately coarse powder using a high capacity grinding machine and stored in a dry environment, at room temperature, in well closed container until used.

Preparation of Ethyl Acetate, Methanol, Chloroform, and Water Extracts for the plant

Each ground dried plant sample (50 g) was extracted by continuous stirring with 500 ml solvent (methanol, chloroform, ethyl acetate, or water) for 72 hours and filtered by vacuum filtration in a large Buchner funnel using Whatman No.4 filter paper. After filtration, solvent was then evaporated until dryness using rotary evaporator under reduced pressure. Finally from 500 ml, the yield of methanol, chloroform, ethyl acetate, and water extracts were 3.14 g, 3.6 g, 1.22 g, 3.8 g respectively [55].

Preparation of Stock Solution

From each crude extract, 0.5 g was dissolved in Dimethyl Sulphoxide (DMSO) (Gibco, USA) until forming homogenous solution, which is considered as a stock solution with concentration of 100 mg/ml. Some extracts (chloroform and water) were sonicated to form homogenous solution. All extracts were kept at -20°C until cytotoxicity tests were carried out.

Culturing of Cell lines

The cancer cell lines used in this study were kindly supplied by Dr. Mam'on Hatmal, The Hashemite University. HCT-116 cell line and fibroblasts (control) were cultured in DMEM high glucose (HyClone; logan, USA) supplemented with 10%(v/v) heat inactivated FBS (HyClone; logan, USA), antibiotics (100U/ml penicillin and 100µg/ml streptomycin) (HyClone; logan, USA), 2mM l-glutamin, while K562, MCF-7, and PC3 cell lines, which were cultured with RPMI 1640 media (HyClone; logan, USA) supplemented with 10%(v/v) heat inactivated FBS (HyClone; logan, USA), antibiotics (100U/ml penicillin and 100µg/ml streptomycin) (HyClone; logan, USA), 2mM l-glutamin in addition to 25µM HEPES buffer (HyClone; logan, USA). Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 in a cell culture incubator (Nuaire,USA).

According to the seeding density, PC3 and HCT116 were seeded with a density of 5000 cell/well, 7000 cell/well with MCF-7 and Fibroblasts, and 3.5x104 cell/well with K562. This number was sufficient to give a reliable reading with MTT assay, which corresponded well with the cell number and was the one that gave exponential growth throughout the incubation period with the plant extracts.

Determining Cell Viability using MTT Assay

The anti-cancer effect of A. palaestinum against various tumor cells was quantified using MTT assay. This test, first described by Tim Mosman (Mosmann, 1983), the tetrazolium dye, MTT, is widely used to assess the viability of cells. The MTT mediated cytotoxicity and cytostatic assay, based upon the ability of living cells to reduce the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) into purple formazan crystals by mitochondrial succinate dehydrogenase in viable cells, which provides a quantitative determination of viable [90,91]. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan product. These crystals are largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells [65,66]. Since

reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. Based on this principle, MTT assays have been extensively used for various medical, microbiological and toxicological approaches including in vitro assessment [65,66].

In the present study, an attempt was made to evaluate the effect of A. palaestinum on the survival of a panel of human tumor cell lines including K562, HCT 116, PC3 and MCF-7. Human fibroblast was used as the model cell line for normal cell.

For the assay, cells with 70-90% confluence were washed three times with phosphate buffer saline (PBS). PBS was decanted and cells detached with 0.025% trypsin-EDTA (Sigma). Media was added to a volume of 10 ml. The cell suspension was centrifuged at 1000 X g for 10 minutes and the pellet was resuspended in 10 ml of medium to make a single cell suspension. Viability of the cells was determined by trypan blue exclusion and it exceeded 90% as counted in a haemocytometer. The cell suspension was diluted afterwards to give the optimal seeding density and 100 µl of the cell suspension was plated in a 96 well plate and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 24 hours the cells were treated with the extracts or pure compounds.

Initial experiments were carried out, each extract (initially dissolved in DMSO), was diluted with the medium and passed through a 0.2 µm filter. 100 µg/ml and 50 µg/ml of each extract was tested initially, and from the results, the stock solution of crude extracts were further diluted in medium to produce ten concentrations (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml) of each extract. 100 µl/well of each concentration was added to the plates in triplicates. Subsequently, they were incubated in 96 well plate, seventy two wells were seeded in each plate. Six wells blank (only cells with media), and six wells blank (cells with media and DMSO), to make sure the inhibition in cancer cell by the extracts not DMSO, three wells for each treatment with different doses. Then cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 72hrs.

Cells were treated also with positive control (doxorubicin hydrochlorid) with different concentrations of (0.024, 0.05, 0.0975, 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 (µM) for 72 hrs.

At the end of the incubation time, cells were washed with PBS, added 100 µl fresh media to get rid of the residual which interacted with MTT solution and could affect the color. After that, cells were incubated further for 4hrs with 15 µl CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit (Promega, Madison, USA). Followed by the addition of 100 µl stop solution after four hours, then incubation overnight to ensure complete dissolution of the formazan crystals. Then Absorbance values were measured at 570 nm using the microplate reader (Tecan, Sunrise, Switzerland). Each treatment was performed in triplicate and IC50 (concentration of drug that inhibits cell growth by 50%) values were determined from the concentration vs percent inhibition curve which was calculated by Prism software (GraphPad). To standardize results, each plate had its own control and cell proliferation was expressed as the percent of control. Control cells were incubated with the culture medium with the dosing (DMSO).

Calculating IC50

IC50 is the concentration of agonist that gives a response half way between Bottom and Top, and represents the concentration that causes 50% of cell growth inhibition in vitro. This is not the same as the response at Y=50. Depending on which units Y is expressed in, and the values of Bottom and Top, the IC50 may give a response near "50". Prism reports both the IC50 and its log [67]. In this study log (inhibitor) vs. response curves were determined using prism program (Graph pad), and the IC50 of the inhibitor was determined [67].

Results

Anti-proliferative effects of A. palaestinum extracts against tested cancer cell lines.

The anti-proliferative effects of A. palaestinum extracts were studied against a panel of human cancer cell lines over 72 hrs exposure. Table 2 and Figure 2 shows the IC50 values of A. palaestinum extracts against the tested cancer cell lines (K562, HCT-116, PC3, MCF-7, and normal human fibroblasts) with those of positive control (doxorubicin) for different extracts (ethyl acetate, methanol, chloroform, and water).

In the US NCI plant screening program, if the IC50 value (concentration that causes a 50% cell kill) in carcinoma cells, following incubation between 48 and 72 hours, is less than 20 µg/ml for the crude extract then is generally represent to have in vitro cytotoxic activity, while it should be less than 4 µg/ml for pure compounds [55,68].

4.2 The comparison between the anti-proliferative effects of different A. palaestinum extracts and doxorubicin (positive control) against tested cancer cell lines.

Figure 3-7 shows the dose response curves for different extracts of A. palaestinum and doxorubicin (positive

control) on the different cell lines. The dose response curve for each extract was made by plotting logarithm of 10 different concentrations (In the range of 3 log cycles) with the cells survival. IC50 was calculated for each of the extracts and doxorubicin against different cell lines using GraphPad prim 5.0 windows (GraphPad software, USA).).

Discussion

A healthy lifestyle and diet can reduce the risk of developing cancer as stated by the World Cancer Research Fund/American Institute for Cancer Research in 2007 [69].

In recent years, there were tremendously increasing trends to explore the potential antioxidant and anticancer properties of plant extracts or isolated products of plant origin [17]. It is well known that many polyphenolic compounds, such as phenolic acids, flavonoids, anthocyanins, and tannins, which possess remarkable antioxidant and anticancer activities, are rich in plant materials [70].

This study was designed to evaluate the antitumor effects of ethyl acetate, chloroform, methanolic and water extracts of A. palaestinum, to our knowledge, this area has not been studied before, our study is aimed to compare between different extracts in the ability to inhibit cancer cell lines evaluated by MTT assay. Interestingly, the present study revealed that ethyl acetate, chloroform, methanolic extracts were more potent than water extract and show a significant activity against leukemia and colon cancer cell lines.

In our study, A. palaestinum had shown no toxicity on normal human skin cells (fibroblasts), which suggests that the bioactive components of this plant trigger responses that are specific for tumor cells; this plant is recommended for their therapeutics values.

Previous studies on the characteristics of secondary metabolites of the Araceae family showed that it has a simple profile of polyphenols and alkaloids with flavone c-glycosides, flavanols, flavones, proanthocyanidins and polyhydroxy alkaloids as main classes that act as anticancer agents []. Some of these compounds such as alkaloids are rich in antioxidant compounds that inhibit certain enzymes in tumor cells inducing apoptosis and block their growth [71]. Few phytochemical and biological investigations have been reported on this plant [72].

The findings of the present study showed that there were differences between these extracts, the hydrophilic compounds like saponins, tannins, terpenoids, flavons and polyphenols as polar constituents of plants can be extracted with polar solvents such as methanol, water and ethyl acetate (the order of polarity is H2O > Methanol > Ethyl acetate > chloroform), the lipophilic compounds can be extracted with low polar and non polar solvents as chloroform and in Table 1 shows some of extracted bioactive compounds by different solvents [55-57].

For different cell lines, all the extracts showed the highest anticancer effect (as shown by IC50) on chronic myelogenous leukemia (K562), and colorectal carcinoma (HCT-116) (IC50 was < 20 µg/ml for all extracts except water extract), followed by prostate adenocarcinoma (PC3), and breast adenocarcinoma (MCF-7), that are agree with Abu-Dahab et al.(2007) for the effect on breast adenocarcinoma (MCF-7) cell line [68], and finally normal dermal fibroblasts (used as controls), were much more resistant with IC50 values exceeding 20 µg/ml for all extracts.

Our findings in table 3 and figures (2-7) showed that on leukemia (K562) cell line, the ethyl acetate extract of A. palaestinum has the greatest anti-cancer effect with an IC50 = 7.28 µg/ml followed by methanolic and chloroform extracts with an IC50 = 11.25 µg /ml and 13.41 µg/ml respectively. Whereas colon cancer cells (HCT-116) showed the highest sensitivity to ethyl acetate extract with an IC50 = 15.57 µg/ml, followed by chloroform and methanolic extracts with an IC50 = 17.87 µg/ml and 18.58 µg/ml respectively, this suggests synergic anti-cancer effects of different components of each extract, where alkaloids (i.e., in ethyl acetate extract) being the major components that show the anti-cancer effects, while anthocyanins, tannins, saponin, terpenoids (i.e., the major components of the water extract) seem to have less important role as anti-cancer effects, that is agree with El-Desouky et al. (2007a, 2007b) reported that alkaloid isolated from A. palaestinum have a significant in vitro cytotoxic activity against some tumor cell lines [40,58].

The significant activity of A. palaestinum extracts against colon cancer is very important because colon is in direct contact with A. palaestinum after oral ingestion, this makes A. palaestinum optimum for the treatment of colon cancer when it is taken orally.

For chronic myelogenous leukemia (CML), it is an interesting finding because it is the second cancer in direct contact with A. palaestinum after absorption from intestines, moreover, leukemia cells often acquire new gene mutations [73], which might make treatments less effective [73], by becoming one of the most apoptosis resistant (least sensitive) cancers to other chemotherapies like doxorubicin [74] as we found in our result, this makes A. palaestinum a promising natural therapy for CML.

Unsurprisingly, doxorubicin (used as positive control) showed much more potent anti-proliferative effects against all tested cell lines. HCT-116 and MCF-7 were the most sensitive with IC50 value of 0.065 µM, and MCF-7

with IC50 = 0.079 µM as shown in table 3 and figures (2-4).
The obtained results could explain the past and current usage of A. palaestinum as food, in folk medicine; also may support its further uses in health (i.e., anti-cancer) and nutrition as a functional food.

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Table 1: Some of extracted bioactive compounds by different solvents [55-57].

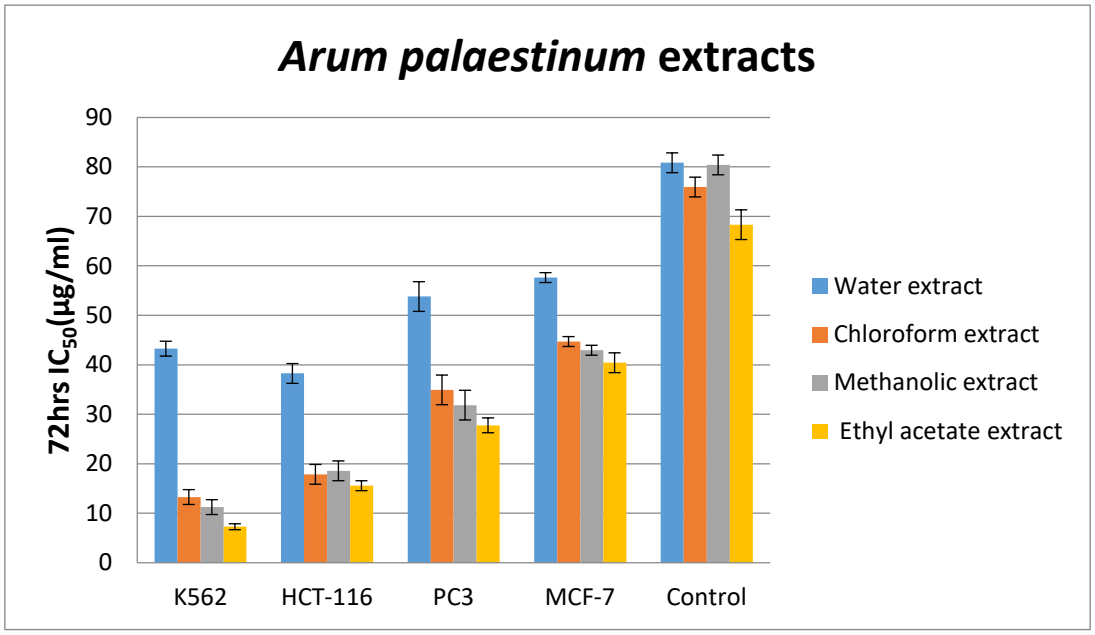
Ethyl acetate	Methanol	Chloroform	Water
Alkaloids, Flavonoids, Terpenoids glycosides, resins glucoseinolates.	Anthocyanins, Tannins, Saponin, Terpenoids, Flavones, Polyphenols.	Flavonoids, Terpenoids.	Anthocyanins, Tannins, Saponin, Terpenoids.

Table 2. In vitro cytotoxic activity (IC_{50} µg /ml ± Standard deviation) of *A. palaestinum* extracts and doxorubicin tested against selected cancer cells.

Cancer cells	***A. palaestinum* extracts**	***A. palaestinum* IC_{50} ± S.D (µg/ml)**	**Doxorubicin IC_{50} ± S.D (µM)**
K562	Ethyl acetate extract	7.28 ± 0.339	0.24 ± 0.034
	Methanolic extract	11.25 ± 0.413	
	Chloroform extract	13.41 ± 0.404	
	Water extract	43.27 ± 0.335	
HCT-116	Ethyl acetate extract	15.57 ± 0.517	0.065 ± 0.009
	Methanolic extract	18.58 ± 0.358	
	Chloroform extract	17.87 ± 0.362	
	Water extract	38.26 ± 0.402	
PC3	Ethyl acetate extract	27.77 ± 0.264	0.35 ± 0.015
	Methanolic extract	31.85 ± 0.356	
	Chloroform extract	34.94 ± 0.264	
	Water extract	53.8 ± 0.409	
MCF-7	Ethyl acetate extract	40.42 ± 0.456	0.079 ± 0.011
	Methanolic extract	42.95 ± 0.437	
	Chloroform extract	44.71± 0.259	
	Water extract	57.63 ± 0.242	
Normal fibroblasts (Control)	Ethyl acetate extract	68.32 ± 0.294	31.21 ± 0.461
	Methanolic extract	80.4 ± 0.199	
	Chloroform extract	75.93 ± 0.294	
	Water extract	80.83 ± 0.347	

Figure 1: Photo of *Arum palaestinum* from the plants garden in the Hashemite University.

(A)



(B)

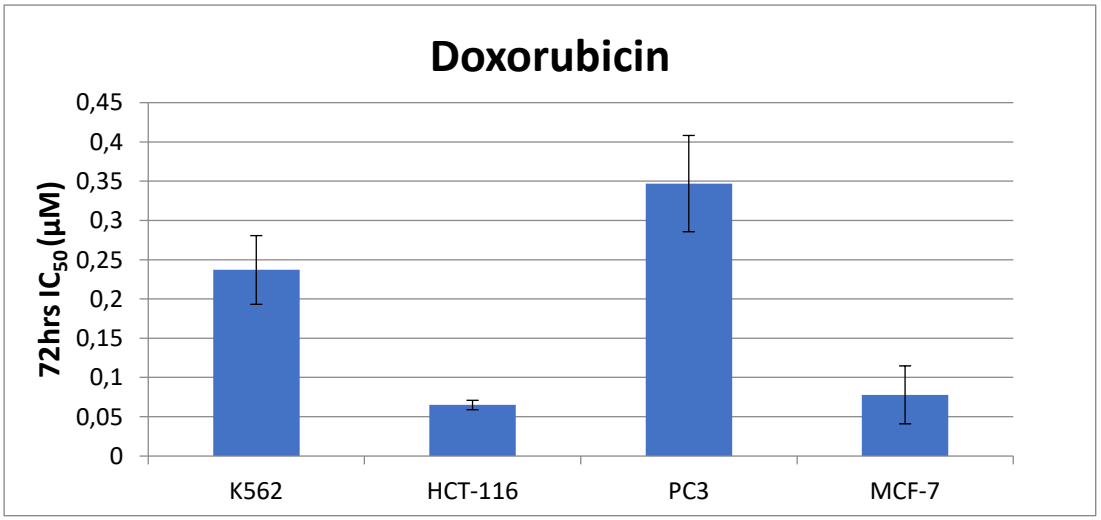


Figure 2: (A) IC_{50} values for *A. palaestinum* extracts against tested cancer cell lines after 72 hrs exposure. (B) IC_{50} values for doxorubicin against the same tested cancer cell lines after 72 hrs exposure. Both assays were determined employing MTT assay. Error bars represent the standard deviation of three measurements.

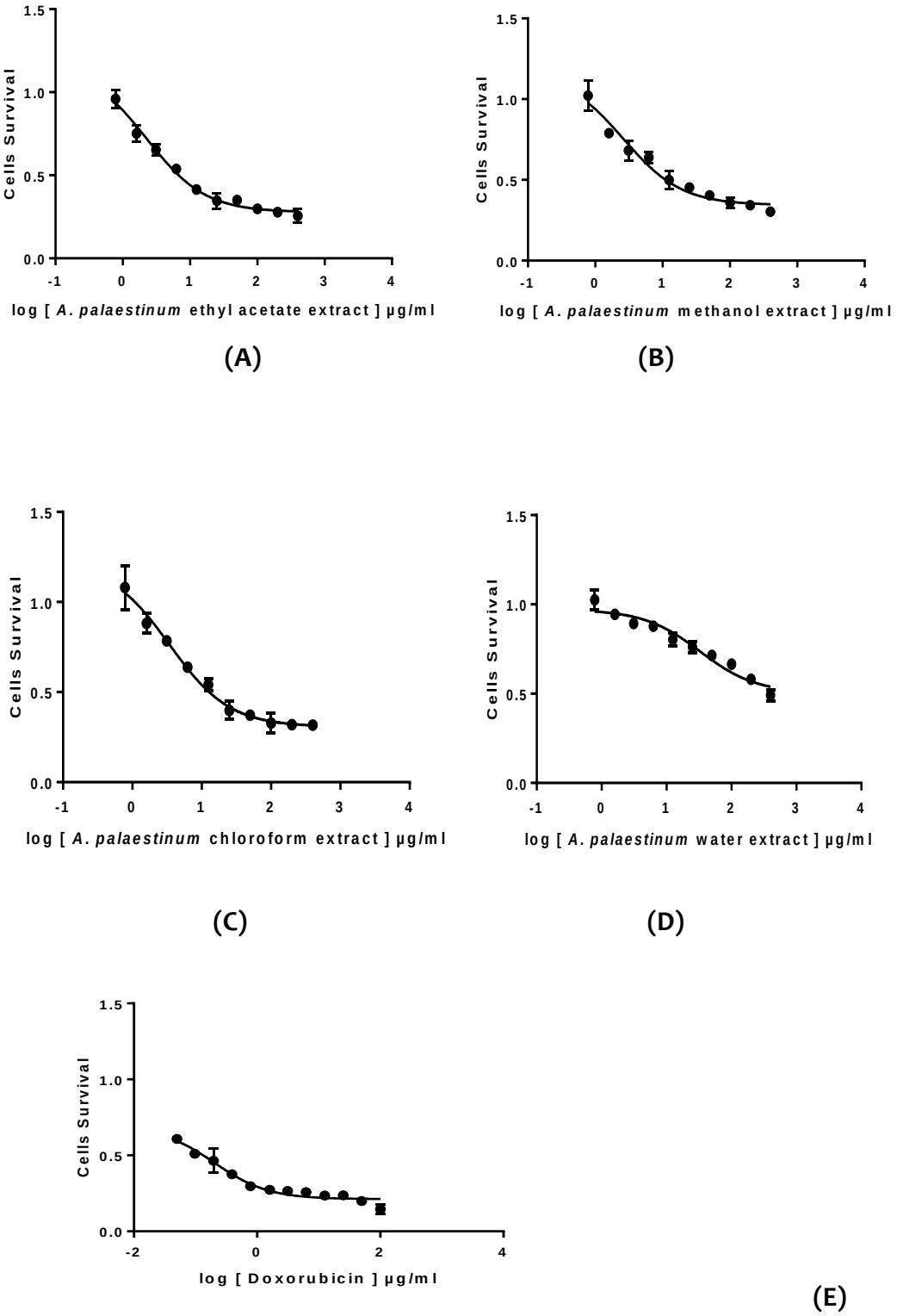


Figure 3: Cells survival of K562 chronic myelogenous leukemia (CML) cancer cells after 72hrs exposure time to *A. palaestinum* extracts. (A-E) show dose response curve determined using MTT assay. The experiments were repeated in triplicates and error bars represent standard deviation of measurements.

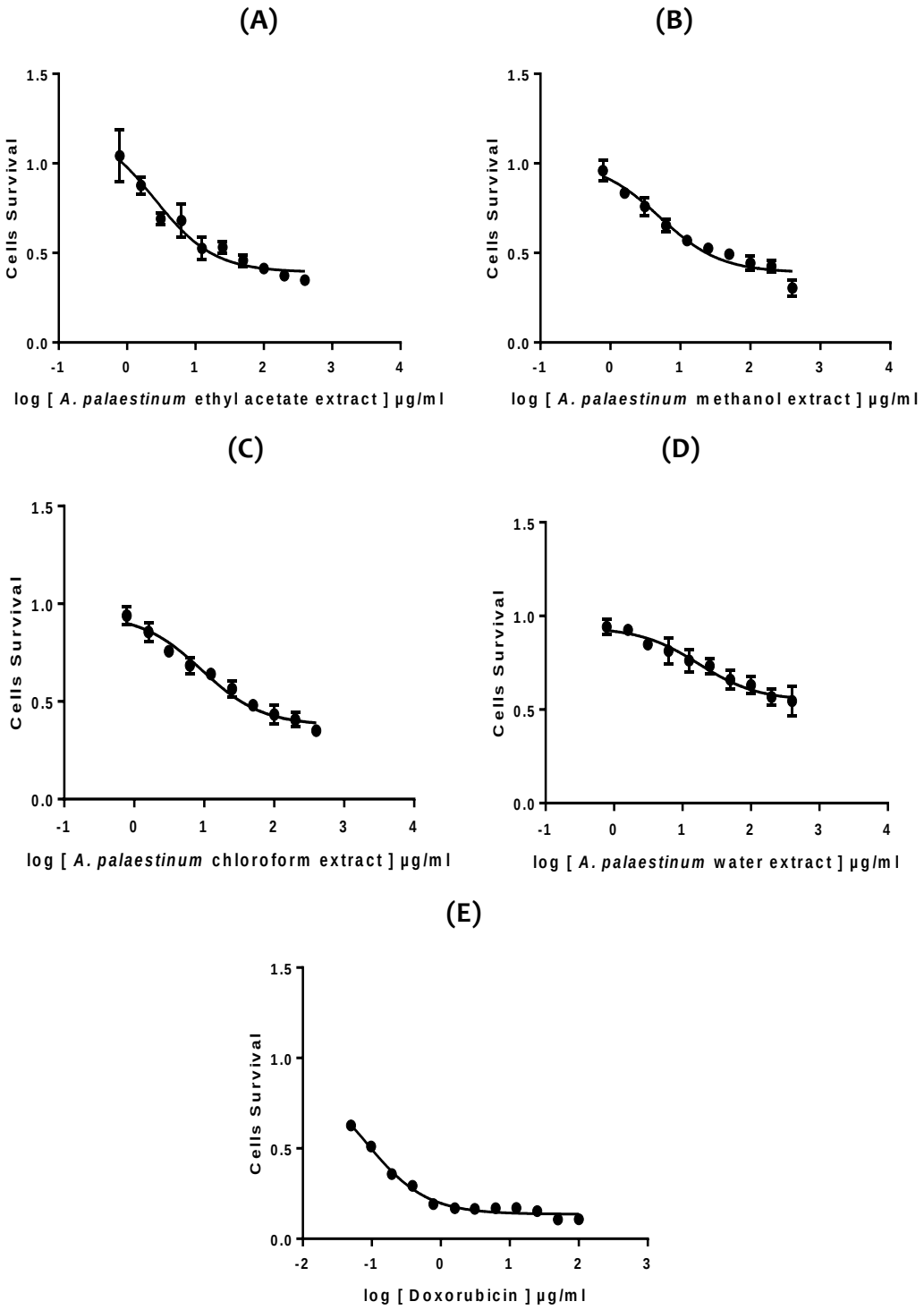


Figure 4: Cells survival of HCT-116 colon cancer cells after 72hrs exposure time to *A. palaestinum* extracts. (A-E) show dose response curve determined using MTT assay. The experiments were repeated in triplicates and error bars represent standard deviation of measurements

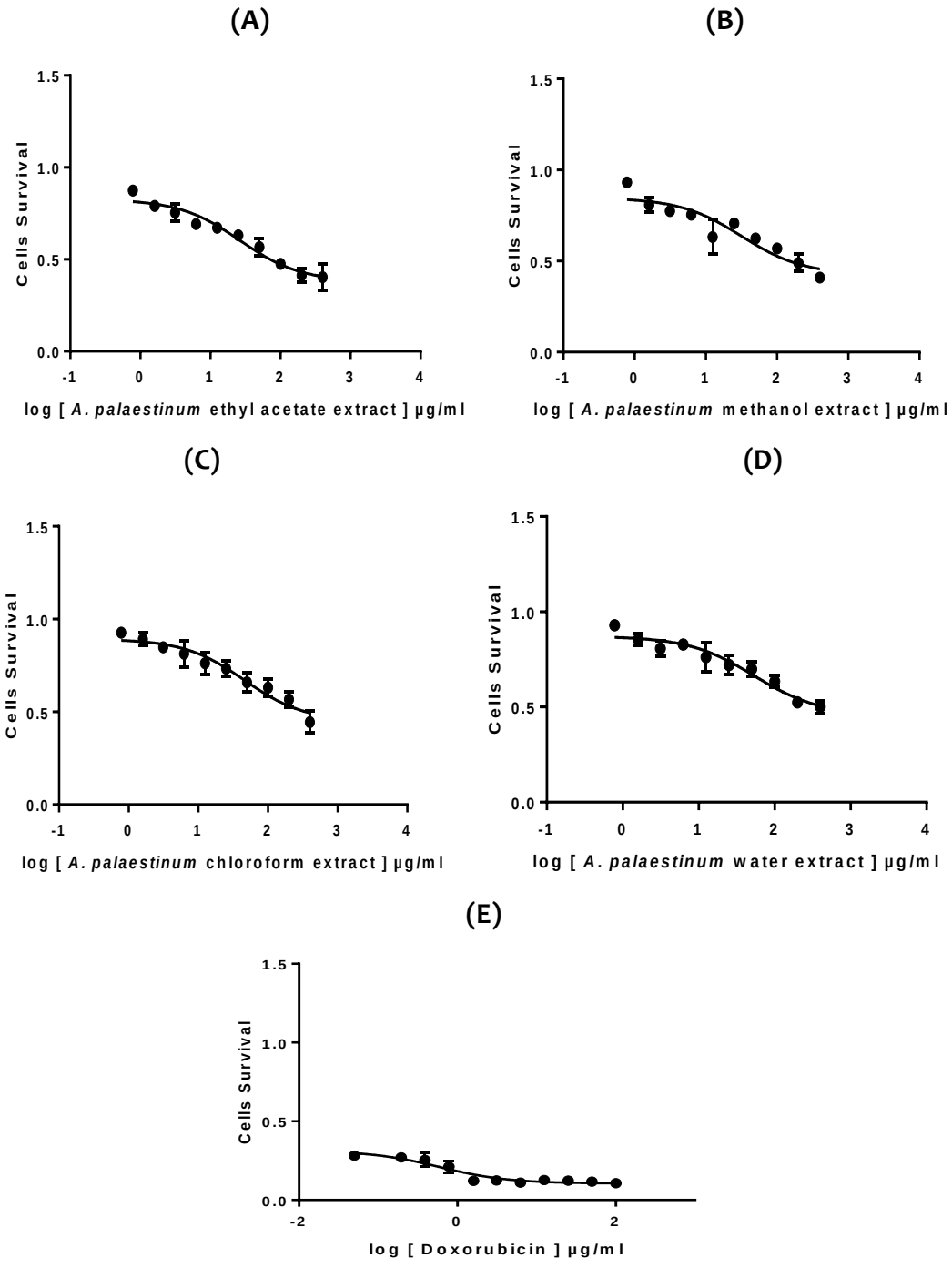


Figure 5: Cells survival of PC3 prostate cancer cells after 72hrs exposure time to *A. palaestinum* extracts. (A-E) show dose response curve determined using MTT assay. The experiments were repeated in triplicates and error bars represent standard deviation of measurements.

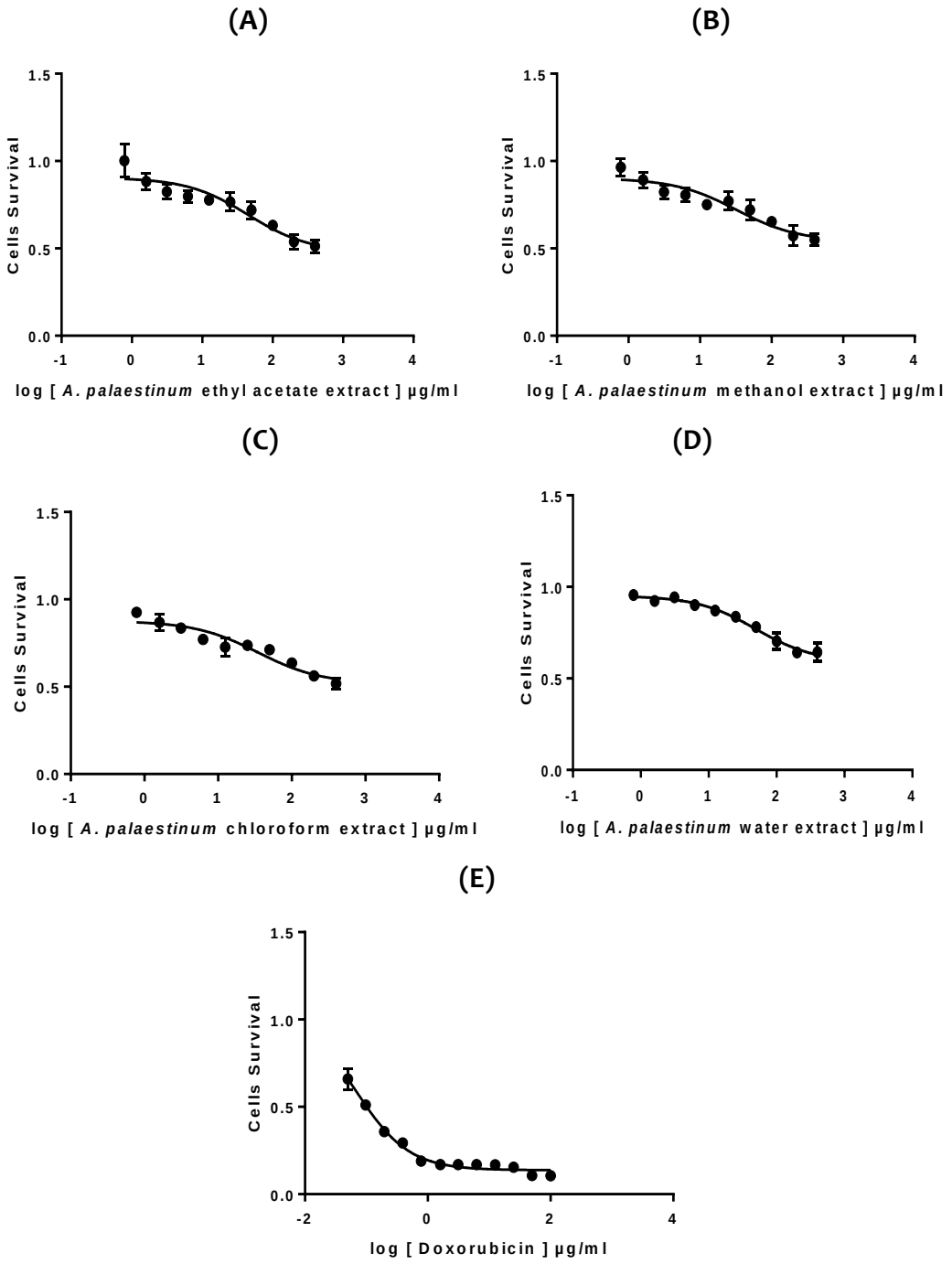


Figure 6: Cells survival of MCF-7 breast cancer cells after 72hrs exposure time to *A. palaestinum* extracts. (A-E) show dose response curve determined using MTT assay. The experiments were repeated in triplicates and error bars represent standard deviation of measurements.

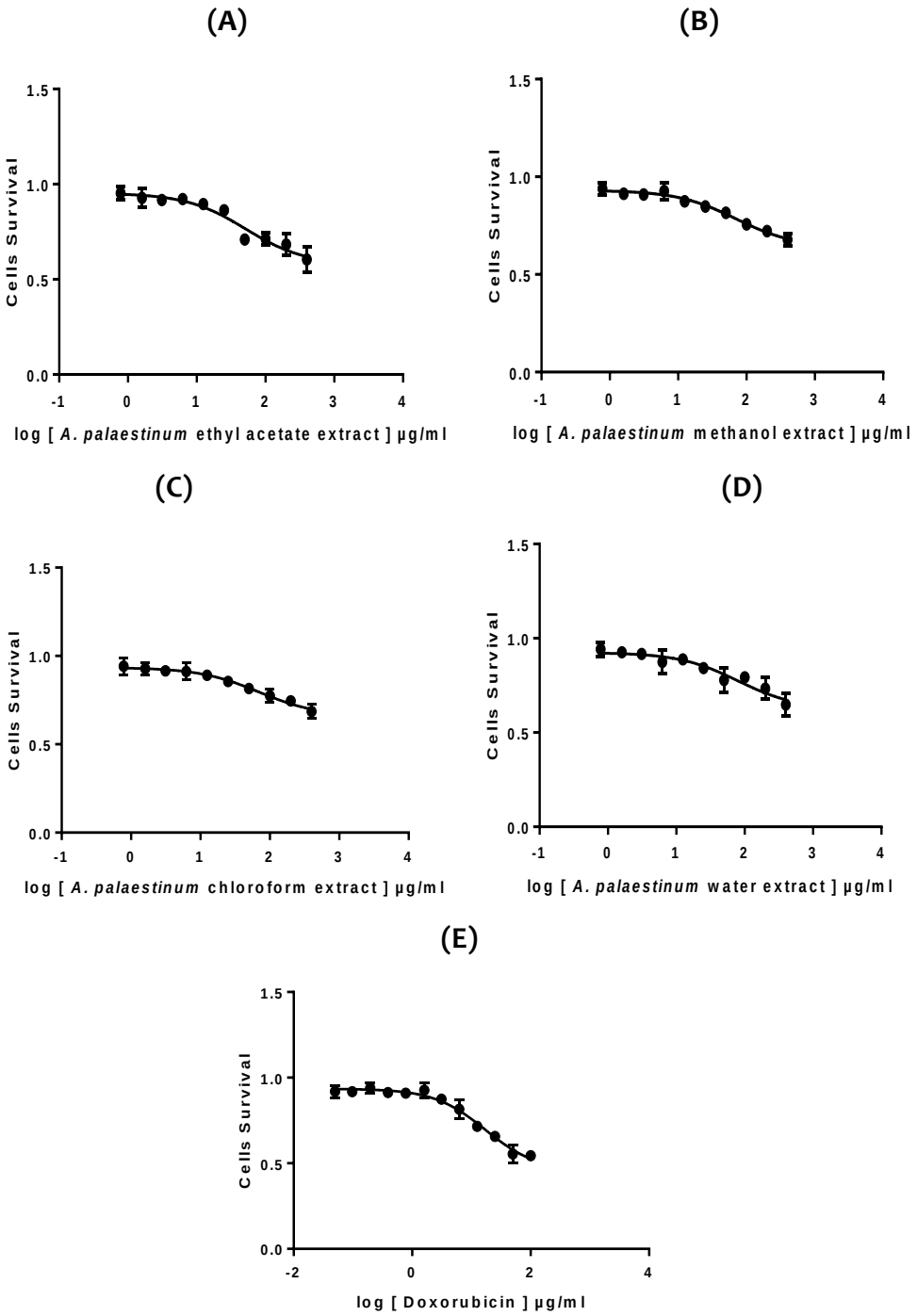


Figure 7: Cells survival of fibroblasts (normal cell) after 72hrs exposure time to *A. palaestinum* extracts. (A-E) show dose response curve determined using MTT assay. The experiments were repeated in triplicates and error bars represent standard deviation of measurements.