An-Najah National University
Faculty of Graduate Studies

In vitro cytotoxic and cytostatic activities of plants used in traditional Arabic herbal medicine to treat cancer in Palestine

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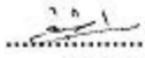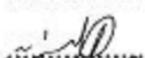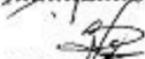**2013**

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This thesis was defended successfully on 20/2/2013, and approved by:

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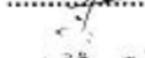
Dedication

To my family, husband, children, and sisters for their support and encouragement, with love and respect.

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الإقرار

أنا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

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In vitro cytotoxic and cytostatic activities of plants used in traditional Arabic herbal medicine to treat cancer in Palestine

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دراسة السمية الخلوية للنباتات المستخدمة في طب الأعشاب العربي الفلسطيني لعلاج السرطان

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Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Students name: اسم الطالبة:

Signature: التوقيع:

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List of abbreviations

TAHM	Traditional Arabic Herbal Medicine
TAHPM	Traditional Arabic Herbal Palestinian Medicine
PA	Palestinian Authority
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.
ATCC	American Type Culture Collection
DMEM	Dulbecco's modified Eagle's medium.
OD	Optical density
SD	Standard Deviation
ELISA	enzyme-linked immunosorbent assay.

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Abstract

The spreading of cancer is increasing over the world and the percentage of deaths caused by this fatal disease is rising especially in the developing countries. Scientists and researchers are now giving more of their attention to the herbal medicine to provide treatment for more difficult diseases like cancer due to the fact that the treatments of cancer patients with chemical therapy have serious side effects. Recently herbal medicines are coming to play a more vital role in the reduction and prevention of cancer. The rapid interest in traditional Arabic herbal medicine (TAHM) worldwide stimulated by many factors mainly, that herbal products are safe and economical.

This current study investigates traditional Arabic Herbal Medicine the *in vitro* cytotoxic and cytostatic of anticancer medicinal plants. Two medicinal plant extracts, *Cyclamen persicum* corm and *Quercus calliprinos,* were prepared using ethanol in water from the leaves of plants, barks and fruits. The selection of these plants was done based on recent survey that revealed their medicinal value in the treatment of cancer. The ethanolic extracts were screened for cytostatic effects against healthy mouse macrophages and both cytotoxic and cytostatic activities against

three cancer cell lines (human lung: fibroblast, mouse -macrophage Raw 264-7, human prostate: cell line PC3) using MTT test. *C. persicum* corm extracts showed cytotoxicity for normal and all three cancer cell lines tested at concentrations than 62.5µg/ml. In comparison to *C. persicum* all three *Q. calliprinos* extracts showed lower cytotoxicity at all tested concentrations. It is concluded that, for these medicinal plants, the traditional uses as antitumor effects are the result may be of cytotoxic but not of cytostatic properties of these plants.

Chapter One

Introduction

Chapter One
Introduction

1.1 Overview

Many people suffer from serious common health problems that are associated with a wide range of effects at both molecular and cellular levels (Amin *et al*., 2007). Research statistics showed that 20% of all the death in the world results from cancer, affecting more than one third of the world population (Toni *et al*., 2010). Cancer belongs to a group of malignant diseases characterized by uncontrolled proliferation of abnormal cells due to imbalance between cell division and cell differentiation, leading to a progressive increase in the number of dividing cells that mass together to form a tumor, or proliferate throughout the body, initiating abnormal growth at other sites by invading other tissue through blood and/or the lymph system.

The cancer cell feeds itself through a process called the angiogenesis process in which the growth of a network of blood vessels will increase production of angiogenesis activators and decrease production of angiogenesis inhibitors (Deshmukh *et al*., 2011). The cancer cells are classified into either benign or malignant. Benign tumors are not cancerous cells. The growth of the benign tumor is limited to restricted areas in the body. On the contrary, a malignant tumor can invade other tissues by metastasis that is the most lethal aspect of carcinogenesis (Becker *et al*., 2009). When cancer cells spread to distant parts of the body, they interfere with the nervous, digestive, and circulatory system, so tumor will appear

when the cells lose their ability to control their regulated cell growth (Sharma *et al.*, 2011).

Among the most common causes of cancer is tobacco smoking; though, it sometimes happens due to certain genetic backgrounds, radiation, chronic viruses that cause chronic inflammation, unhealthy food, and other environmental factors (Anand, 2008; Ames *et al.*, 1995).

Human cancer is classified among two hundred different disease types that may affect many parts of the body. Most of these types are classified according to the cells in which they were initiated (Cooper, 1990). All cancer types are put into three groups: the carcinomas appear in 90% of all cancers, such as lung, breast, and colon cancer; the sarcomas are present in the cells that support connective tissue, bone, and cartilage; Finally, the leukemias arise from the cells of lymph nodes and blood.

The transformation of normal cells into cancerous ones is referred to as abnormalities in the DNA of affected cells. The selective accumulation of mutated cell through a multistep and long process will induce the development of cancer through an initiation process. The promotion stage is enhanced through the proliferation of initiated cells associated by a selection of cells, exhibiting stimulated growth traits, followed by progression of tumor cell (Figure 1). The tumor progress may occur through a combination of DNA mutation and epigenetic events. Many factors could contribute to increasing the chance of cancer cell via activating oncogenes that code abnormal amounts of protein that in its turn

stimulates cell proliferation. The decrease in tumor suppressor gene expression (e.g.p53) through the destruction of p53 can no longer trigger apoptosis in the cells with damaged DNA.
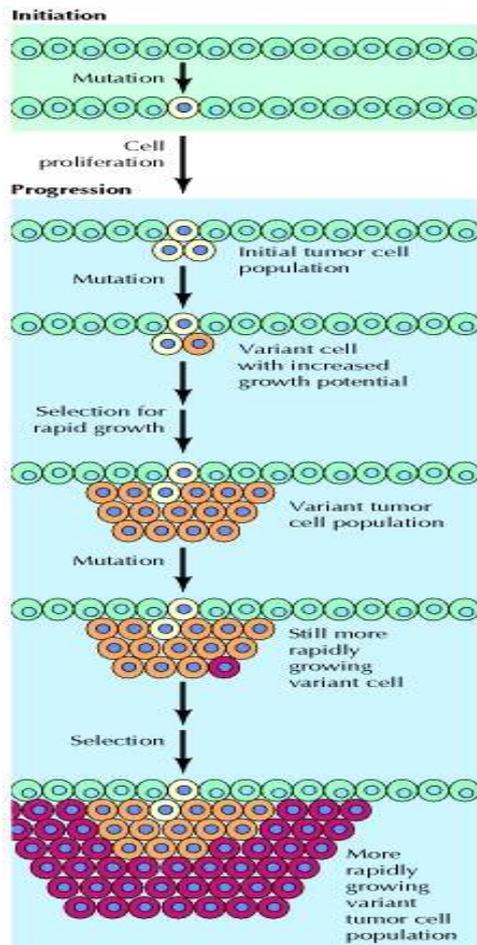
Figure (1.1): Stages of tumor development (Cooper, 2000).

A cancer cell has the ability to divide continuously. While a normal cell waits a special chemical transduction signal, the tumor cell ignores such signal sent to it by other tissue. Moreover, a cancer cell has traits of immortality even *in vitro* but normal cells undergo apoptosis through halting of cell division after 50-70 generations (Deshmukh *et al*., 2011).

The main forms of cancer treatment is via surgery and radiation before any cancer progress happens, but the advanced tumor cases are

treated by chemotherapy that can provide a temporary relief of symptoms a likely prolongation of life. However, the cancer patients are exposed to many drawbacks such as drug resistance and may suffer many side effects. The history of cancer treatment tells that the interest in cancer treatment goes back to the times of Islamic renaissance scholar Ibn Sina (980-1037)**.** The famous scholar suggested "When cancer starts, it is possible to keep it as is, so that it will not increase and keep it non-ulcerated. It may happen sometimes that the starting cancer may be cured. But when it is advanced, verily it will not" (Zaid *et al*., 2010).

Modern medical research focuses on finding new anticancer agents in order to reduce the existing resistance mechanisms. Herbs and other natural plant products have become the main source for this purpose. Herbal medicines play a vital role in the prevention and treatment of cancer. This is because they are always available and mostly of low cost (Saad *et al*., 2008).

Traditional Arab-Islamic herbal-based medicines might be promising for new cancer therapeutics with low toxicity and minimal side effects (Saad *et al*., 2006., Saad *et al*., 2008; Said *et al*., 2008).

The present study will concentrate on alternative medicinal plants and will try and prove if these plants have anticancer activities. The studied plants are *C persicum* (corm) and *Q calliprinos*. Samples were taken from the leaves, fruits, and barks.

1.2 Medicinal plants

Since the earliest times, Herbal medicine has been taken as a source of medicines for the treatment of several diseases and do not show signs of toxicity as in the case of synthetic drugs. Palestine is unique and diverse in its geographical location and cultural practices including Traditional Arabic Palestinian Herbal Medicine (TAPHM) (Ali-Shtayeh, & Jamous, 2008). About 340 plant species belonging to 93 families, are currently used in TAPHM in the West Bank and Gaza (Ali-Shtayeh, & Jamous, 2006). This kind of medicine has a long-standing history in Palestine and continues to provide useful means for treating various ailments including cancer (Ali-Shtayeh *et al*., 2000; Ali-Shtayeh, & Jamous, 2006, 2011). Nearly 50% of prescribed modern drugs in clinical use are of natural products; many of those have the ability to control the proliferation of cancer cells (Rosangkima *et al*., 2004). Crude extracts of medicinal plants in the form of syrups, teas, infusion, ointments, and powders are derived from plant species (Saad *et al*., 2005). Approximately 80% of the population according to (WHO) depend on complementary and traditional medicine (CAM) for their primary health needs (Farnsworth *et al*., 1985). It was reported in previous studies that 60% of cancer patients concentrate in their treatment on herbs or vitamins (Madhuri and Pandey, 2009; Sivalokanathan *et al*., 2005). A recent ethnopharmacological survey in the PA estimated the popularity of herbal therapies used by cancer patients as 60.9 % (Ali-Shtayeh *et al*., 2011). The study revealed that a large number of indigenous plants are used by this group of patients.

Despite the wide progress of pharmaceutical and many synthetic drugs nowadays, many people prefer to use medicines derived from plants which are considered as a natural source as a drug. This preference is due to the fact that plant remedies do not have any serious side effects. Moreover, they play a vital role in the treatment and prevention of cancer. They are also safer and not expensive. Some herbs protect the body from cancer by the anchorage detoxification function of the body through antioxidant activity. Cancers release specific hormone that change body function, and certain medicinal herbs can inhibit the growth of cancer by modulating the activity of specific enzymes and hormones (Deshmukh *et al*, 2011).

This type of medicine derived from plants is called Herbal Medicine or Alternative Herbal Medicine. Many ancient civilizations used this type of medicine in the past. The Arabs and Muslims introduced many plants that could be used to cure many diseases (Zaid *et al*., 2011). The holy Quran mentions that many plants used in Muslim's foods have much the nutritional and health values. In Islam the healthy diet is considered a good food habit to protect from many diseases. Prophet Mohammad, peace be upon him, said" Food is the source of illness, and the diet is the source of health"(Zaid *et al*., 2010).

1.2.1.a *Zamatot (Cyclamen persicum* Miller)

The plant was traditionally classified in the family <u>Primulaceae</u> but recently it was placed in the family of <u>Myrsinaceae</u> (Debussche *et al*., 2000).

A   B
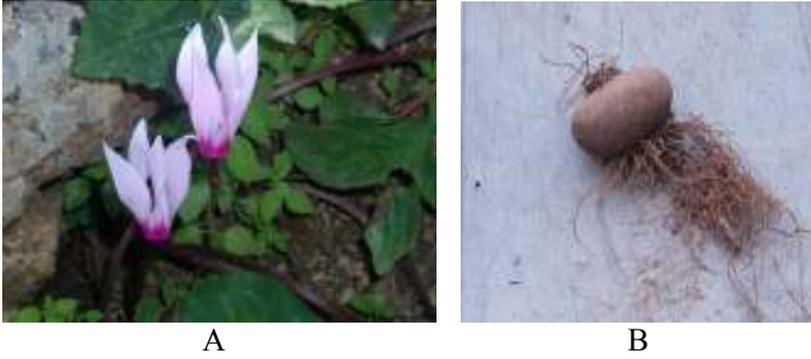
Figure (1.2); A. *Cyclamen persicum* **growing in nature. B.** *Cyclamen persicum* **corm**

This plant is a species growing from tubers, which remain in soil up to 20 years and this tuber can protect themselves from animals, since they include toxic substances known as Jalikosid Alsalamin. The corm is between 4-15cm diameter, brown, of circular shape.

1.2.1.b The medicinal uses of *C. persicum* (Ali-Shtayeh *et al*., 2008)**.**

1. *C. persicum* corm is used to treat hemorrhoid.

2. The root of this plant is used as an ointment to reduce the pain of arthritis.

3. For respiratory, *C. persicum* is used in treating dyspnea, and the root for treatment of sputum.

4. The leaves are used as a lotion in the treatment of the skin uncompromising. Roots are also used in the treatment of burns and feud boils.

5. The leaves are cooked and eaten and the flake corm is cut into pieces and boiled then filtered and the product is drunk to treat cancer.

The plants selection was based on previous studies conducted by Khaleeliah (2001). He worked on two prostate cell line and one breast cancer cell line to examine the anticancer activity of *C. persicum* Miller, who found that this plant was most active against the breast cancer cell line.

1.2.1.c *Palot (Quercus calliprinos* L. Kermes Oak)

The *Q calliprinos* belongs to Fagaceae family. It is used commercially as well as in medical field, especially the fruits and the barks.

| A | B | C |
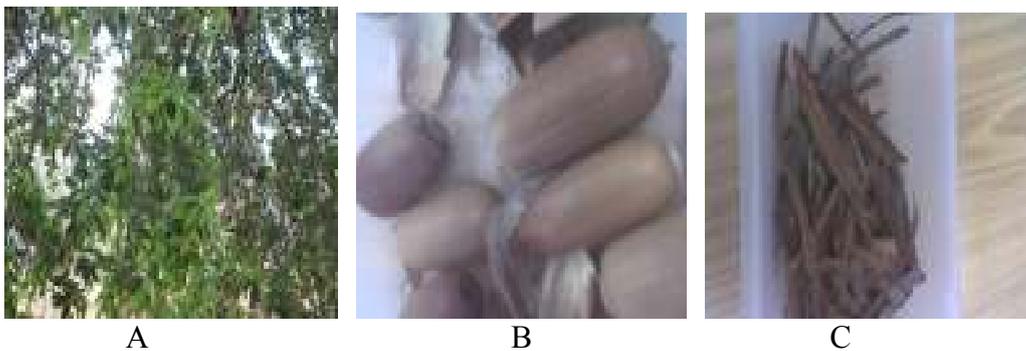
Figure (1.3); A. The general form for *Quercus calliprinos*. B. *Qurecus calliprinos* fruits. C. *Quercus calliprinos* barks.

1.2.1.d The medical uses for *Q. calliprinos*

Oak bark is used to treat diarrhea, urinary tracts, eye infections, blood diseases, skeletal systems, cancer, reproductive tracts, skin, wounds and hair diseases (Ali-Shtayeh *et al*, 2008)**.**

According to a study carried out by Khaleeliah, (2001) *Q calliprinos* was found to stimulate some of cancer cell line. Significantly, stimulations of LNCaP and MCF-7 were observed with *Q. calliprinos* which are used as popular remedies.

1.3 Anticancer activity of medicinal plants

Medicinal herbs are of great benefit to individuals as well as to communities. The therapeutic value of these plants lies in their bioactive photochemical constituents that contain substances that give a specific physiological action on the human body (Edeoga *et al*., 2005**).** The anticancer activities result from immunomodulatory and antioxidant properties that present in medicinal plants (Pandey *et al*., 2006; Caragay, 1992). The most great bioactive compounds are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many others (Pascaline *et al*., 2011). 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 (Deshmukh *et al*,. 2011).

A phytochemical is a chemical compound naturally present in plants. It actually plays a role in disease prevention (Al-Akhras *et al*., 2007). Phytochemicals are divided into two parts, of primary and secondary constituents. Primary constituents are common in amino acid, sugars, proteins and chlorophyll; but whole secondary constituents contain alkaloids, terpenoids, and phenolic compounds (Krishnaiah *et al*., 2009). Flavonoids and phenolics and their derivatives are present in most plants. They work as a cell-wall support materials (Wallace, 1994) and as colorful

attracters for insects and birds aiding in seed dispersal and pollination (Harborne, 1994). 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 (Caragay, 1992; Steinmetz & Potter, 1991). Studies carried out by Ahmad, (2000) indicated the presence of flavonids and catechins in medicinal plants were responsible for inducing apoptosis in human carcinoma cells. So any agent that has the ability to stimulate programed cell death can be used as antitumor treatment.

1.4 Methods used in the study of cell viability

The most important method used nowadays for determining the cell viability and cell proliferation have been developed in 96 well plate in a micro plate reader. This assay allows many samples to function in quick and synchronized procedure.

1.4.1 General Background

MTT assay is defined as a laboratory test and a standard colorimetric assay that is used for measuring the activity of enzymes. It can determine the toxicity of potential medicinal agents and other toxic material. This assay is based on the conversion of yellow tetrazolium bromide (MTT) to purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells (Figure1.4) (Bresica and Banks, 2009).
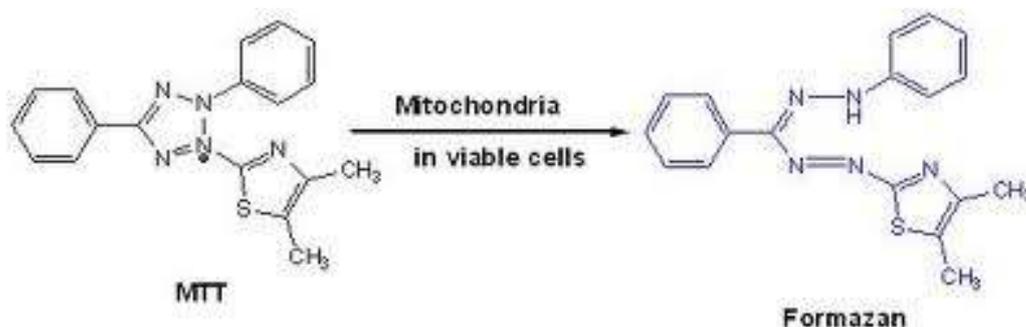
Figure (1.4): MTT reduction in live cells by mitochondrial reductase results in the formation of insoluble formazan, characterized by high absorptivity at 570 nm..

A solvent is added to dissolve the insoluble formazan crystals that are formed. The formazan dye can then be quantified by measuring it at a specific wavelength by a spectrophotometer. The absorbance appears direct correlates to the cell number (Sieuwerts *et al.*, 1995).

MTT, the tetrazolium dye, is widely used for quantification cell viability and cell proliferation in cell population. Also, is used for plant extracts biosafety (Saad *et al.*, 2006; Mosmann, 1983). This assay measures cell respiration and the amount of formazan produced in proportion to the number of living cells present in culture.

The main application for this assay measurement of cell proliferation in response to growth factors, mitogens, cytokines, and nutrients (Huang *et al.*, 1998). Also it is used as anticancer drugs and other pharmaceutical compounds through analysis of cytotoxic and cytostatic compounds (Gergel *et al.*, 1995; Wong, & Goeddel, 1994).

The use of MTT offers many advantages: 1- Safety: without radioisotope that is produced. 2- High degree of precision 3- Rapid: allows

more than one plant extracts to be processed by the use of a multiwall-plate reader. 4- Simplicity. 5- And sensitive (Riss and Moravec, 1993).

The main drawbacks of this method is required volatile organic solvent that needs insolublization steps to dissolve the formazan crystals (Promega, 1996).

1.5 The aim of the present study

At present, very little experimental data on the bioactivity of herbal remedies used to treat cancer in Traditional Arabic Palestinian Herbal Medicine, (TAPHM), is available (Kaleeliah *et al*., 2001; Zaid *et al*., 2011).

The objectives of this study

Studying the anticancer activity of two plants used in traditional medicine in Palestine: *C. persicum* corm, and *Q. calliprinos* leaves, fruits, and bark, by assessing their *in vitro cytotoxic* activity in cancer cell lines.

The following three cell line were used: human prostate cancer (PC3 cell line), human lung cancer (Fibroblast cell line), and mouse macrophage cell line (Raw 264-7).

Chapter Two

Material and Methods

Chapter Two
Material and Methods

2.1 Chemicals

Reagents used in this *in vitro* study are shown in Table 2.1 and Table 2.2:

Table (2.1): Reagents used in this study

No.	Items	Company	Cat.No#
1	Trypsin-EDTA solution C	Bilogical Industries	03-54-1A
2	Isopropanol	Sigma Company	10398
3	Formic acid	Sigma Company	S32351-102
4	Trypan blue solution0.5%	Biological Industries	03-102-1B
5	Thiazolyl- blue tetrazolium bromide(MTT)	Sigma Company	M5655-1G
6	DMSO	Sigma Company	D2650
7	Ethanol	Merck Company	K28744386

Table (2.2): Cell culture media used in this *in vitro* study

No.	Items	Company	Cat.No#
1	DMEM medium	Biological Industries	01-055-1A
2	RPMI medium1640	Biological Industries	01-104-1A
3	Penicillin-Streptomycin solution.	Biological Industries	03-031-1B
4	Fetal calf serum (FCS)	Biological Industries	04-121-1A
5	L-Glutamine solution	Biological Industries	03-020-1B
6	MEM-non-essential amino acids solution	Biological Industries	01-340-1B
7	Amphotericin B Solution	Sigma Company	A2942

2.2 Cell lines

Cell lines are widely used in biological research. Cells from cell lines are immortal, e.g., cancer cells. The following cell lines were used in this study: lung carcinoma (Fibroblast), Leukemia cancer (RAW264-7), prostate adenocarcinoma (PC-3), and primary isolated cells rat -peritoneal

macrophages. All these cell lines were obtained from the American Type Culture Collection (ATCC) and were provided by Prof. Bashar Sa'ad from the Arab American University, Jenin.

2.3 Experimental Part

2.3.1 Plant materials

The plants were collected from different locations in the northern part of the West Bank (Palestinian Authority, PA.). The following plants were used: *C. persicum* corm and *Q. calliprinos* (leaves, fruits, and bark). The plants were identified by Prof. M. S. Ali-Shtayeh, from the Department of Biology at An-Najah University, Nablus, Palestine. Voucher specimens were deposited at the Biodiversity and Environmental Research Center, BERC, Herbarium.

2.3.2 Preparation and storage of plant extracts

Plant extracts were prepared according to the procedure described by (Saad *et al* ., 2006). In Brief:

1. 10 g from each part of the plant were used , 100ml of 50% ethanol in distilled water were added to the mixture.

2. The mixture was boiled carefully for 10 minutes. After cooling, The supernatant was centrifuged at 2500 rpm for 10 minutes to get rid of the impurities. The centrifuge step was repeated twice. The extracted solutions were filtered and evaporated under vacuum by means of a rotary evaporator.

3. The concentration of plant extract was determined by drying 5ml from each extract in a petri dish, and weighing the remaining part.

2.3.3 Preparation of stock solution

Each plant extract was dissolved in full D-MEM to a final concentration of 1 mg/ml and the prepared plant stock solution was stored at – 20°C until use. To prepare stock solution, the calculations needed were as follows:

1. *C. persicum* corm concentration: 32mg/ml.

2. *Q. calliprinos* bark concentration: 48mg/ml.

3. *Q. calliprinos* leaves concentration: 80mg/ml.

4. *Q. calliprinos* fruits concentration: 28mg/ml.

2.3.4 Cell culture

Cell lines of lung carcinoma and leukemia cancer (RAW264-7) were cultured in a sterile T75 flask (Costar) and were grown in Dulbecco's modified Eagle's medium (D-MEM) (Gibco), with a high glucose content (4.5g/ml) supplemented with fetal bovine serum (10%, v/v), 1% non essential amino acid, 1% l-glutamine,1% pencillin, and 1% streptomycin in 5% CO2-95% humidified air at 37°C. Prostate adenocarcinoma(PC3) were maintained in a mix media (DMEM and RPMI1640) with all of above supplements were added to media as the previous two cell lines. The culture medium was changed twice a week.

2.3.5 Cell harvesting and counting

1. Cells form Fibro, RAW, and PC3, were trypsinized with 3ml of trypsin-EDTA solution °C for each flask, and were incubated at 37°C for 5%CO2.

2. After 10 minute, 3 ml media were added and cell suspension was centrifuged for 5minutes at a speed of 2000rpm.

3. Then, the supernatant was removed and 3ml media were added to the pellet and made resuspension of the cell with media.

4. 100 µl of these cells were taken in an Eppendorff tubes and were added to 100 µl of trypan blue, then they were mixed well and left for 4 minutes where cell viability was assessed by trypan blue. This dye blue color stains the dead cells which will be excluded when the cell will be seeded.

5. 10 µl of mixer were put in haemocytometer for counting it under microscope (Fig2.1).

A.   **B.**
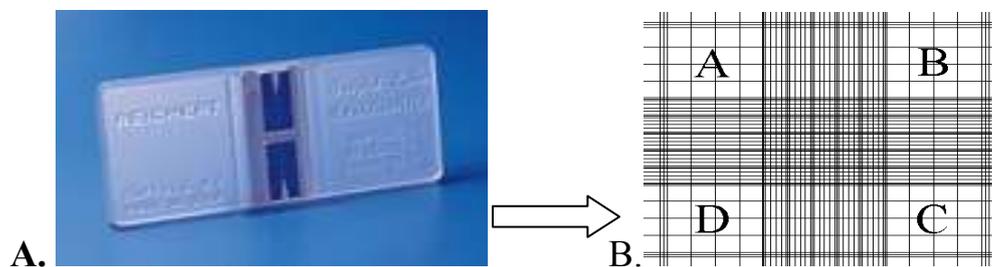
Figure (2.1): A. Haemocytometer: used for cell counting. B. Under microscope

6. For cytotoxic experiments 20.000 cell/well were seeded, whereas in cytostatic experiments, 10.000 cell/well were seeded.

7. The cells then were seeded in 96-well plate (Fig 2.2) at a density of 10.000/well for cytostatic and 20.000 cell/well cytotoxic in 100μl of full DMEM, then incubated at 37°C in an atmosphere of 5% CO_2, the pH of the media was maintained at 7.4.

   1. (1-3) *C.persicum* corm extracts.

   2. (4-6) *Q.calliprinos* leaves.

   3. (7-9) *Q.calliprinos* fruits.

   4. (10-12) *Q.calliprinos bark* extracts.

A diagram of a 96-well plate divided into units of 16 samples, or one run.
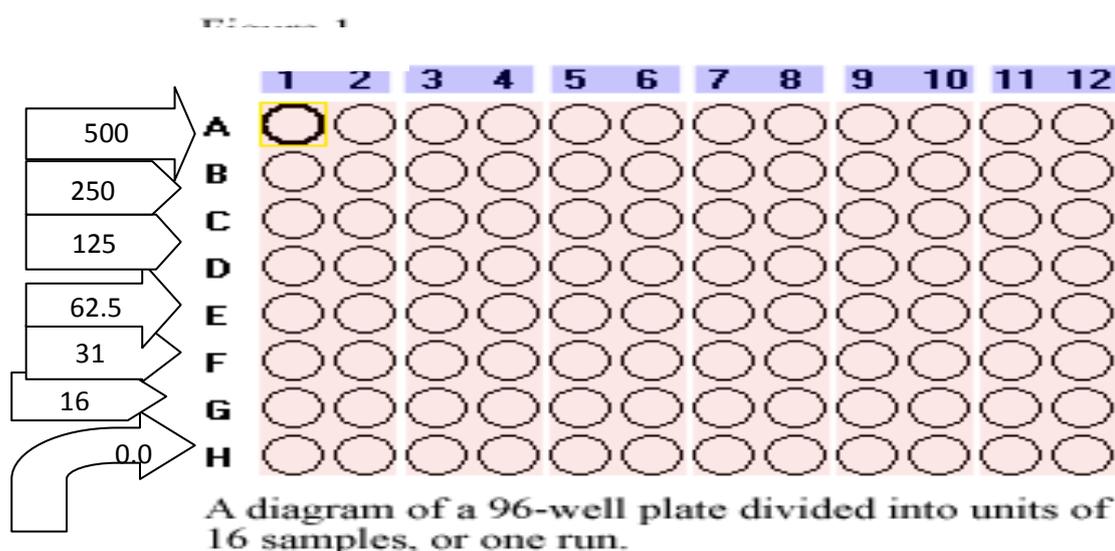
Figure (2.2): 96-well plate used for seeding.

8. After 24hours of cell seeding, when the cells had attached well to the surface of the flask, the medium was taken outside from the well and the cells were exposed to various concentration of serial dilution of plant extracts in fresh full medium as in (Fig2.2). The concentrations of plant extracts were 500, 250, 125, 62.5, 31, 16, and 0.000μg as a control and incubated for 48h at 37°C.

9. All experiments were repeated three times in triplicates formation for each plant extracts.

2.3.6 MTT Assay

Cell proliferation was evaluated by monitoring the conversion of the tetrazolium bromide dye MTT to the dark purple formazan. This reduction is catalyzed by mitochondrial dehydrogenase enzymes that is a measure of cell viability.((Mosmann, 1983)

Cells were plated in 100 μl DMEM in 96-well plate and treated for 48h with various concentrations of plant extract as mentioned previously. The extract were stilled for 48h at 37°C. After removal of plant extract, 70μl of MTT (0.5 mg/ ml) was added and put in incubator for 4hours.

Then, The medium was removed and 70μl of acidic isopropanol were added to dissolve the formazan crystals. The plate was covered with tinfoil for 15 minute and agitated on orbital shaker for 5 minute. Then, the Optical Density (OD) of the MTT formazan was determined at 570 nm in an enzyme-linked immunosorbent assay (ELISA) reader.

Finally, cell viability was defined as the ratio (in %) of absorbance of treated cells to untreated cells (control).

2.3.7 Cytotoxicity assay

Cytotoxicity assay was performed according to the established method of Rahman *et al*. (2001). At 70-80% confluence, cells were

detached from the cultured flask by treatment with 0.05% trypsin- EDTA and a suspension of 2×10^4 cell/ml viable cells was seeded in a 96-well microtitre plate and incubated for 24 h. At this cell density, cells were in confluent monolayer. When cells reached >80% confluence, the medium was replaced and cells were incubated with stock solutions of crude extracts serially diluted to reach concentrations of 500.0, 250., 125, 62.5and 31 μg/ml. After 48 h of incubation, 70μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) solution (0.5mg/ml) were added to each well and incubated at 37 ∘C for 4 h. The medium then aspirated, and the formazan product was solubilized with 70μl acidified isopropanol (0.4N HCl).

2.3.8 Determination of cytostatic effects

For the determination of cytostatic effects, cells were seeded at lower cell density at which cells were at about 50-70% confluency. Therefore, $1.0X10^4$ were plated in 100 μl D-MEM in 96-well-plate and were treated with the plant extract in different concentrations as mentioned in the previous section then incubated for 48 hours at 37°C. Following the removal of plant extracts from each well, the cells were incubated in D-MEM to which MTT (0.5 mg/ ml) was added to each well (70 μl), and then cells were incubated for 4h at 37°C. The medium was removed and 70 μl of isopropyl alcohol were added to dissolve formazan crystals. The plate was then covered with tinfoil and agitated on orbital shaker for 5 min.

Chapter Three

Results

Chapter Three
Results

MTT is a widely-used test to evaluate the metabolic activity of cultured cells by measuring the activity of the mitochondrial succinate dehydrogenase enzyme. MTT is a specialized test used for quantification of both cell viability and cell cultures in 96-well plates. This test is widely used to evaluate the *in vitro* cytotoxic and cytostatic activities of medicinal plants. In the present *in vitro* study, we used this test to assess the cytotoxic as well as the cytostatic effects of our test plants. Therefore, cells from human lung cancer cell line (Fibroblast), human prostate cancer cell line (PC3) and murine macrophages cell line (Raw 264-7) were exposed to increasing concentrations of the two plant extracts (16-500 μg/ml of culture medium) for 48h. Following removal of plant extracts from each well, the MTT was carried out as described in the Material and Methods section. Primary isolated peritoneal macrophages were used as control cells in order to assess the specificity of the plant extracts towards cancer cells.

3.1 Evaluation of effects on cell viability using MTT assay:

3.1a Fibroblast cell lines
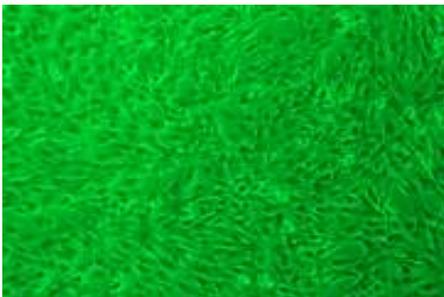
Figure (3.1): Fibroblast cell lines under microscope

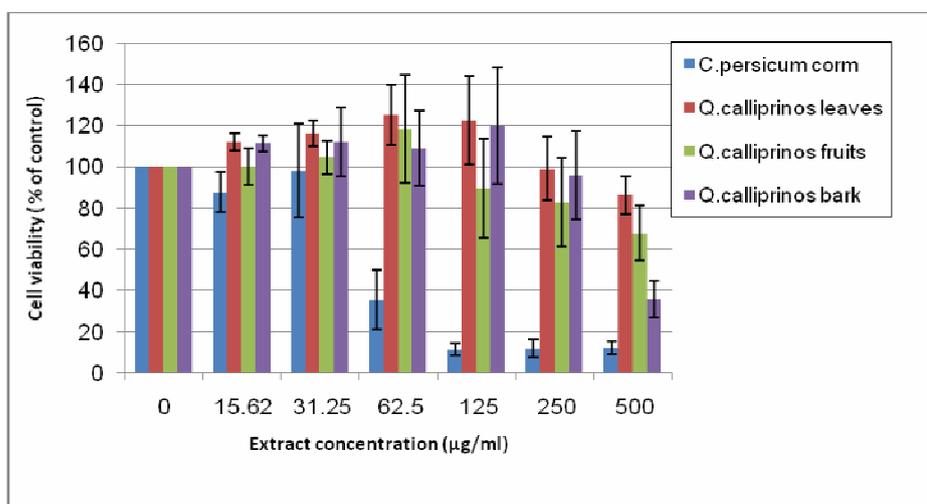**Cytotoxic experiment:** Extracts from *C. persicum* corm exhibited no sign effects at concentrations of 16 and 31 µg/ml but the same extracts reduced the cell viability at concentrations higher than 62.5 µg/ml (Figure 3.2). Cell viability was decreased with increasing extract concentrations of *C. persicum* corm. All three extracts from *Q. calliprinos* (leaves, fruits and bark) showed no appearance of cytotoxicity on the fibroblast (Figure 3.2).

Figure (3.2): *Cytotoxic experiment with fibroblasts.*

For the determination of cytotoxic effects of extracts from *Q. calliprinos* and *C. persicum* on fibroblast cell line, 20.000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytostatic experiment: Extracts from *C.persicum* corm exhibited no cytostatic effects at concentrations of 16 and 31 µg/ml; but the same extracts reduced the cell viability at concentrations higher than 62.5 µg/ml (Figure 3.3). Cell viability was decreased with increasing extract

concentrations of *C. persicum* corm. An extract from *Q. calliprinos* leaves has no effects on cell viability but *Q. calliprinos* fruits and bark decreased cell viability at concentrations of 500 µg/ml (Figure 3.3).
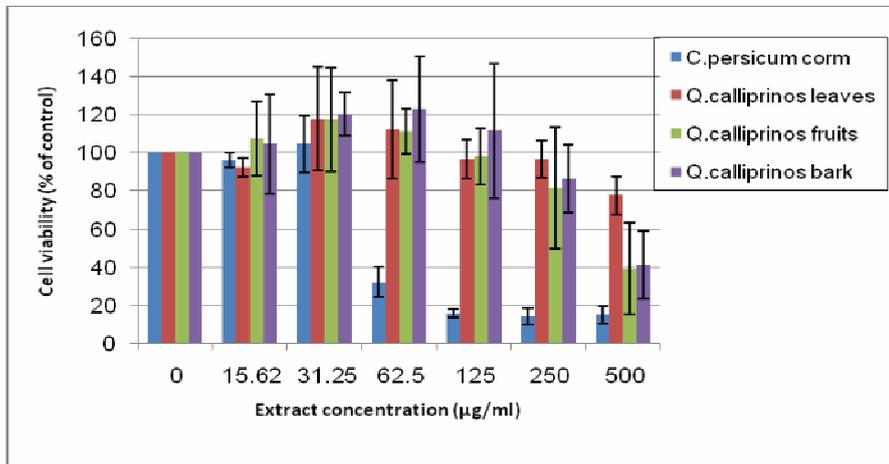
Figure (3.3): Cytostatic effects *of Q. calliprinos* and *C. persicum* extracts on cells from fibroblast cell line.

For the determination of cytostatic effects of plant extracts from *Q. calliprinos* and *C. persicum* on fibroblast cell line, 10.000 cells/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

3.1b Raw cell lines
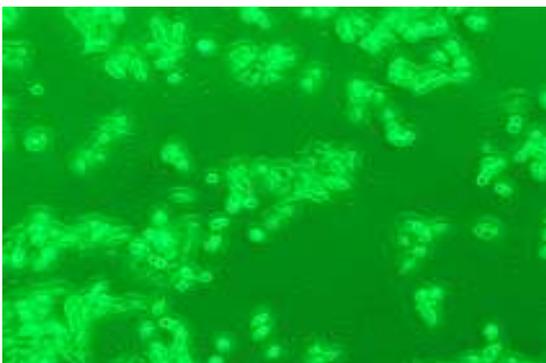
Figure (3.4): HCT Raw cell lines under microscope.

Cytotoxic experiments: No reduction of cell viability was observed in Raw cell line after treatment with 16 and 31 μg/ml of extracts from *C. persicum* corm. The same extracts induced cytotoxic effects at concentrations higher than 62.5μg/ml as seen in (Figure 3.5). Cell viability was decreased with increasing extract concentrations of *C. persicum* corm. All three extracts from *Q. calliprinos* (leaves, fruits and bark) showed no sign of cytotoxicity in the Raw cell lines as shown in (Figure 3.5).


Figure (3.5): Cytotoxic experiment with *Raw264-7*.

Cytotoxic effects of extracts *Q. calliprinos* and *C. persicum* on Raw264-7cell line were determined by the MTT assay. 20.000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytostatic experiment: At concentration below 31μg/ml the extracts from *C. persicum* corm displayed no cytostatic effects but the same extracts minimized the amount of MTT at concentrations higher than 62.5μg/ml as

presented in (Figure 3.6). Cell viability was decreased by increasing extract concentrations of *C. persicum* corm. Extracts from *Q. calliprinos* leaves and fruits decreased cell viability at concentrations of 500 µg/ml while *Q. calliprinos* bark has no effects on cell viability (Figure 3.6).
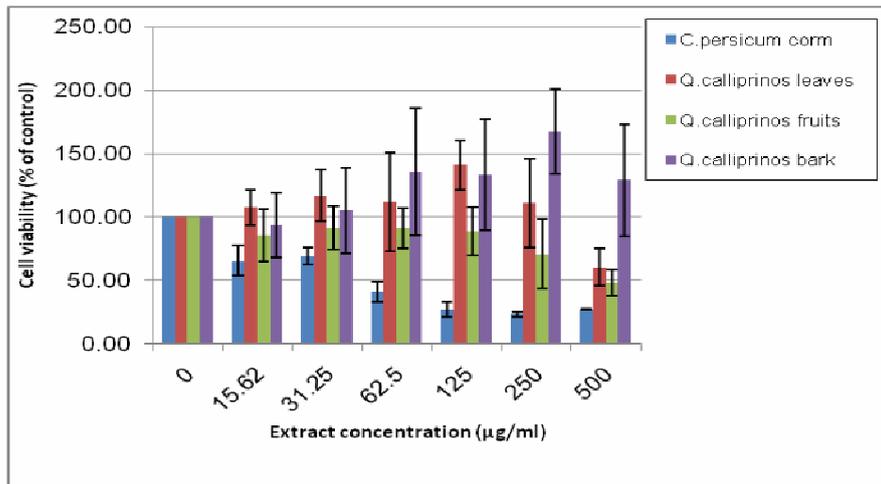
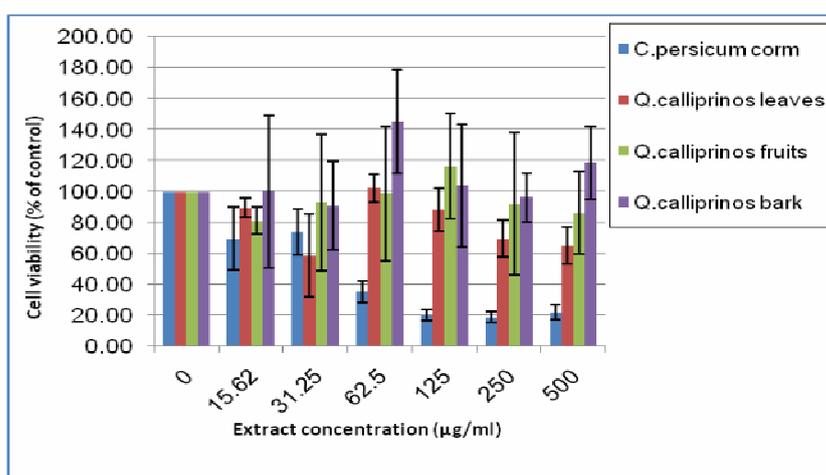**Figure (3.6): Cytostatic effects of *Q. calliprinos* and *C. persicum* extracts on fibroblast cell line.**

Cytostatic effects of plant extracts from *Q. calliprinos* and *C. persicum* on Raw264-7cell line were tested with the aid of MTT assay. 10000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three separate experiments carried out in triplicates.

3.1c PC3 cell lines

Figure (3.7): PC3 cell lines

Cytotoxic experiment: Cell viability was decreased by increasing extract concentrations of *C. persicum* corm. *Q. calliprinos* (leaves, fruits and bark), the three extracts did not give any sign of cytotoxicity on the PC3 as presented in (Fig 3.8).
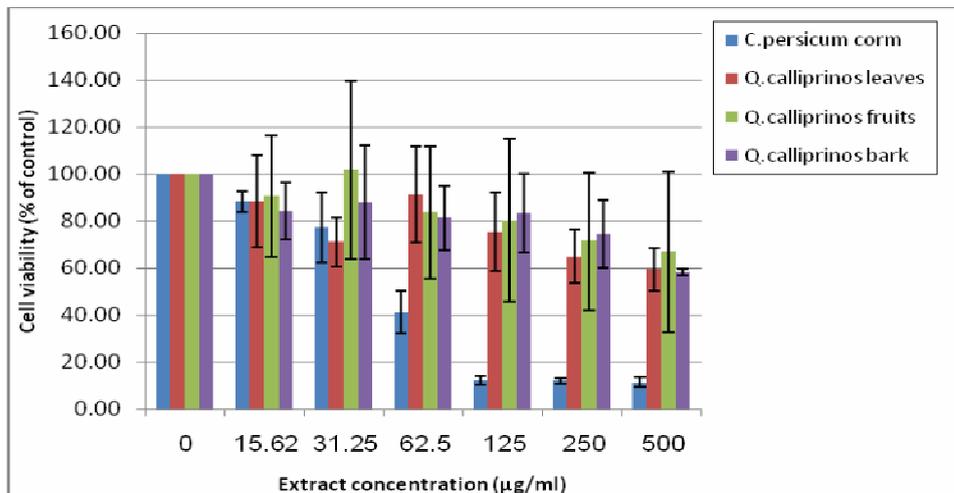

Figure (3.8): Cytotoxic experiment with PC3.

Cytotoxic effects of extracts from *Q. calliprinos* and *C. persicum* on PC3 cell line were tested for their ability to inhibit the proliferation of the PC3. 20000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. The

lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytostatic experiment: At a concentration less than 62.5 μg/ml for *C. persicum* corm indicated no cytostatic effects on PC3 cell line; but the same extracts showed a significant cytostatic effects at concentrations higher than 62.5μg/ml (Figure 3.9). All three extracts from *Q. calliprinos* (leaves, fruits and bark) did not indicate any form of anticancer activity on the PC3 (Figure 3.9).
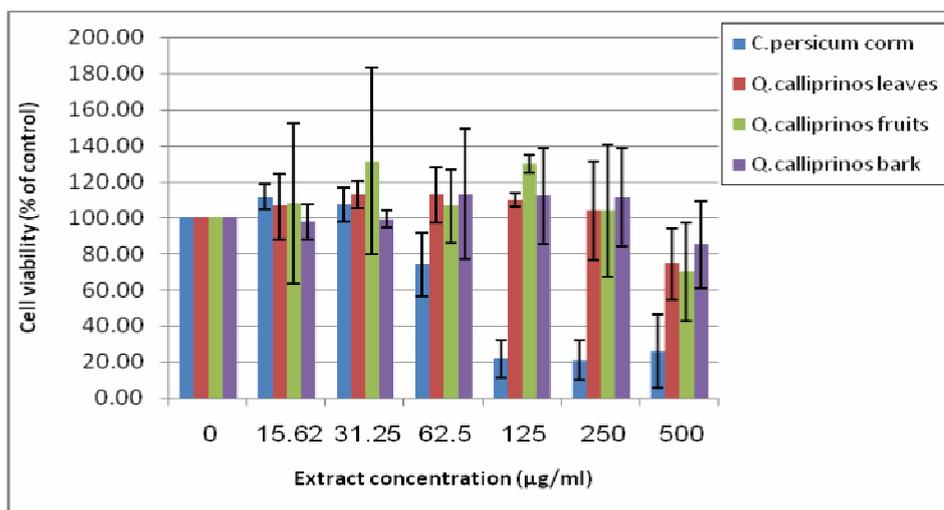
Figure (3.9): Cytostatic effects *Q. calliprinos* and *C. persicum* extracts on PC3 cell line.

Cytostatic effects of extracts from *Q. calliprinos* and *C. persicum* on PC3 cell line were examined using cell viability assay. 10.000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

3.2 The effect of each plant extracts on the three cell lines

Cytotoxic experiment: Figure 3.10 shows the cytotoxic effects of *C. persicum* on the three cell line tested. Extracts from *C. persicum* corm revealed no effects at concentrations below 62 μg/ml, but the same extracts revealed the highest toxicity at concentrations higher than 62.5μg/ml (Figure 3.10). The three cell types were affected at the same level and no cell specific effects were observed.
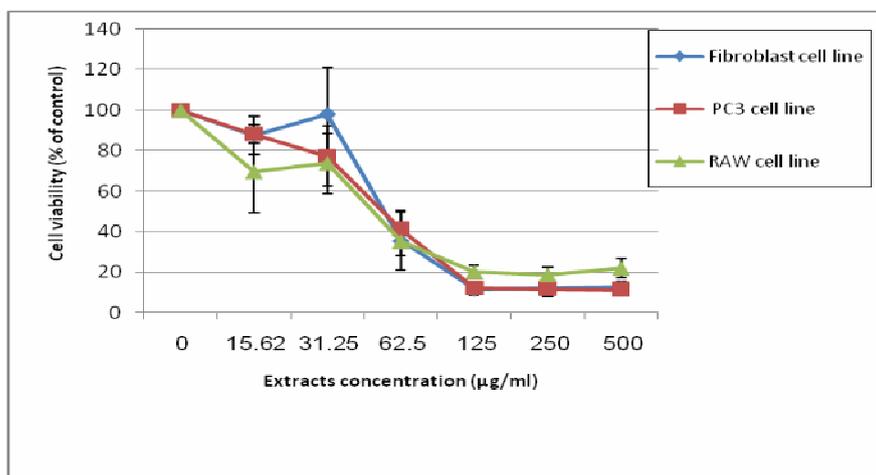
Figure (3.10): Cytotoxic experiment with fibroblast, PC3 and Raw264-7cell line

MTT assay on the three cell lines after overnight treatment with various concentrations of extract from *C. persicum* for 48h. The absorbance of the MTT formazan was determined at 570nm in an ELISA reader. The values given represent the mean ± SD of three independent experiments carried out in triplicates.

Cytostatic experiment: Figure 3.11 depicts the cytostatic effects of *C. persicum* on the three cell line tested. Extracts from *C. persicum* corm revealed no cytostatic effects at concentrations lower than 62.5 μg/ml but

the same extracts reduced the MTT amount at concentrations higher than 62.5μg/ml (Figure 3.11). All the cell types have changed at the same level and no cell specific effects were noticed.
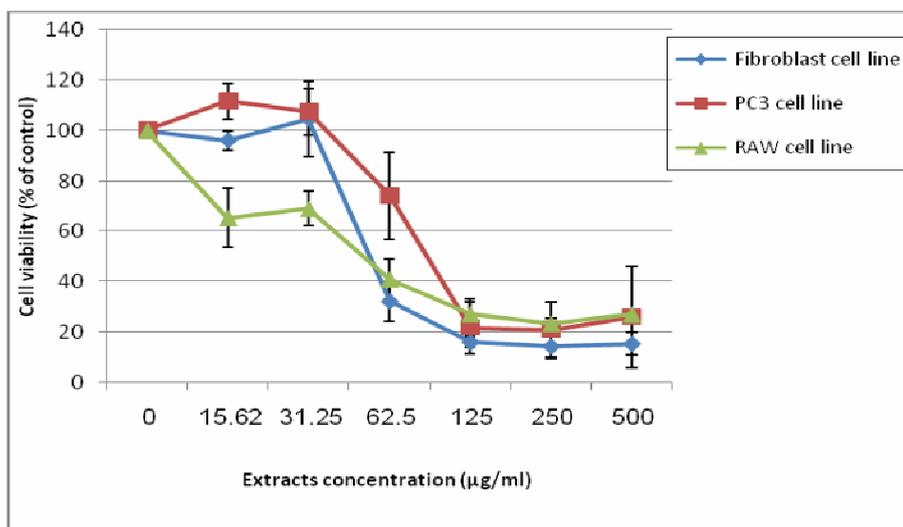
Figure (3.11): Cytostatic effects of *C. persicum* extracts on fibroblast, Raw264-7and PC3 cell line

For the restriction of cytostatic effects of extracts on Fibroblast, PC3 and Raw264-7 cell line, 10000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates .

Cytotoxic experiment: Extracts from *Q. calliprinos* leaves showed no positive effects at 16 and 31 μg/ml, but the same extracts slightly reduced the MTT amount at concentrations more than 62.5μg/ml for Fibroblast cell lines as seen in (Figure 3.12). Both PC3 and *Raw264-7* cell line were affected when treated with *Q. calliprinos* extract but without cytotoxic effects on cell line (Figure 3.12).
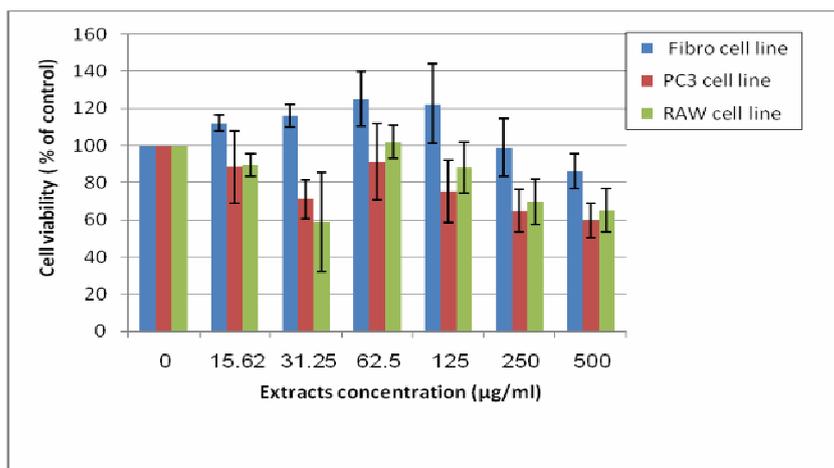
Figure (3.12): Cytotoxic experiment with fibroblast- Raw264-7 and PC3 cell line.

In order to determine the cytotoxic effects of *Q. calliprinos,* extracts of leaves were added on Fibroblast, PC3 and Raw264-7 cell line, 20000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytostatic experiment: The three cell lines were affected nearly at the same level after being treated by extract of *Q. calliprinos* leaves (Figure 3.13).
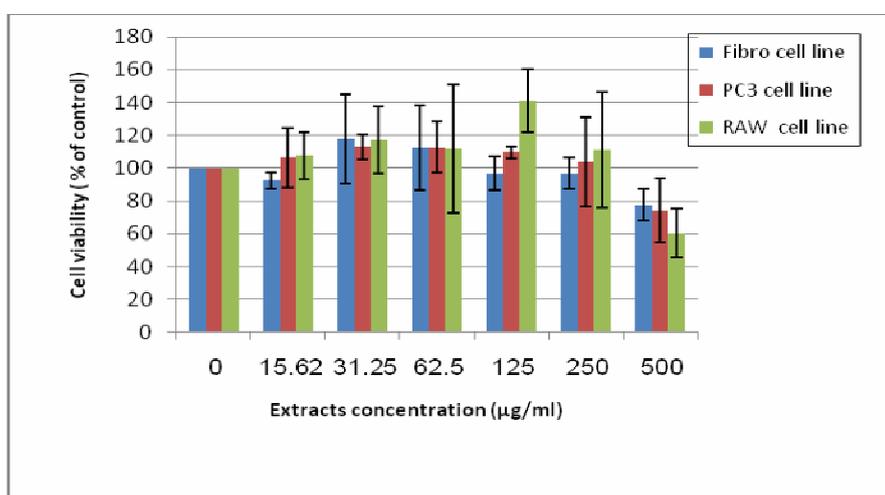
Figure (3.13): Cytostatic effects *Q. calliprinos* leave extracts on fibroblast, Raw264-7 and PC3 cell line.

For the determination of cytostatic effects of *Q. calliprinos* leave extracts on Fibroblast, PC3 and Raw264-7 cell line, 10000 cell/well were seeded in 96 well plate for 24h and were treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytotoxic experiment: No significant reduction in MTT amount of both fibroblast and Raw cell line after being treated with various concentration from *Q. calliprinos* fruits was found. However, a slight decrease was observed at concentration of 500μg/ml of both PC3 and fibroblast cell line. (Figure 3.14).

Figure (3.14)*: Cytotoxic experiment with fibroblast, Raw264-7 and PC3 cell line.*

Cytotoxic effects of *Q. calliprinos* fruits extracts on Fibroblast, PC3 and Raw264-7 cell line were tested using MTT assay. 20000cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability

was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicate.

Cytostatic experiment: No anticancer activity was observed on the three cell lines after being treated with *Q. calliprinos* fruit extracts at concentrations of 16 and 31 µg/ml but the same extracts slightly decreased the cell proliferation at concentrations higher than 62.5 µg/ml for all cell lines (Figure 3.15).

Figure (3.15): Cytostatic effects *Q. calliprinos* fruit extracts on fibroblast, Raw264-7 and PC3 cell line.

For the determination of cytostatic effects of plant extracts from *Q. calliprinos* fruits on Fibroblast, PC3 and Raw264-7 cell line, 10000cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.
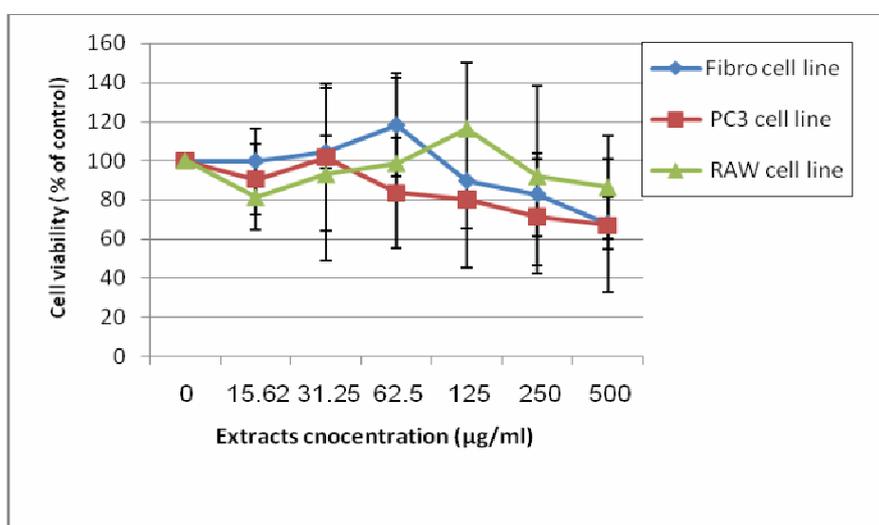
Cytotoxic experiment: *Q. calliprinos* bark has no effect at 16 and 31 μg/ml for all cell lines; but the same extracts slightly reduced the cell viability at concentrations of 500 μg/ml for both fibroblast and PC3 cell lines (Figure 3.16).
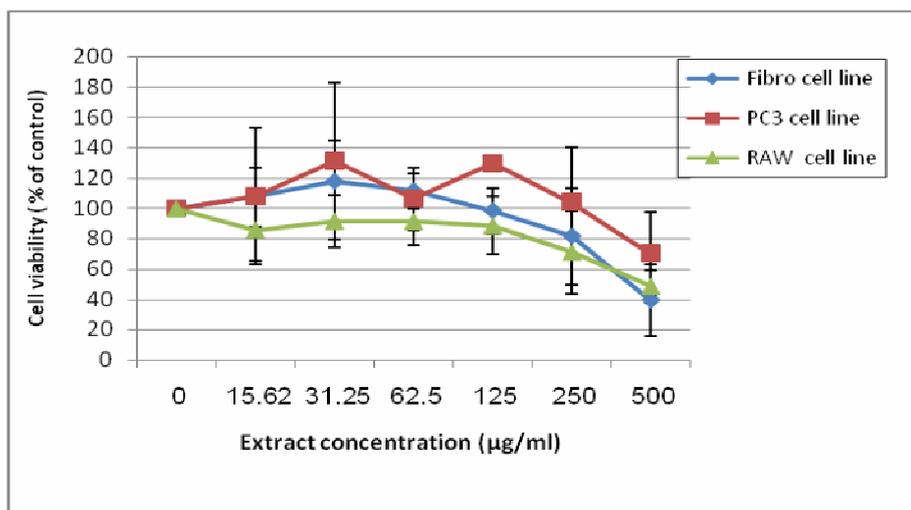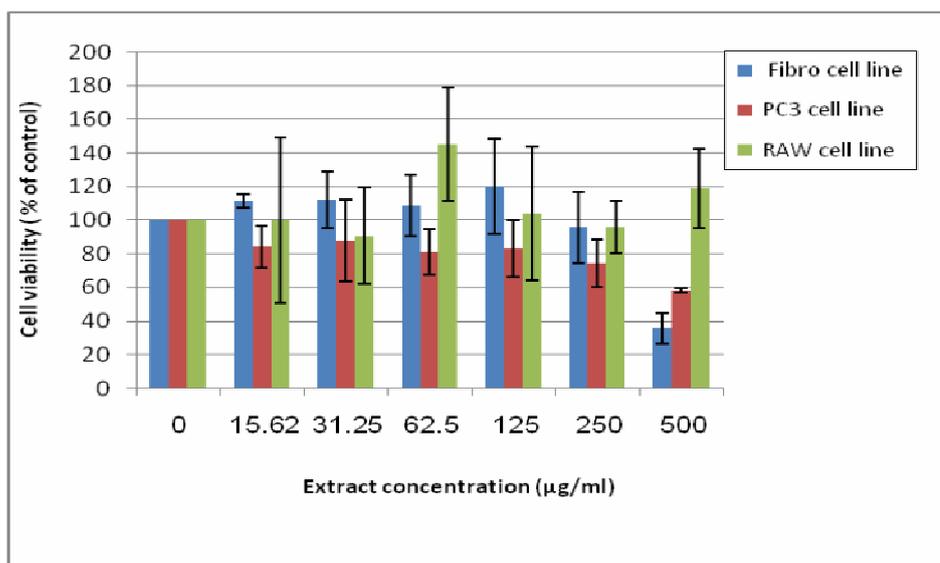

Figure (3.16): Cytotoxic experiment with fibroblasts, Raw264-7 and PC3 cell line

Cytotoxic effects of *Q calliprinos* bark extracts on Fibroblast, PC3 and Raw264-7 cell line were investigated by using MTT reduction assay. Therefore, 20.000cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. The cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytostatic experiment: Extracts from *Q.calliprinos* bark revealed no cytostatic effects at all concentrations of both PC3 and *Raw264-7* cell lines. A slight but not significant reduction in the MTT amount at concentrations of 500 μg/ml (Figure 3.17) for fibroblast cell lines.
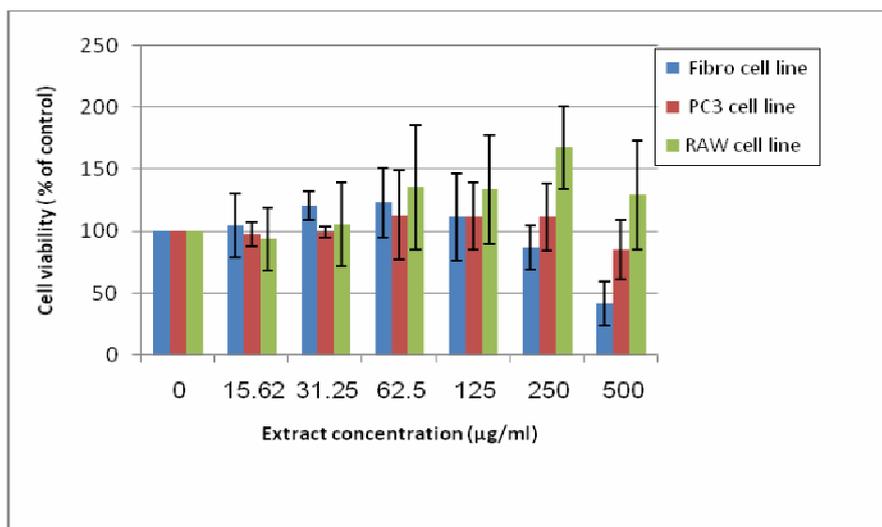
Figure (3.17): Cytostatic effects *Q. calliprinos* **bark extracts on fibroblast, Raw264-7 and PC3 cell line.**

For the restriction of cytostatic effects of plant extracts from *Q. calliprinos* bark on Fibroblast, PC3 and Raw264-7 cell line, 10000cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16,31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

3.3 Cytotoxic Vs cytostatic effects of plant extracts

Cytotoxic and cytostatic experiment for fibroblast cell lines: No difference in both cytostatic and cytotoxic effects were noticed for the extracts from *C. persicum* corm on fibroblast cell lines. Both of them showed no effects at concentrations of 16 and 31 μg/ml and the same extracts reduced the MTT amount at concentrations higher than 62.5 μg/ml in both experiments (Figure 3.18). Cell proliferation was decreased with increasing extract concentrations of C. *persicum* corm.
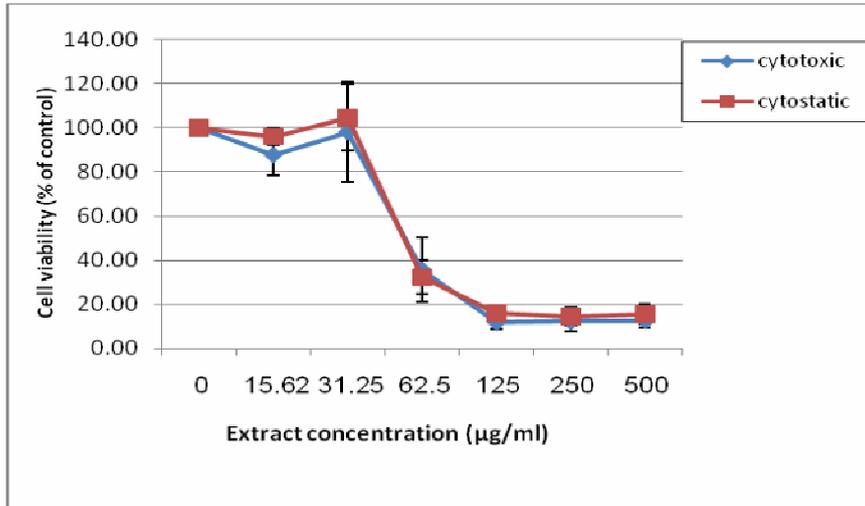
Figure (3.18): Cytotoxic and cytostatic effects of *C. persicum* extracts on fibroblast cell lines.

To compare between cytostatic and cytotoxic effects of *C. persicum* corm extracts on fibroblast cell line, 10000 and 20000 cell/well respectively were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined by using the MTT test. The lines represent the mean ± SD of three independent experiments carried out in triplicates.

Cytotoxic and cytostatic experiment: Extracts from *Q. calliprinos* as shown in the graph revealed no cytostatic and cytotoxic effects at concentrations Less than 125 μg/ml. A little reduction in the cell viability for *Q. calliprinos* leaves and fruits at concentration higher than 250 μg/ml but the bark from the same plants decreased at concentration of 500 μg/ml for both experiments (Figure 3.19).
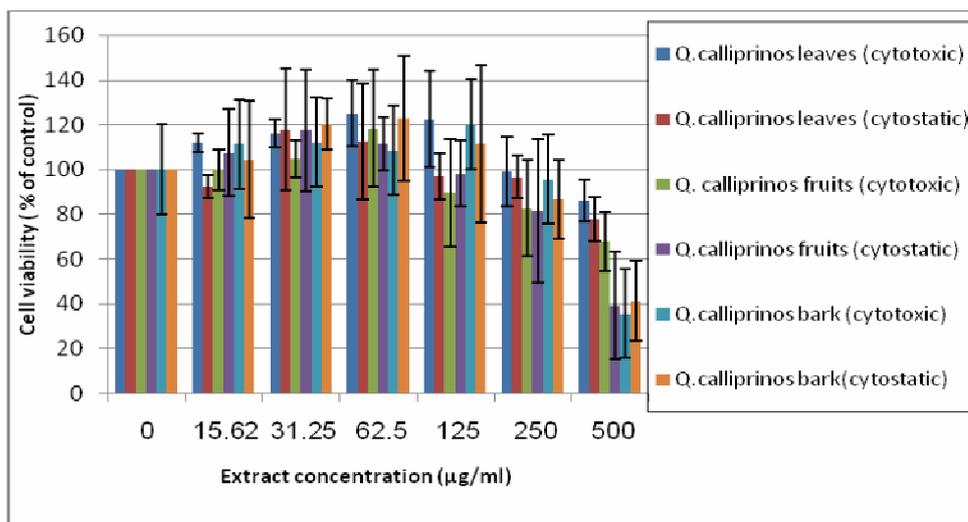
Figure (3.19): Cytotoxic and cytostatic effects of *Q.calliprinos* (leaves, fruits and bark) extracts on fibroblast cell lines.

For the determination of cytostatic and cytotoxic effects *Q. calliprinos* leaves, fruits and bark extracts on Fibroblast cell line, 10000 and 20000 cell/well respectively were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. Cell viability was determined by using the MTT test. The graph represents the mean of three independent experiments carried out in triplicates. Values given represent the mean ± SD.

Cytotoxic and cytostatic experiment for *PC3* cell line: No effect on cell viability was seen for the PC3 cell line when treated with concentration lower than 62.5 μg/ml in the two experiments, but significant decline in cell viability for the same cell line was observed when treated with concentration higher than 62.5 μg/ml (Figure 3.20).
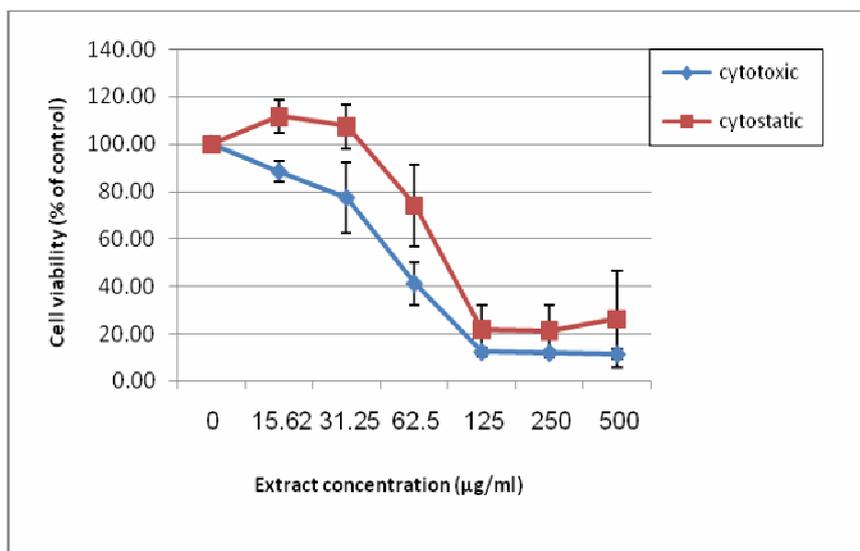
Figure (3.20): The effect of *C. persicum* corm extract on PC3 cell line (cytotoxic & cytostatic).

Cytostatic and cytotoxic effects of *C. persicum* corm extracts on PC3 cell line were tested by MTT assay. Therefore, 10000 and 20000 cell/well respectively were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. Figure 3.20 presents the mean of three independent experiments carried out in triplicates. Values given represent the mean ± SD.

Cytotoxic and cytostatic experiment: when comparing between cytotoxic and cytostatic experiment for the *Q. calliprinos* extract, no tangibel reduction was approximately seen in cell viability for all concentrations (Figure 3.21).
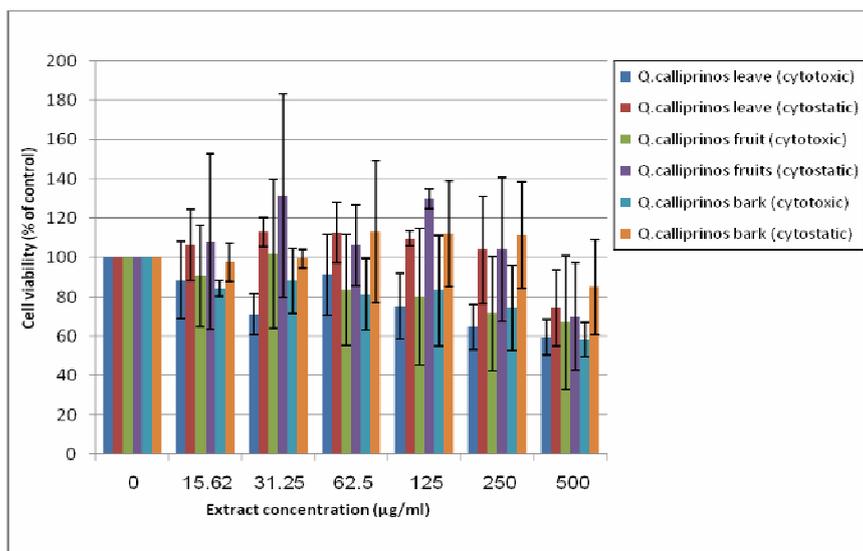
Figure (3.21): The effect of *Q. calliprinos* leaves, fruits, and barks on PC3 cell line. (cytotoxic & cytostatic).

The effects of cytostatic and cytotoxic *Q. calliprinos* leaves, fruits and bark extracts on PC3 cell line were investigated by using cell viability assay. Therefore, 10000 and 20000 cell/well respectively were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500μg/ml) for 48h. Cell viability was determined by using the MTT test. The graph represents the mean of three independent experiment carried out in triplicates. Values given represent the mean ± SD.

Cytotoxic and cytostatic experiment for *Raw264-7* cell line: no change on cell viability was seen for the *Raw264-7* cell line when treated with concentration 16 μg/ml and 31 μg/ml. A considerable reduction in cell viability for the same cell line when treated with concentration higher than 62.5μg/ml in the two experiments was observed (Figure 3.22).
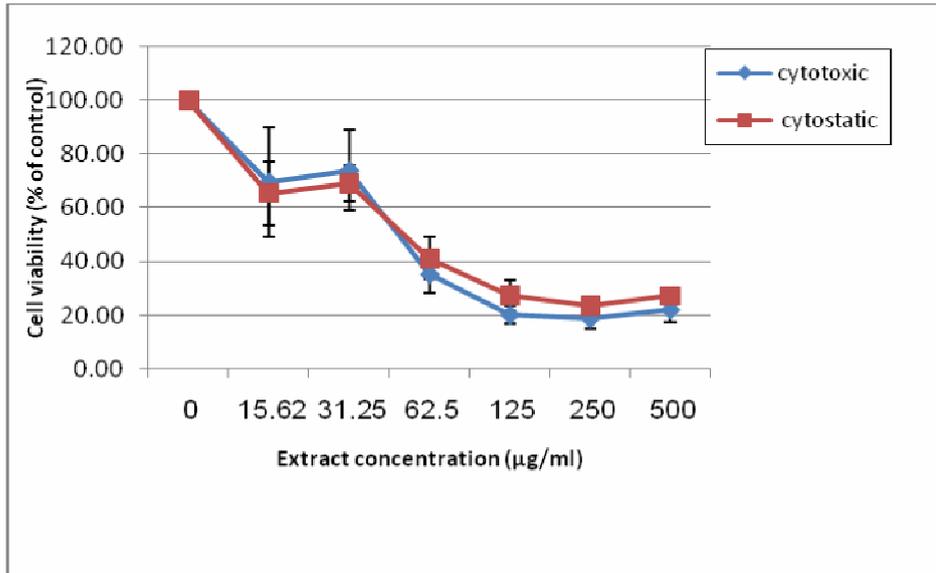
Figure (3.22): The effect of *C. persicum* corm on RAW cancer cell line. (cytotoxic & cytostatic).

For the determination of cytostatic and cytotoxic effects *C. persicum* corm extracts on RAW cell line, 10000 and 20000cell/well respectively were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 μg/ml) for 48h. The absorbance of the MTT formazan was determined at 570nm in an ELISA reader. Values given represent ± SD of three independent experiment carried out in triplicates.

Cytotoxic and cytostatic experiment: Figure 3.23 depicts the cytostatic and cytotoxic effects of *Q. calliprinos* on *Raw264-7* were tested. No significant reduction in MTT amount for *Raw264-7* was observed when treated with *Q.calliprinos* extracts at all concentration.
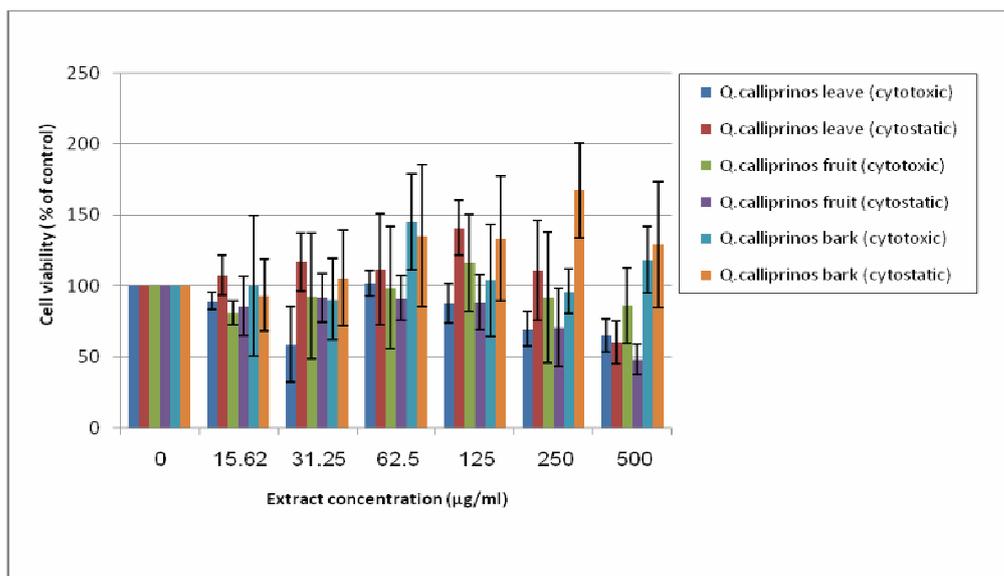
Figure (3.23): The effect of *Q. calliprinos* leaves, fruits and bark extract on RAW cell line, (cytotoxic and cytostatic experiment).

For the determination of cytostatic and cytotoxic effects *Q. calliprinos* leaves, fruits and bark extracts on RAW cell line, 10000 and 20000 cell/well respectively were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (16, 31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. The graph represents the mean of three independent experiment carried out in triplicates. Values given represent the mean ± SD.

Cytostatic experiment: A considerable reduction in cell viability was seen. The primary isolated cells rat-peritoneal macrophages was treated with *C. persicum* corm.
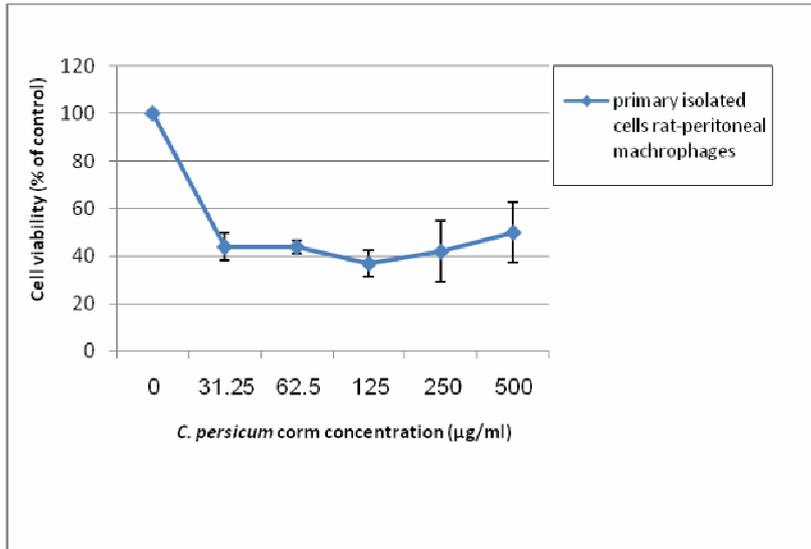
Figure (3.24): The effect of *C. persicum* corm on primary isolated cells rat-peritoneal machrophages. (cytostatic experiment).

Cytostatic effects of plant extracts from *C. persicum* on machrophages were examined by using cell viability assay. 10000 cell/well were seeded in 96 well plate for 24h and treated with increasing concentrations of plant extracts (31, 62.5, 125, 250, 500 µg/ml) for 48h. Cell viability was determined by using the MTT test. The graph represents the mean of three independent experiments carried out in triplicates. Values given represent the mean ± SD.

# Chapter Four

Discussion

Chapter Four
Discussion

4.1 Anticancer medicinal plants

Many scientific researchers have drawn attention to anticancer properties of medicinal herbs (Saad *et al.*, 2008; Ali-Shtayeh *et al.*, 2008). Several *in vitro* and *in vivo* experimental studies have revealed important phytochemical constituents derived from traditional medicinal plants that inhibit or decrease some type of cancers. The term phytochemical is defined as any chemical compound that naturally exists in plants which have biological effects in the treatment of disease (Zaid *et al.*, 2011). Some of them have physiological function as those present in fruits and vegetables that are involved in metabolic pathways and antioxidant properties (Brown, & Arther, 2001). Many researches are directed towards the discovery of phytochemicals that give high antitumor properties with the least side effects and that can be considered the typical phytochemical and have well known mechanism of action. Experiments that have been conducted on *in vitro* and *in vivo* showed that medicinal plants possess several mechanism in cancer prevention such as; induction of apoptosis, antiangiogenesis, antimetastasis effect, and anti-inflammatory/ antioxidant properties (Zaid *et al.*, 2010). Several studies reported strong association between consumption of vegetables and reduction in cancer risk. For example, one study revealed that consuming some garlic and onions was linked with reduction in stomach cancer (Setiawan *et al.*, 2005), nearly 30-50% reduction in prostate cancer risk (Colli, & Amling, 2009; Hsing *et al.*,

2002). Many cancer patients prefer herbal medicine for their illness when standard cancer treatment fails, or they use it in addition to conventional treatments. Despite the rapid understanding of cellular and molecular mechanisms of cancer, the average of deaths from this disease is still high (Zaid *et al.*, 2011). In the present *in vitro* study, I tested cytotoxic and cytostatic effects of *C. persicum* corm and *Q. calliprinos* (leaves, fruits, and bark) on cells from three cancer cell line, namely, human-lung carcinoma cell line (Fibro), rat macrophages (Raw 164-7) and human prostate adenocarcinoma (PC3). Primary rat peritoneal macrophages were taken as a control sample to test the cancer specificity of the plant extracts. We choose this cancer type, because epidemiological studies on cancer have revealed that several cancer types are more common among people as: lung, prostate, breast, and colon cancer. The Hadith, "The one who caused the disease created the remedy". And " For every disease, God has given the cure, This has encouraged me to carry out this study and to look for potential anti-cancer properties of medicinal plants used in traditional Arab and Islamic medicine (Zaid *et al.*, 2011).

4.2 Cytotoxic Vs Cytostatic properties

The death of the cell as a result of a compound that controls cell division through interrupting DNA synthesis is called cytostatic agents. A constant percent of the total number of tumor cells will be killed through a high effective dividing process of high fraction of cancer cells. This agent may also be toxic to normal cells such as bone marrow, hair, and mucosa

(Dorr, & Fritz, 1980), and this depends on the intensity of the dose from these agents. Many therapeutic agents developed today to inhibit cancer-special receptor, or to be a pathway that leads to stop the progress of cancer and the death of the cell. But a largely, exhibited cytostatic effect by stopping the cancer progress rather than the death of the cell through target invasion, proliferation, and migration (Blakelely, & Grossman, 2012).

Differences between cytotoxic and cytostatic effects which are presented in the following.

Table (4.1): Differences between cytotoxic and cytostatic effects

Cytotoxic	Cytostatic
Treating cells with cytotoxic agent have several fates: 1. Cell may undergo cell lysis. 2. A decrease in cell viability. 3. Or apoptosis.	Inhibitory growth effect with their ability to induce cell cycle arrest.
The number of cancer cell line is high 20.000cell/well	The number of cancer cell line is 10.000 cell/well
cytotoxic agent will kill both cancer and normal cell with many side effects	Cytostatic to cancer cell with less side effects.

4.3 Medical uses of *Cyclamen persicum*

C. persicum is a species of the family Myrsinaceae. The plant is native to rocky hillsides, shrubland, and woodland above sea level, from south-central Turkey to Palestine and Jordan. Wild plants have heart-shaped leaves which grow from tuber which is considered as the storage organ. Many recent studies have focused on the medical importance of its extract, since it was proved in previous studies for its activity against several diseases. *C. persicum* leaf and bulb are used to treat external skin

and ear infections (Said *et al*., 2006 ; Ali-Shtayeh *et al*., 2000; Azaizeh *et al*., 2006). The bulbs are used to treat genitourinary tract, sterility of men and women, prostate and cystitis (Abu-Rabia, 2005).

4.3.1 *In vitro* Anticancer activity of *C. persicum* against PC3, Fibroblast and Raw 164-7

According to a study conducted by Khaleeliah, in 2001 on *C. persicum* against three cell line which are: PC3, MCF-7 and LNCaP indicted that *C. persicum* corm was the most active extract against the cell lines tested with high inhibitory effects. The present study pointed that *C. persicum* corm has cytotoxic and cytostatic effects against PC3, Fibroblast and Raw164-7 with high inhibitory effects at concentration (62.5, 125, 250, 500µg/ml), but no significant effects at both concentration (16, 31.25µg/ml).

4.3.2 Anticancer properties of *C. persicum* corm

C. persicum corm extracts have cytotoxic and cytostatic activities. This result is in agreement with that of Khaleeliah (2001), which attributed the anticancer of the plant to the presence of some alkaloids, diterpenes and sesquiterpenes 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 (Khaleeliah, 2001).

4.3.3 Primary isolated cells rat- peritoneal macrophages against *C. persicum* corm

Anti-proliferative activities are reported about the current study on the control normal cell sample as macrophages on *C. persicum* corm at concentration more than 62.5μg/ml. This indicates that *C. persicum* corm extract has cytostatic effects on both cancer and normal cells not specific to cancer cells.

4.4 Medical uses of *Quercus calliprinos*

Q. calliprinos is a species of the Fagaceae family . It spreads to the east of the Mediterranean region and southwest Asia, from northern Algeria and Turkey east across the Middle East. Previous studies demonstrated the bioactivity of *Q. calliprinos* bark, stem and fruits that are used to treat fever, ulcer and high blood pressure (Zaid *et al*., 2010). This plant was reported for the first time in a survey for treating diabetes in our region (Said *et al*., 2002). Additional uses of fruits and bark decoction from these plants will treat bed wetting, ulcer, diabetes and skin disease (Zaid *et al*., 2010 and Said *et al* 2002). Another study done by Ali-Shtayeh *et al,*. (2000) showed that *Q. calliprinos* can be considered the most active in addition to other plants for the treatment of prostate disorders, including prostate cancer.

4.4.1 *In vitro* **anticancer activities of** *Q. calliprinos* **against PC3, Fibroblast and Raw164-7 cell lines**

Previous studies showed that *Q. calliprinos* extract stimulate of LNCaP and MCF-7 cancer cell line **(**Khaleeliah, 2001**)**. The findings from current study indicate that neither significant cytotoxic nor cytostatic effects were reported on three parts of *Q. calliprinos*: The leaves, fruits and bark against PC3, Fibroblast and Raw164-7 by using MTT assay which means that this plant is safe and has no toxicity to cancer cells tested.

References

Abu-Rabia, A. (2005). **Herbs as a food and Medicine Source in Palestine**. Asian Pacific Journal of Cancer Prevention, 6: 404-407.

Ahmad, N., Gupta, S., Mukhtar, H. (2000). **Green tea polyphenol epigallocatechin − 3 - gallate differentially modulates nuclear factorkappa B in cancer cells versus normal cells**. Arch. Biochem. Biophys.: 338-376

Al-Akhras, M., Amin, A., Mohammad, K., Al-Haddad, F., and Hamza, A. (2007). **In vitro studies on the effect of phototoxicity of a new photosensitizer extracted from flowers and aerial parts of Cichorium Pumilum**. Amer J Pharmacol Toxicol, In Press.

Ali-Shtayeh, M. S., & Jamous, R. M. (2006). **Ethnobotany of Palestinian herbal medicine in the Northern West Bank and Gaza Strip: Review and a comprehensive field study**. BERC, Palestine, Nablus, Til, 4: 1-22.

Ali-Shtayeh, M. S., Jamous, R. M. (2008). **Traditional Arabic Palestinian Herbal Medicine, TAPHM**. Til, Nablus, Palestine, Biodiversity and Environmental Research Center, BERC.

Ali-Shtayeh, M. S., & Jamous, R. M. (2011). **Herbal preparation use by patients suffering from cancer in Palestine**. Complementary Therapies in Clinical Practice,17: 235-240.

Ali-Shtayeh, M. S., Jamous, R. M., Al-Shafie', J. H. *et al*. (2008): "*Traditional knowledge of wild edible plants used in Palestine (Northern West Bank): a comparative study*," **Journal of Ethnobiology and Ethnomedicine**, volume 4.

Ali-Shtayeh, M. S., Yaniv, Z., & Mahajna, J. (2000). **Ethnobotanical survey in the Palestinian area: Aclassification of the healing potential of medicinal plants**. Journal of Ethnopharmacology, 73: 221-232.

Ames, B. N., Gold, L. S. & Willett, W. C. (1995). **The causes and prevention of cancer.** Proc. Natl. Acad. Sci. USA, 92: 5258–5265.

Amin, A. & Mousa, M. (2007). Merits of anti-cancer Plants from the Arabian Gulf region. Cancer Therapy, 5: 55-66.

Anand, P., Kunnumakkara, A. B. (2008). **"Cancer is a Preventable Disease that Requires Major Lifestyle Changes"**. *Pharmacy Research*, 25 (9): 2097–2116.

Azaizeh, H., Saad, B., Khalil, K., and Said, O. (2006). **The state of the Art of Traditional Arab Herbal Medicine in the Eastern Region of the Mediterranean: A Review**. eCAM, 3(2): 229-235.

Becker, W. M., Kleinsmith, L. J., Hardin, J. And Bertoni, G. P. (2009). **The world of the cell**. 7nd Edition. person eduction, Inc., publishing as person Benjamin Cummings, San Francisco: 757-790.

Blakeley, J. & Grossman, S. A. (2012). **Chemotherapy with cytotoxic and cytostatic agents in brain cancer**. Brain cancer program, John Hopkins Hospitals, Baltimore, USA: 104.

Brescia, P., M. Sc. & Banks, P. Ph. D. (2009). **Quantifying Cytotoxicity of Thiostrepton on Mesothelioma Cells using MTT Assay and the Epoch™ Microplate Spectrophotometer**. BioTek Instruments, Inc., Winooski, VT.

Brown, K. M; Arthur, J. R. (2001). "**Selenium, selenoproteins and human health: a review**". Public health nutrition, 4 (2B): 593–599.

Caragay, A. B. (1992). **Cancer-preventative foods and ingredients**. Food Technol., 46: 65–68.

Colli, J. L, Amling, C. L. (2009). **Chemoprevention of prostate cancer**: what can be recommended to patients? Curr Urol Rep, 10(3): 165-171.

Cooper, G. M. (1990). **Oncogenes (The cancer cell)**. Chapter one.page4.

Cooper, G. M. (2000). **The development and Causes of cancer**. The cell: A molecular approach. 2nd edition Sunderland (MA): Sinauer Associates.

Debussche, M., Debussche, G., Grandjanny, M. (2000). **Distribution of cyclamen repandum sibth. & sm. Subsp repandum and ecology in Corsica and continental France**. Acta Botanica Gallica 147(2): 123-142.

Deshmukh, V. N., and Sakarkar, D. M. (2011). **Ethnopharmacological Review of Traditional Activity**. International Journal of pharm Tech Research, 3(1): 298-308.

Dorr, R. T., & Fritz, W. L. (1980). **Cancer Chemotherapy Handbook**. Elsevier North-Holland, New York.

Edeoga, H. O, Okwu, D. E., & Mbaebie, B.O. (2005). **Phytochemical constituents of some Nigerian medicinal plants**. African Journal of Biotechnology, 4 (7): 685-688.

FAO, (2004). **Trade in Medicinal Plants**, Economic and Social Department, Food And Agriculture Organization of United Nations, Rome, Italy.

Farnsworth, N. R., O. Akelere, A. S. Bingel, D. D. Soejarto and Z. Guo, (1985). **Medicinal plants in therapy**. Bull World Health Org, 63: 965-981.

Gergel, D., Misik, V. Ondrias, K., & Cederdaum, A. (1995). **Increased cytotoxicity of 3-morpholinosynosydnonimine to HepG2 cells in the presence of superoxide dismutase**. J. Biol. Chem., 270(36): 20922-20929.

Harborne, J. B. (1994). **The Flavonoids: Advances in Research Since 1986**. London, UK: Chapman & Hall.

Hill, A. F. (1952). **Economic Botany**. Textbook of useful plants and plant products. 2nd edn. McGraw-Hill Book Company Inc, New York.

Hsing, A. W, Chokkalingam, A. P, Gao, Y. T. (2002). **Allium vegetables and risk of prostate cancer: a population-based study**. J Natl Cancer Institute, 94(21): 1648-1651.

Huang, R-P., Fan, Y., deBelle, I., Ni, Z., Matheny, W., & Adamson, E. D. (1998). **Egr-1 inhibits apoptosis during the UV response**: correlation of cell survival with Egr-1 phosphorylation. Cell Death and Differentiation, 5: 96-106.

Jeruto Pascaline, Mutai Charles, Catherine Lukhoba & Ouma George (2011). **Phytochemical constituents of some medicinal plants used by the Nandis of south Nandi district**, **Kenya**. **Journal of Animal and Plant Sciences**, 9 (3): 1201-1210.

Kaileh, M., Vanden Berghe, W., Boonec, E., Essawi, Tamer, Haegeman, G. (2001). *Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity*. **Journal of Ethnopharmacology**, 113: 510–516.

Kaur, R., Kapoor, K., and Kour, H. (2011). **Plants as a source of anticancer agents**. J. Nat. Prod. Plant Resource, 1 (1): 119-124.

Khaleeliah, W. (2001). **Screening for Anticancer Activity of Palestinian Plants**. MSc Thesis, An-Najah National University, Palestine.

Krishnaiah, D., Devi, T., Bono, A., and Sarbatly, R. (2009). *Studies on phytochemical constituents of six Malaysian medicinal plants*. **Journal of medicinal plants research**, 3(2): 067-072.

Lev, E., Amar, Z. (2002). **Ethanopharmacological survey of traditional drugs sold in Kingdom of Jordan**. J Ethanopharmcol, 82:131-145.

Madhuri, S. and Pandey, G., (2008). **Some dietary agricultural plants with anticancer properties**. Plant Arch, 8: 13-16.

Madhuri. S, and Pandey, G., (2009). **Some anticancer medicinal plants of foreign origin**. Current science, 96 (6): 779-783.

Mosmann, Tim. (1983). "*Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays*". **Journal of Immunological Methods,** 65 (1–2): 55–63.

Pandey, G. & Madhuri, S. (2006). **Medicinal Plants: better remedy for neoplasm**. Indian Drugs, 43: 869-874.

Pascaline, J., Charles, M., Lukhoba, C., & George, O. (2011). *Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya*. **Journal of Animal & Plant Sciences**, 9(3): 1201-1210.

Promega Corporation (1996). **Aqueous non-radioactive proliferation assay**. http://www.promega.com/.

Rahman, A., Choudhary, M.I., Thomsen, W.J., (2001).**Bioassay Techniques for Drug Development**. Taylor & Francis Group, Netherlands: 34–35.

Riss, T., & Moravec, R. (1993). **Comoarison of MTT, XTT, and anovel tetrazolium compound MTS for in vitro proliferation and chemosensitivity assays**. Biomedical Products, 18: 66-68.

Rosangkima, G. and Prasad, S. B.,(2004). **Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dolton's lymphoma**. Indian J. Exp. Biol., 42: 981–988.

Saad, B., Azaizeh, H., & Said, O. (2008). **Arab Herbal Medicine**. Botanical Medicine in Clinical Practicem, 4: 31.

Saad, B., Azaizeh, H., Abu Hijleh, G., and Said, O., (2006). **Saftey of Tarditional Herbal Medicine**. eCAM; 3(4): 433-439.

Saad, B., Azaizeh, H., Said, O. (2005). **Tradition and Perspectives of Arab Herbal Medicine**. Evid Based Complement Alternat Med, 2(4): 475-479.

Saad, B., Dakwar, S., Said, O., Abu Hijleh, G., Albattah, F., Kmeel, A. S., & Azaizeh, H. (2006). **Evaluation of medicinal plants hepatotoxicity using co-cultures of hepatocytes and monocytes**. eCAM, 3: 93 - 98.

Said, O., Fulder, S., Khalil, K., Azaizeh, H., Kassis, E., & Saad, B. (2008). **Maintaining a physiological blood glucose level with 'glucolevel', a combination of four anti-diabetes plants used in the traditional arab herbal medicine**. Evid Based Complement Alternat Med; 5: 421-428.

Said, O., Khalil, K., Fulder, S., & Azaizeh, H., (2002). **Ethanopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region**. J Ethanopharmacol; 83: 251-265.

Sakarkar, D. M., & Deshmukh, V.N. (2011). **Ethnopharmacological Review of Traditional Medicinal Plants for Anticancer Activity**. Int. J. Pharmacy Technology Research , 3(1): 298-308.

Setiawan, V. W., Yu GP, Lu QY, et al. (2005). **Allium vegetables and stomach cancer risk in China**. Asian Pac J Cancer Prevention, 6(3): 387-395.

Sharma, H., Parihar, L., & Parihar, P., (2011). **Review on cancer and anticancerous properties of some medicinal plants**. Journal of Medicinal Plants Research, 5(10): 1818-1835.

Sieuwerts, A. M., Klijn Jan, G. M., Peters, H. A., & Foekens, J. A. (1995). **The MTT Tetrazolium Salt Assay Scrutinized**: How to Use this Assay Reliably to Measure Metabolic Activity of Cell Cultures in vitro for the Assessment of Growth Characteristics, IC50-Values and Cell Survival. Division of Endocrine Oncology Department of Medical Oncology, 33: 813-823.

Sivlokanathan, S., Ilayaraja, M. & Balasubramanium, M. P. 2005. **Efficacy of Terminalia arjuna (Roxb) on N-nitrosodiethylamine induced hepatocellular carcinoma in rates**. Indian J. Exp. Biol., 43: 264-267.

Steinmetz, K. A. & Potter, J. D.(1991). **Vegetables, fruits and cancer, 1. Epidemiology**. Cancer Causes Control, 2: 325-357.

Toni, I., Flamini, E., Mercatali, L., Sacanna, E., Serra, P., & Amadori, D. (2010). **Pathogenesis of osteoblastic bone metastases from prostate cancer**. Cancer, 116(6): 1406-1418.

US FDA, Guidance for Industry: **Evidence-Based Review System for the Scientific Evaluation of Health Claims**.

Wallace, G., Fry, S. C. (1994). **Phenolic components of the plant cell wall**. Int. Rev. Cytology., 151: 229–267.

Wong, G. & Goeddel, D. (1994). **Fas antigen and p55 TNF receptor signal apoptosis through distinct pathways**. J. Immunology, 152: 1751-1755.

Zaid H, Rayan A, Said O, & Saad B. (2010). **Cancer Treatment by Greco-Arab and Islamic Herbal Medicine**. The open Nutraceuticals Journal, 3: 203-213.

Zaid, H., Silbermann, M., Ben-Arye, E., & Saad, B. (2011). **Greco-Arab and Islamic Herbal-Derived Anticancer Modalities: From Tradition to Molecular Mechanisms**. Evidence-Based Complementary and Alternative Medicine: 1-13.

جامعة النجاح الوطنية

كلية الدراسات العليا

دراسة السمية الخلوية للنباتات المستخدمة في طب الأعشاب العربي الفلسطيني لعلاج السرطان

إعداد

ميسر محمد محمود بشارات

إشراف

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أ. د. بشار سعد

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير فـي العلـوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2013

ب

دراسة السمية الخلوية للنباتات المستخدمة

في طب الأعشاب العربي الفلسطيني لعلاج السرطان

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الملخص

انتشار السرطان في تزايد في أنحاء العالم، ونسبة الوفيات الناجمة عن هذا المرض الفتاك في ارتفاع وخاصة في البلدان النامية. العلماء والباحثين يعطون الآن اهتمامهم إلى الأدوية العشبية لتوفير العلاج لأمراض أكثر صعوبة مثل السرطان يرجع ذلك إلى حقيقة أن العلاجات لمرضى السرطان بالعلاج الكيميائي لديها آثار جانبية خطيرة. حديثا الأدوية العشبية تأتي للعب دور أكثر حيوية في الحد والوقاية من السرطان، والاهتمام السريع في طب الأعشاب العربي التقليدي في جميع أنحاء العالم يحفزه عوامل أساسية كثيرة، أن المنتجات العشبية آمنة واقتصادية.

هذه الأطروحة الحالية تتناول دراسة نباتات عشبية تقليدية عربية لها دور في إحداث السمية الخلوية مخبريا أو تثبيط الخلايا من النباتات الطبية المضادة للسرطان. تم تحضير خلاصة من كل نبات الزعمطوط (الكورم) والبلوط وذلك باستخدام الايثانول في الماء من أوراق النباتات واللحاء والفواكه. وقد تم اختيار النباتات على أساس دراسة حديثة كشفت أن لها قيمة طبية في علاج السرطان. تم فحص مستخلصات الايثانول لآثار تثبيط ضد الخلايا العادية وكذلك تثبيط ضد ثلاث أنواع من الخلايا السرطانية باستخدام اختبار MTT. وأظهرت الدراسة وجود سمية لنبات الزعمطوط لكلا الخلايا العادية والسرطانية على بعض التراكيز. مقارنة بالزعمطوط، مستخلص البلوط سواء الأوراق واللحاء والثمرة تظهر اقل سمية على جميع التراكيز. ومن ذلك نستنتج أن لهذه النباتات الطبية، والاستخدامات التقليدية ضد السرطان ناتج عن سمية الخلايا ولكن ليس لكونها نباتات مضادة للسرطان بتثبيط الخلايا.