An-Najah National University
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In Vitro Evaluation of Apoptotic Induction of *Hypericum triquetrifolium* and *Arum palaestinum* Plant Extracts on Cancer Cell lines

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Dedication

To My Amazing Parents

Who have raised me to be the person whom I am today and whose words of encouragement, push for tenacity ring in my ears. It's their unconditional love that motivates me to set higher targets

To My Sweet Sisters

Who have provided me with a strong love shield that always surrounds me and never lets any sadness enter inside

I Present This Work

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الاقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل عنوان:

In Vitro Evaluation of Apoptotic induction of _Hypericum triquetrifolium_ and _Arum palaestinum_ Plant Extracts on Cancer Cell lines

التقييم المخبري لنباتي داذي واللوف الفلسطيني على تحفيز الموت المبرمج للخلايا السرطانية

أقر بأن ما اشتملت عليه هذه الرسالة هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وإن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

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

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List of Abbreviations

µg	Microgram
µl	Microliter
A549	Lung cancer cell line
AP	*Arum palaestinum*
APAF1	Apoptosis Protease-Activating Factor 1
A. Palestinian	*Arum palaestinum*
AO	Acridin Orange
AV	Annexin V-CY3
CAM	Complementary and Alternative Medicine
DMEM	Dulbecco's Modified Eagle's Medium
DR	Death Receptor
DW	Distilled Water
ELISA	Enzyme-linked immunosorbent assay
HCL	Hydrochloric acid
HCT116	Colon cancer cell line
HT	*Hypericum triquetrifolium*
H. *triquetrifolium*	*Hypericum triquetrifolium*
L6	Muscle normal cell line
Ml	Milliliter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium Bromide
NCEs	New Chemical Entities
NSCLC	Non Small Cell Lung Carcinoma
Nm	Nanometer
PBS	Phosphate Buffered Saline
SCLC	Small Cell Lung Carcinoma
WHO	World Health Organization

In Vitro Evaluation of Apoptotic induction of *Hypericum triquetrifolium* and *Arum palaestinum* Plant Extracts on Cancer Cell lines

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Abstract

Herbal medicines in treatment of cancer as complementary and alternative therapy are accepted increasingly with growing scientific evidences of biomedical research and clinical trials. Anticancer drugs discovered from herbal medicines have a long history and some of them have been used in clinical setting as the conventional anticancer drugs.Actually, herbal medicines are a source for anticancer drug discovery and drug development.

The traditional Arab-Islamic herbal-based medicines might be promising candidates for new cancer therapeutics, especially natural herbal products with low toxicity and minimal side effects. This study aimed to investigate the anti-cancer effect of two medicinal plants extracts: *Arum palaestinum* (AP) and *Hypericum triquetrifolium* (HT).

Two cancer cell lines: Colon and Lung (HCT-116, A549) and one normal (control) cell line (skeletal muscle, L6) were selected to test the efficacy of AP and HT different extracts in apoptosis induction. The cells were treated with an increasing concentration of(distilled water, 50%water-50% ethanol, and hexane) plant extracts (0, 8, 16, 32, 62, 125, 250, 500,

1000 and 2000µg/ml) for 24h. Then we used MTT assay to test cytotoxicity of the extracts, Annexin V-Cy3 to test apoptosis and ELISA kit specific for cytochrome c release. Results show that none of the three extraction methods of HT has a toxic effect on all treated cell lines at all concentrations tested. However, they had induced apoptosis in colon cancer cell line (HCT116), muscle cell line (L6), and lung cancer cell line (A549) at the concentration 500µg/ml through mitochondrial dependent pathway by releasing cytochrome c at the concentration 250µg/ml. Surprisingly, AP(distilled water and 50%water/50% ethanol) had no cytotoxic or apoptotic effect in all selected cell lines at non-toxic concentration up to 1000 µg/ml, whereas hexane extract had induced apoptosis at the concentration 500µg/ml through mitochondrial dependent pathway by releasing cytochrome c in the three cell line at non-toxic concentration 250µg/ml.

Taken together, these results indicate that HT (but not AP) might be an effective herbal candidate in cancer chemotherapy.

Chapter One

Introduction

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Introduction

1. Introduction

1.1 General background

Cancer is a growing health problem around the world. It is considered a second leading cause of mortality after heart disease (1). Statistics show that cancer in some form strikes more than one third of the population, accounts for more than 20% of all deaths around the world(2). The prevalence of cancer increases parallel to the increase in life expectancy and found to be more in developing countries compared to developed countries (3). Half of the 10 million cancerous cases worldwide were in developed countries in 2004 (4). The American Cancer Society estimates that 27 million newly cancer cases will be diagnosed and 17.5 million cancer related deaths globally by 2050 (5).According to the annual report published in (2012) by Palestinian Health Information Center (PHIC), 1802 new cancer cases were reported in West Bank (6).

Cancer is usually fatal if it is not treated. Diagnosis of cancer at early stages is a vital step for treatment. Moreover, identification of persons with high risk factors of cancer before its development is the main objective of cancer research.

Cancer, known medically as a malignant neoplasm, is a broad group of various diseases with many possible factors (Biological, Physical and environmental factors). It is uncontrolled cell growth occurred due to multiple mutations in genes that controlling cell growth and division leading to dysregulation of the normal cellular pathways for cell division, which results in loss of balance between cell division and cell death, leading into a population of tumor cells that proliferate and metastasize throughout the tissues of the body and it may progress and cause death (7-9).Therefore, anticancer drugs involve pharmacological effects of cytotoxicity, anti-proliferation, induction of differentiation, anti-inflammation, induction of apoptosis and/or autophagy, cell-cycle arrest, anti-invasion and Anti-metastasis. Cancer is a type of tumor (abnormal growth of tissue).

Tumors can be divided into benign and malignant (cancer). Benign tumors are those whose cells lose the ability to metastasis, therefore they are not cancerous. Usually, benign tumors can be removed without coming back.

Cancerous cells use the circulatory system and lymphatic system to migrate throughout the body, attacking the normal healthy tissues and destroy them in a process called invasion. During the invasion process, the cell divide, grow, and induce the body to form new blood vessels to feed itself, this process called angiogenesis. When a cancerous cells success to move and spread from its primary site to the secondary site, invading and

destroying other normal tissues, it is called metastasized and the mechanism called metastasis. Once cancer cells reach this stage (metastasis), a serious condition will be happened, and will be very difficult to treat. When cancer cells reach their secondary sites (metastases), they often named after these sites. For example, a cancerous cell that spread from the liver to pancreas is called pancreatic cancer (10).

Benign tumors don't spread to nearby tissues, don't metastasis to other parts of the body and usually don't need to be removed. On the contrary, malignant tumors have the ability to spread to the nearby tissues and metastasis to other parts of the body. Sometimes, malignant tumors can be removed but may grow back. Therefore, based on the definition, the term "cancer" applies only to malignant tumors.

There are numerous physiological and biochemical carcinogens, for example, ionizing radiation; asbestos and tobacco smoke, infections by microorganisms such as viruses and parasites ,ultraviolet and some kinds of cancer are due to oxygen-centered free radicals and other reactive oxygen species because overproduction of such free radicals can cause oxidative damage to biomolecules such as lipids, proteins, DNA (11).According to the estimations of international agency for research on cancer, there is a prevalence of 27 types of cancers which is threatening to the mankind. The most frequent types of these cancers among women are breast, lung, colorectal, stomach, and cervical, while among men are lung, stomach, liver, colon (colorectal), and prostate (12).

1.2 Lung cancer

Lung cancer is ranking at the first malignant tumor with the highest mortality rate worldwide. It is responsible for nearly 1.4 million deaths yearly (13), often because it is hard to be detected until there has been substantial progression of the cancer, which leads to a significant reduction in quality of life of the patient (14). For example, among the 1.6 million new cases of lung cancer diagnosed each year, nearly 220,000 are diagnosed and157, 000 deaths in the United States (15). Thus, there is a general call for the development of novel approaches and effective anticancer strategies to prolonged survival of lung cancer and early being pursued.

According to epidemiology studies (16), Lung cancer is more common in men than in women and it is twice the death rate of the prostate cancer in men.

Lung cancers are categorized into two main histological groups which are non small cell lung carcinoma (NSCLC), and small cell lung carcinoma (SCLC). NSCLC is found to be responsible for 85% of all lung cancer cases including large cell carcinoma, and adenocarcinoma, whereas, SCLC represents the rest of the lung cancer cases which are 15% (17).

Lung cancer is known with its early metastasis once it's established which makes it hard to treat and a big threat to human life. If lung cancer is not diagnosed early, it may spread and metastasis into nearby tissues and

later to other parts of the body till reached its secondary sites. The most common secondary sites for lung cancer metastasis are brain, adrenal glands, bone, and liver (18).

There are different risk factors contribute in lung cancer formation, these include: smoking cigarette, negative smoking, radiation, indoor and outdoor air pollution, and exposure to some agents like arsenic, nickel, and asbestos (19). The most important risk factor is Cigarette smoking which contributes to (NSCLC) (20). Nonetheless, the number of cases of lung cancer diagnosed in never-smokers has also increased in recent times (21).

Several therapies have been used in lung cancer treatment; among these therapies is herbal-based therapy. Herbal medicines have been proven to be useful for lung cancer patients in several ways such as prolonging the survival time, improving the quality of the life, sensitizing conventional agents, and preventing side effects of chemotherapy (21). Moreover, several studies showed that many plants and vegetables are associated with a lower risk of lung cancer especially those that contain leafy green and yellow/orange parts (22).

1.3 Colon cancer

Globally, colon cancer is responsible for more than 9% of all cancer cases. Its incidence rate among men and women are similar and is currently classified at the fourth commonest malignant tumor after lung, breast and prostate (23). Colon cancer is also known as a colorectal cancer

since the final portion of colon which is the rectal can be affected. Statistics showed that the highest prevalence of colon cancer occurs in North America, Europe and the lowest incidence occurs in Central and South America, Asia and Africa (18).

Most colon cancers are adenocarcinomas-tumors that develop from the glands lining the colon's inner wall. Several risk factors are associated with the incidence rate of colon cancer such as age, hereditary factors and lifestyle especially the diet (24).

Colon cancer is currently treated by surgery, chemotherapy alone or in combination with radiotherapy. However, these therapies are not highly effective against colon cancer (25). Therefore, new treatments for advanced or metastatic colon cancer are highly needed. Epidemiological studies show that Diet-based strategies hold promise for both prevention and treatment of colon cancer (26).

1.4 Cancer treatment

Several evolutionary changes have occurred in cancer treatment in order to achieve the main goal of the treatment that is the complete removal of the cancer cells without harming the normal ones. Several factors should be considered once choosing any treatment such as the medical condition of each person, the type and stage of cancer, and age. The most Common treatments involve surgery, chemotherapy, radiation therapy, and biological therapies (27).

1.4.1 Chemotherapy

Chemotherapy is one of the most common modes of the treatment of cancer patients that depends on using anticancer drugs and chemical substances to damage cancer cells (28). Chemotherapy may be used alone or in combination with other treatments depending on the cancer types. In combination with radiation for example, they cause severe side effects and toxicity (29). There are several drawbacks associated with chemotherapy treatment including lack of selectivity that damage the normal cells in addition to cancer cells, the drug-resistant of cancer cells, and the reduction the effectiveness of drugs(28) . In addition, the cost of the synthetic drugs is very high (30). Some of the chemotherapy drugs are naturally occurring compounds that have been identified in different plants. These products have been known with their strong biological activity, low toxicity and minimal side effects (31). This in turn, encouraged the scientists to study the biological activity of some plants and identify the compounds they contain. Thus, the need for development of natural medicines has been high.

The process that depends on using natural, biologic chemical or synthetic agents or their mixture to reverse, suppress, or prevent the progression and invasion of the cancer is often called chemoprevention (3). Several medicinal plants and natural products like grains, fruits, and vegetables are well known with their effects on cancer prevention and lowering its risks and they have been proven as one of the important

sources of anticancer drugs (32). The contribution of natural products to cancer therapy is more evident considering the fact that they were involved in the development of roughly 75% of novel anticancer agents between 1981 and 2010 (33). Thereby, it is an important aspect to study, and characterize the natural products and plants with anticancer activity.

The use of the medicinal plant in complementary and alternative medicine (CAM)was found to be widespread especially in those diseases that are not amenable to modern methods (10). Statistics showed that less than 10% to more than 60% of cancer patients have used CAM (34, 35). Moreover, in a survey conducted in 2002 indicated that 80% of cancer patients used an alternative or complementary modality (36). CAM use is also used among patients with gynecological (37), hematological malignancies (38), and pediatric (39).

Based on numerous reports describing anticancer activity published by the National Cancer Institute, there are about 40 eatable plants were identified to have cancer-preventive properties (40). In addition, there are more than 400 species of traditional Chinese medicinal herbs associated with anticancer (41). Identification and characterization of Plants, fruits, and vegetables revealed more than 5000 individual phytochemicals (11).

There are many medicinal plants available in nature which has the anticancerous properties and the majority of them are still to be exploited. So, there is an urge for these plants to be discovered so that the cancer could be totally eradicated (10).

1.5 Medicinal plants

The use of medicinal plants to treat various diseases and maintain health has began before the invention of modern medicine and several cultures throughout the world have depended on them since the antiquity time (42). It has been estimated that about 80-85% of global population rely on traditional medicines for their primarily health care needs and it is assumed that the use of plant extracts or their active ingredients is a major part in the traditional therapy (43).

The easy availability and cheaper cost of the traditional ways of treatment make people especially rural one more disposed to them. Several factors contributed to develop the knowledge about medicinal plant and their properties and later to the formation of the traditional medical systems and therapies such as Greco-Arab medicine and naturopathy, these factors include anecdote, personal experimentation, local custom, and folk tradition (44).

Nowadays, there is an extensive use of medicinal plant in primary health care across the world particularly in the developing countries. Several researches groups, including us (45, 46)investigates the action mechanism of medicinal plants in treating diseases and working on purifying active natural compounds for the treatment of human diseases such as infectious diseases and cancer (47).

Epidemiological studies show that the Mediterranean diet has a strong protective effect against cardiovascular disease and cancer (46). Recently, there is an increase interest in using traditional Greco- Arab-Islamic herbal medicine in cancer treatment in the scientific researches (48). In addition, herbal medicine is still used among the Arab and Islamic societies especially throughout the Mediterranean region. Moreover, during the Islam period, the use of herbal medicine was the first choice in treating several diseases such as depression, epilepsy, infertility, and cancer (46).

1.5.1 Herbal-Based Prevention and Therapy

Several curative bioactive compounds and aromatic substances such as saponins, alkaloids, coumarins, flavonoids have been detected in medicinal plants and found to be valuable as primary or supplemental therapies to treat various human diseases including liver diseases, diabetes, skin, and cancer (42, 49). Flavonoids are probably the best known of these substances due to their antiviral, anti-inflammatory, antiallergenic, anti-proliferative and antioxidant properties (10).

Today, several drugs that are used in modern medicine are derived from plants. In addition to their active agents, plants and herbs are also important source for but not limited to vitamins, minerals, volatile substances, and glycosides (49).

Recently, the World Health Organization (WHO) has identified more than 20000 medicinal herbs, and about 250 of these herbs had been

analyzed to detect their bioactive chemical ingredients (42). In Middle East area particularly, nearly 200–250 of more than 2600 known plant species are being used in the reduction of the risk factors and treatment of several diseases (10), and around 286 compounds of the traditionally used ones are derived from herbs (50).

The advantage of using herbal-based molecules- which are usually called phytochemicals- for cancer treatment and prevention is their relatively low/nontoxic nature (51). Several wild plant derived agents have clinically proved their antitumor activity such as camptothecin derivatives, vinblastine, vincristine, taxol, and topotecan (52, 53). In spite of the use of these antitumor agents in targeted mechanism-based pathways, their exact effects on manipulation the extrinsic and the intrinsic apoptosis pathways are still being investigated.

1.5.2 Medicinal plants and drugs

In general, the use of natural products as a source of drug production has been great in science. Many compounds that are produced from natural products have used in many applications in the fields of pharmacy and medicine (31). Different kinds of natural products have been identified in a dietary plant such as fruits, vegetables in significant levels, and proved to be effective in the reduction and prevention of several human chronic diseases (3). However, many studies have revealed that plant exhibit a wide spectrum of biological activities such as, antioxidant, antiviral, anti-inflammatory, and anticancerous (11).

Nowadays, there is a general call for the development of new drugs that possess low toxicity, show high effectiveness, and have a little impact in the environment. Several plants including Traditional herbal medicines appear to be a remarkable source, good candidate, and offer opportunities in the drug development and discovery (11). Actually, half of the modern drugs are derived from herbs (11). Therefore, there is an increasing interest around the world in studying the traditional medicinal plants and investigate their effects on the variety of diseases. Moreover, the combination between traditional medicine and other new biotechnological tools have to be established in order to make new drug development (54).

The studies reported that of the 877 small molecule new chemical entities (NCEs) introduced between 1981 and 2002 nearly the half (49%) were natural products, semi-synthetic natural products, semi-synthetic natural products analogues or synthetic compounds based on natural products (55).

1.5.3 Anticancer activity of medicinal plants

The dependence on Herbal medicines in treating cancer has increased widely parallel to the increased scientific evidences in biochemical research and clinical trials (29). The contribution of phytochemical products in the discovery of novel anticancer drugs have been significant in the recent two decades; indeed 69% of anticancer drugs confirmed between the 1980s and 2002 were either natural products or developed based on knowledge gained from natural products , and over

60% of currently used anti-cancer agents are derived from natural sources (29, 56).

The main disadvantages of synthetic drugs in cancer treatment are the associated side effects, toxicity, multi-drug resistance which in turns, result in decreasing the ability of modern medicine to cure different kinds of cancer effectively. Whereas natural therapies, such as the use of the plants or plant derived products are more natural and more accessible and safer than manufactured drugs (57, 58). This may indicate that drug development based on the components with lead structures of potent bioactivity isolated from medical plants has been a major strategy for developing new anticancer drugs from herbal medicines.

There are now several useful antitumor agents have been proved clinically and are derived from wild plants such as taxol, vinblastine, vincristine, camptothecin derivatives lingzhi, and irinotecan (3, 59).

Diet has showed crucial role in cancer prevention and treatment, in fact, it attributed for about 35% of all human cancer related mortality (11, 48). Based on several laboratory studies, the results have showed an inverse relationship between the high dietary intake and the risk factors of specific cancer. Moreover, the consumption of vegetables such as cabbage, cauliflower, and tomatoes, and fruits such as, apples and grapes, is strongly associated with reduced risk of cancer (3, 60).

Different kinds of phytochemicals especially those derived from traditional medicinal plants have showed a significant effects on prevention the establishment and reduction of some types of cancer, as well as, inhibition the development and spread of tumors in test animals (46). So, considering the facts, it is strongly recommended that there is an urge for these plants to be discovered so that the cancer could be totally eradicated.

In Palestine particularly, the screening of flora for pharmacological active compounds started in the late sixties (61). There are lots of medicinal plants available in a very small geographical area, this large number is due to the diversity of the soil and climatic conditions (62) and it is considered as a major advantage of studying the Palestinian flora. Therefore, two of these plants that are widely used to treat cancer patients (*Arum Palaestinum*and *Hypericum triquetrifolium*) were selected to test their efficacy on cancer cell lines in vitro (Apoptosis induction).

1.6 Cancer and Apoptosis

Cancer is a complex disease occurring due to mutations in proto-oncogenes or tumor suppressor genes that can be developed due to alteration of signaling pathways which will lead to dysregulation of the normal cellular program and cell division (63). It is characterized by uncontrolled growth and reproduction of cells, loss of contact inhibition, and evading apoptosis (64). Apoptosis is a crucial physiological process that allows the cell to commit suicide and getting rid of damaged or infected cells that may interfere with normal function (65, 66). It describes

the orchestrated collapse of a cell characterized by membrane blabbing, cell shrinkage, condensation of chromatin, fragmentation of DNA, and loss of adhesion to neighbors or to extracellular matrix (67).

Apoptosis is becoming widely known as being an essential tissue protection against carcinogens by inhibiting survival and controlling the growth of precancerous cell populations and tumors at different stages of carcinogenesis (68). In general, apoptosis is activated through two main pathways, the extrinsic (death receptor (DR) -mediated) and intrinsic (mitochondrial mediated) pathways (69).The intrinsic pathway, also known as the mitochondrial mediated pathway, is found to be activated by a wide range of signals; including radiation, cytotoxic drugs, and cellular stress, and involves the release of proteins such as cytochrome *c* from the mitochondrial membrane space (70). Mitochondria play a crucial role in the induction of apoptosis. Once cytochrome *c* is released, it joins with an adaptor molecule called apoptosis protease-activating factor 1(APAF1), and also with an inactive initiator caspase called procaspase-9 to form a multiprotein complex called the apoptosome. This in turn initiates the activation of caspase-9, which then activates a cascade of caspase (caspase-3, caspase-6, caspase-7) activation, resulting in the physiological and biochemical changes associated with apoptosis (71, 72). Imperfections in apoptosis are common phenomena in many types of cancer and are found to be a critical step in tumor development and resistance to therapy (73).

Many studies have indicated that the most common anticancer mechanism has been used by many cancer therapies is induction of apoptosis in cancer cells (74). Cancer chemotherapy however, causes several side effects (29).Therefore; several researchers nowadays have performed anti-cancer studies on herbal extracts as well as natural compounds based on their biochemical properties of apoptosis.

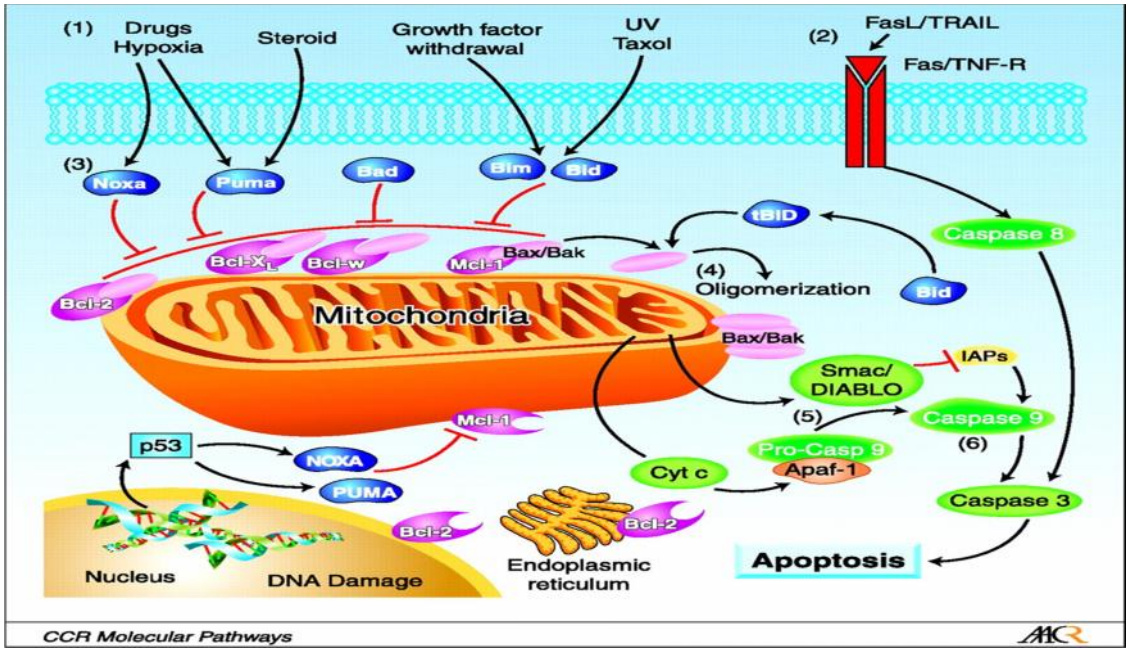


Figure 1.1: Mitochondrial apoptotic pathway (75).

1.7 *Arum palaestinum* (Palestinian Arum)

Figure 1.2: *Arum palaestinum* plant.

Arum palaestinum is a species of flowering herbaceous perennial plant in the family Araceae. It has been used traditionally as a vegetable for Palestinian people. In the traditional medicine, according to surveys conducted in 2008 and in 2012, it was found that the Arabs attribute cancer fighting properties to this plant (76, 77). Moreover, it was reported that this plant contains moderate levels of antioxidants (78). Also, pervious study reported that an alkaloid derived from *Arum palaestinum* suppresses proliferation of breast and lymphoblastic leukemia cells (79). In addition, it showed effectiveness against internal bacterial infections, poisoning and circulatory system (80).

However, in the supervisor lab (unpublished data), the water-alcoholic extract of *Arum Palaestinum* was not neither toxic nor apoptotic inducer when applied on lung, prostate, and colon cell lines up to 1mg/ml. Yet, it is traditionally in use as anti cancer therapy and some other research groups, as stated above, had reported its toxic effect. Hence, we thought to

test the effect of hexane extract from *Arum Palaestinum* on apoptosis induction, as it might include different (hydrophobic) active compounds.

1.8*Hypericumtriquetrifolium*

Figure 1.3: *Hypericumtriquetrifolium* plant.

Hypericum triquetrifolium, native to Eastern Europe and the Mediterranean area, is aherbaceous perennial plant that grows in open dry stony/sandy ground. *Hypericum triquetrifolium* have been used in traditional Arab herbal medicine to treat various inflammatory diseases. Results from recent studies reporting the antinociceptive (81), anti-inflammatory (82), antioxidant (83), antibacterial (84), antifungal (85), and cytotoxic (86) activities of *H. triquetrifolium* demonstrate the significant potential of this species for use as a medicinal plant. The methanolic extract of the aerial parts of Hypericum species has been reported to contain many bioactive compounds, namely the naphthodianthrones, hypericin and pseudohypericin (87), the phloroglucinol derivatives hyperforin and adhyperforin, flavonoids (88), essential oils (89), xanthones (90), tannins (91), procyanidins, and other water-soluble components (92) that possess a

wide array of biological properties. Unfortunately, previous studies show that *H. triquetrifolium* is no longer used within the practitioner communities in West Bank. This in turn reflects an extinction process of important elements of the Arab herbal medicine heritage (93).

Several clinical studies indicated the possible role of Flavonoids in preventing cardiovascular diseases and several kinds of cancer (94). However, the apoptotic effect of *Hypericum triquetrifolium* and its mechanism of action needs more investigations.

1.9 The aim of the study

Based on the worldwide trend and interest toward medicinal plants, the current study was undertaken to evaluate the effect of the aqueous (distilled water and 50% ethanol) and hexane extracts of two medicinal plants on the viability of three cell lines. Thus, the efficacies of their hydrophobic vs. the hydrophilic extracts were also tested. These medicinal plants(*Arum palaestinum* and *Hypericum triquetrifolium*) are recommended by the traditional therapists for the treatment of cancer. So we assessed their efficacy in apoptotic induction after examining their cytotoxic activity on three cell lines and investigated their role on mitochondrial cytochrome c release.

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1 Plant material

Plants (*Arum palaestinum* and *Hypericum triquetrifolium*) were collected from different places in Tulkarm and Jenin (Palestine) in the beginning of the spring.

2.2 Preparation of Plant Extracts

One-hundred grams of air dried parts were added to 1L of either, distilled water (DW), 50% Ethanol and50% distilled water, or hexane. They were heated up to 60◦C for 10 min. After that, the extracts obtained were filtered using filter paper, aliquoted and frozen at −70°C until use (93).

2.3 Cell culture

2.3.1 Cell lines

Two cancer cell lines and one normal cell line were used in the experiment, the cancer cell lines were colon cancer (HCT116, ATCC number: CCL-247, human, from the epithelial tissue of the colon), lung cancer cells (A549, ATCC number: 86012804, from the epithelial tissue of the lung) and normal muscle cells (L6, ATCC number: CRL-1458, from the skeletal muscle). These cell lines were grown in RPMI, RPMI-1640, Dulbecco's modified Eagle's medium (DMEM), and alpha MEM,

respectively, supplemented with 10% fetal calf serum, 1% penicillin streptomycin,1% amphotricine B, 1% nonessential amino acids and 1% L-glutamin, all chemicals were purchased from SIGMA company. Cell lines were incubated in a humidified atmosphere of 95% air, 5% CO2 at 37°C.

2.4 Determining of cell viability

2.4.1 Cytotoxicity, using MTT Assay

The tetrazolium dye, MTT, assay is broadly used to measure the cytotoxic effects of drugs on cell lines in vitro by evaluating the viability and/or the metabolic state of the cells. MTT is based on the reduction of the yellow 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT) into purple formazan crystals by mitochondrial succinate dehydrogenase in living cells, which provides a quantitative determination of viable cells (93, 95). Cells with 70-80% confluence were detached from the cultured flask by treatment with 0.05% trypsin- EDTA and a suspension of 100 µl (2.0×10^4 cell/well) of viable cells were seeded/well in a 96-well plate and incubated for 24 hrs. Cells then were incubated with stock solutions of crude extracts from plants serially diluted to reach concentrations of 2000.0, 1000.0, 500.0, 250.0, 125.0, 62.5, 31.25, µg/ml. After 24 hour of incubation, 100 µl 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 hours. MTT solution was then removed, and the formazan product was solubilized with acidified isopropanol (0.1N HCl).The plate were covered with tinfoil and agitated on

orbital shaker for 20 min. The optical density (OD) of the MTT formazan then was determined at 570 nm in an enzyme-linked immunosorbent assay (ELISA) reader (BIO-RAD, model: 680, serial NO: 10170).

2.4.2 Cytostatic effect, using MTT Assay

In order to test the cytostatic effect of the extracts, less number of cells were seeded in each well. 1.0×10^4 cells/well, which were plated in 100 µl of the medium in 96-well plate for 24 hours were treated with the plant extracts in different increasing concentrations as mentioned in the previous section and then were incubated for 24 hours at 37°C. Following the removal of plant extracts from each well, the cells were incubated in DMEM to which MTT (0.5 mg/ml) was added to each well (100 µl), and then the cells were incubated for 4h at 37°C. After that, the medium were removed and 100 µl of isopropyl alcohol were added to dissolve the formazan crystals. The plate was covered with tinfoil and agitated on orbital shaker for 20 min. Then, the optical density (OD) of the MTT formazan was determined at 570 nm in an enzyme-linked immunosorbent assay (ELISA) reader.

2.4.3 Mitochondrial Cytochrome c release.

In order to investigate the release of the mitochondrial cytochrome c protein, we used the kit Quantikine ELISA Human Cytochrome c Immunoassay from R&D system, catalog NO: DCTC0. Cells were seeded with growth medium into 6- well plate at 500,000 cell/well and incubated

for 24 hours. Then cells were treated with two plant concentrations of each extract: (A) 0 mg/ml, (B) 0.250 mg/ml and incubated for 24 hours. After that, cells were washed three times with phosphate buffered saline (PBS) and re-suspended in Cell Lysis Buffer 2 to a concentration of $1.5x10^6$ cells/ml and incubated for 1 hour at room temperature with gentle mixing. Then, the cells lysates were centrifuged at 1000 x g for 15 minutes and the supernatants were tested for Cytochrome c appearance as follows.

After that, 100 µL of Calibrator Diluent RD5P was added to each well (in 6 well plate) followed by 100 µL of Standard, control, or sample per well and then, incubated for 2 hours at room temperature after covering the wells with adhesive strip. The wells were then washed with wash buffer for 4 times. After the washing step, 200 µL of human cytochrome c conjugate was added to each well and incubated for 2 hours at room temperature. Later, 200 µL of substrate solution was added to each well and incubated for 30 minutes at room temperature in dark. After 30 minutes of incubation, 50 µl of stop solution was added to each well and the optical density of each well was determined at 450 nm and 570 nm using microplate reader and the final readings were obtained by subtraction readings at 570 nm from the readings at 450 nm.

2.4.4 Apoptosis: using Annexin V-Cy3 protocol for the staining of cells

Apoptosis induction by *Hypericum triquetrifolium* and *Arum palaestinum* extracts was determined by using Annexin V-Cy3 protocol purchased from Sigma Aldrich, catalog NO: APOAC. We used two plant

concentrations of each extract to determine apoptosis, cells were seeded with growth medium into 6- well plate at 200,000 cell/well and incubated for 24 hours, then cells were treated with two concentrations of each extract: (A) 0 mg/ml, (B)0.5 mg/ml. after 24 hours we washed the cells with 500 µl of PBS. After trypsenization with 500 µl trypsin, cells were centrifuged at 1500*g for 5minutes at room temperature and re-suspended in 500 µl of binding buffer.

After centrifugation at 1500*g for 5 minutes, cells were subjected to staining with 1 µl Annexin V-Cy3 (AV) and were incubated at room temperature for 20 minutes, then 1 µl of Acridine Orange (AO) from Sigma Aldrich, catalog NO: A6014, was added. The cells were then visualized by fluorescence microscopy (Olympus IX51) and images were recorded by Olympus DP70 camera, using an SWB filter (96).

Chapter Three

Results and Discussion

Chapter Three

3 Results and Discussion

Since cancer is a growing health problem around the world and because of the side effects of chemotherapy and based on the worldwide trend and interest toward medicinal plants, we selected two medicinal plants, *Arum palaestinum* and *Hypericum triquetrifolium,* based on Ethno botanical studies that reported an anti-cancer therapeutic usage of these two medicinal plants. Therefore, we selected the two medicinal plants to show their anti-tumor on different cancer cell lines, colon cancer cells(HCT116), lung cancer cells (A549) and a control normal cell line which was muscle cell line (L6).

3.1 Plants different extracts concentrations

Results show that *Arum palaestinum* water extraction method produced 40.8 mg/ml, 50% ethanol extraction produced 31.9 mg/ml, and hexane extraction produced 2.52 mg/ml, whereas the *Hypericum triquetrifolium,* water extraction produced 19.2 mg/ml, 50% ethanol extraction produced 26.3 mg/ml, and hexane extraction produced 6.0 mg/ml. These crude extracts were liquated and freezed in -80^{o}C and used in the next experiments.

3.2*Hypericumtriquetrifolium*

3.2.1 Cytotoxic effect of *Hypericum triquetrifolium*

Cells with 70-80% confluence were seeded on 96 well plate. For cytotoxicity, 2.0×10^4 cell/well were seeded, whereas for cytostatic less cell number were seeded (1.0×10^4 cells/well), and left for 24 hours. Then the cells were treated with the plant extracts (0, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/ml), after 24 hours of incubation, cytotoxic and cytostatic effects of plant extracts were measured using MTT assay. After four hours of adding MTT, isopropanol was added for 15-20 minutes in dark to dissolve the formazan crystals, then absorbance was measured at 570 nm using ELISA reader.

Although herbal medicines containing *Hypericum triquetrifolium* (HT) extracts have been used in traditional Arab herbal medicine to treat various inflammatory diseases (97) and their polyphenolic compounds exhibited potent antioxidant activity (83), our results show that none of the three different extraction methods (distilled water, 50%ethanol, and hexane) for *H. triquetrifolium* was toxic at all concentrations tested on the selected cell lines (HCT-116, L6 and A549) (Table 3.2.1).

HT(1000 µg/ml) water extract led to, 13%, 5% and 8% cell death in colon cancer cell line(HCT-116) (figure 3.2.1.1), lung cancer cell line (A459)(figure 3.2.1.2) and normal muscle cell line (L6) (figure 3.2.1.3) respectively. HT (1000 µg/ml) 50% ethanol extract led to a similar results

in the 3 above cell lines, (figures 3.2.1.1, 3.2.1.2 and 3.2.1.3). HT hexane extract (1000 µg/ml) led to a higher death percentage, 24%, 17% and 17% in colon cancer cell line(HCT-116)(figure 3.2.1.1), lung cancer cell line (A459)(figure 3.2.1.1) and normal muscle cell line (L6)(figure 3.2.1.1) respectively.

Previous study showed that *H. triquetrifolium* methanolic extract exhibited cytotoxic activity against brine shrimps with an LC50 of 22 mg/mL (98). We found in the literature reviews that few studies on the potential anticancer activity of *H. triquetrifolium* had been undertaken. However, according to study conducted in 2007(86), it showed that the methanol extract of *H. triquetrifolium* did not show significant cytotoxic activity against the hepatocellular carcinoma cell line HepG-2, renal cell adenocarcinoma ACHN, the amelanotic melanoma cell line C32 and normal human foetal lung MRC5with the exception of the large cell lung carcinoma cell line COR-L23 (IC50 of 44.81 µg/mL). In addition, the water extracted *H. triquetrifolium* had no sign of any negative effects after treatment with concentrations up to 250 µg/ml against the human monocyte cell line (97).However, according to our results, we can conclude that *H. triquetrifolium* different extracts are non- toxic on all cancer cell lines tested (HCT116, A459, and L6).

Table 3.2.1.1:The cytostatic and cytotoxic effects of three different extraction ways of *H. triquetrifolium*on the viability of three cell lines (L6, HCT, A459) at the concentration 1000 µg/ml.

Cell Viability % / Extraction Way	Cell Viability % of L6		Cell Viability % of HCT116		Cell Viability % A459	
	Cytostatic	Cytotoxicity	Cytostatic	Cytotoxicity	Cytostatic	Cytotoxicity
DW Extraction	82% ± 3.0	93% ± 4.2	88 % ± 3.85	87% ± 2.0	89% ± 1.0	96% ± 2.86
50% Ethanol Extraction	80% ± 3.7	82% ± 3.85	82% ± 4.0	83.5% ± 3.0	92% ± 2.0	93% ± 2.0
Hexane Extraction	88% ± 1.7	84% ± 2.0	83% ± 4.5	77% ± 4.2	83% ± 2.5	83% ± 3.7

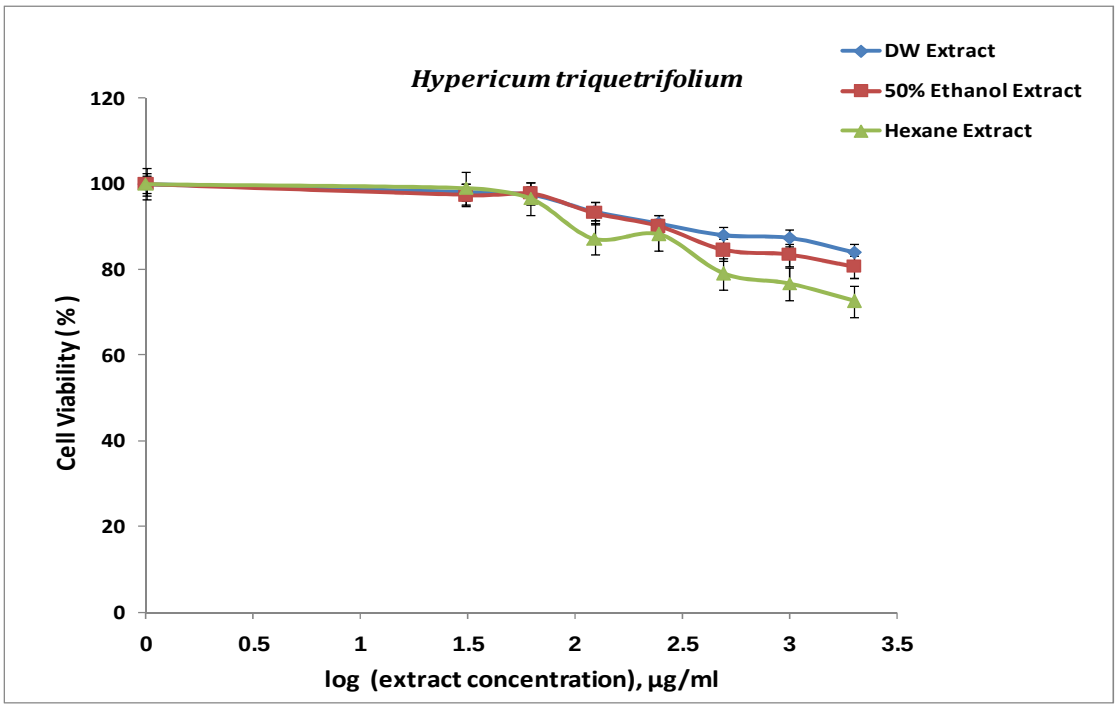


Figure 3.2.1.1:Effect of different concentrations, in logarithmic scale, (0-2000 µg/ml) of *H. triquetrifolium* different extracts on cell survival of colon cancer cell line (HCT116) obtained by MTT colorimetric assay.

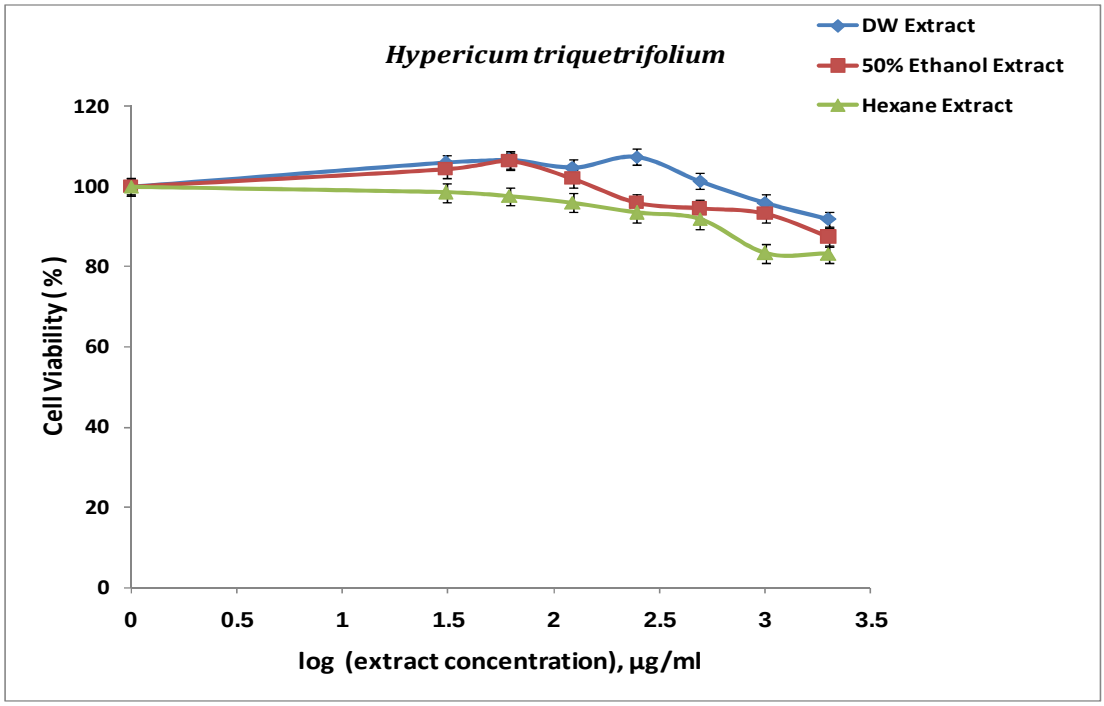


Figure 3.2.1.2: Effect of different concentrations, in logarithmic scale, (0-2000 µg/ml) of *H. triquetrifolium* different extracts on cell survival of lung cancer cell line (A459) obtained by MTT colorimetric assay.

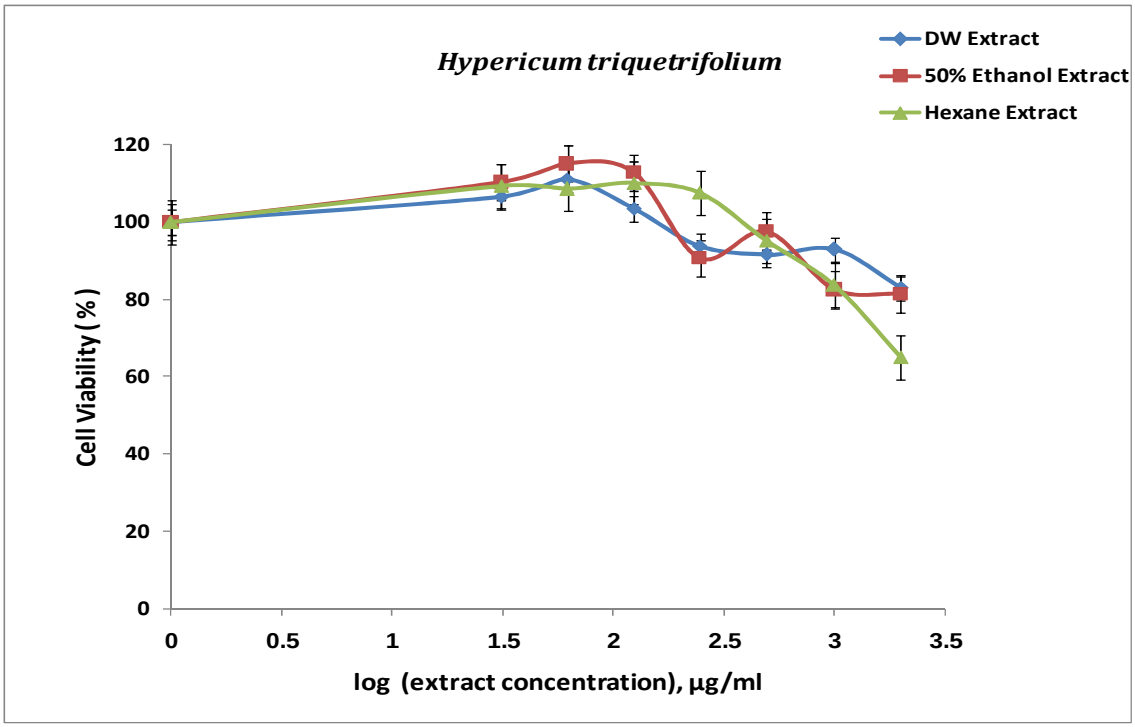


Figure 3.2.1.3: Effect of different concentrations, in logarithmic scale, (0-2000 µg/ml) of *H. triquetrifolium* different extracts on cell survival of normal muscle cell line (L6) obtained by MTT colorimetric assay.

3.2.2 Apoptotic effect of *Hypericum triquetrifolium*

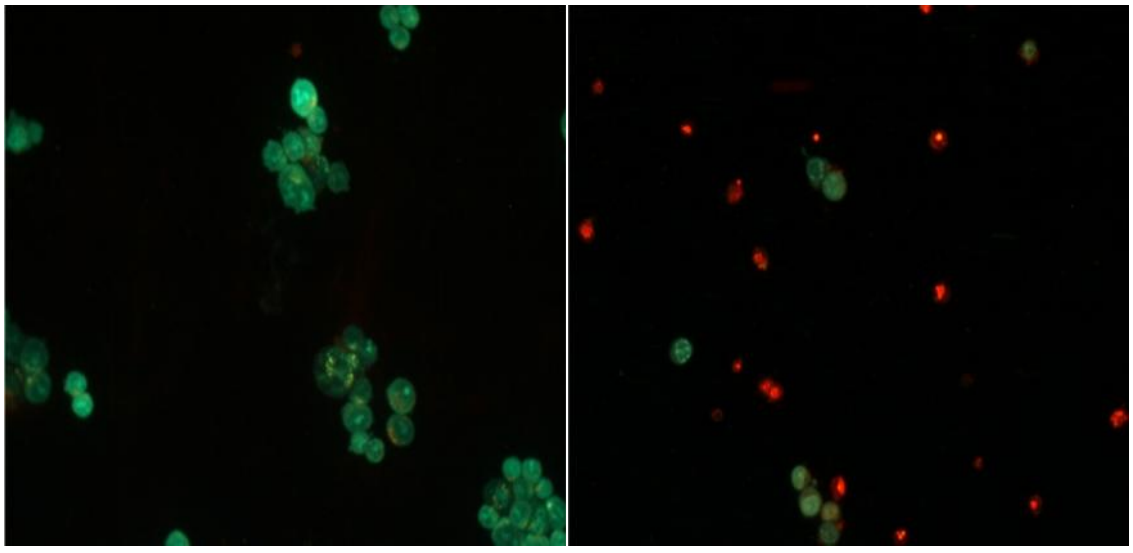
Apoptosis induction by *Hypericum triquetrifolium* was determined by using Annexin V-Cy3 protocol. The three extraction methods show apoptotic effect in the three cell lines at a non-toxic concentration 500 µg/ml with different percentages of dead cells. For hexane extraction method, the percentage of dead cell for HCT116 was 46% (figure 3.2.2.1) and for 50% ethanol extraction method, the percentage was 19% (figure 3.2.2.2 and Table 3.2.2.1). This difference in the percentage of dead cells between apoptosis and MTT is due to the fact that MTT measures the dead cells, while apoptosis measures the dead cells and the cells that start

preparing for programmed cell death (early apoptotic cells).Hence, the death detection in apoptosis is more than in MTT.

So, we can conclude that *H. triquetrifolium* different extracts have an apoptotic effect on colon cancer cell line(HCT-116), lung cancer cell line (A459) and normal muscle cell line (L6) at non-toxic concentrations.

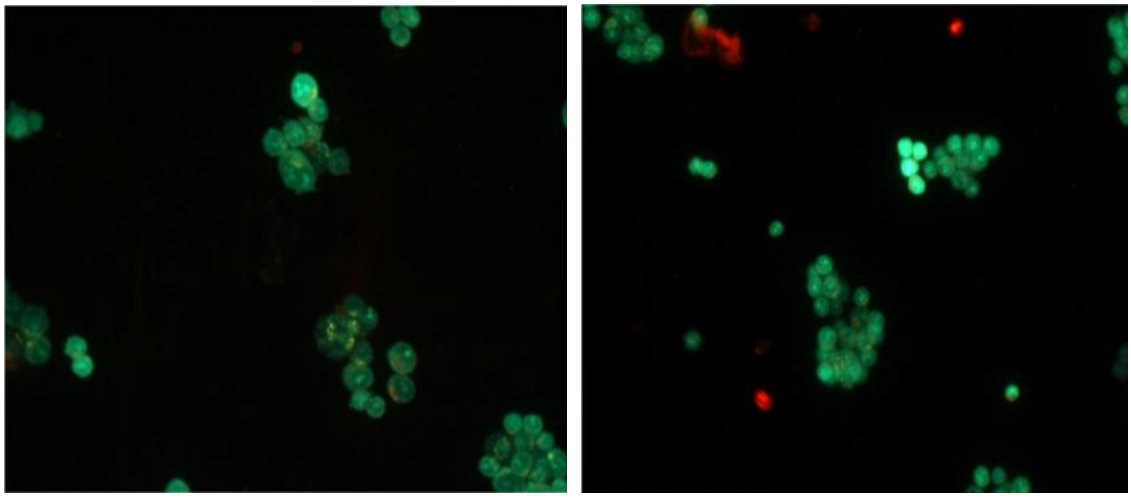
Table 3.2.2.1:Apoptotic effect of *H. triquetrifolium* different extracts on colon cancer cell line (0, 500 µg /ml) after using Annexin v-cy3 under the florescent microscope.

Extraction Way / Cell line	Hexane Extraction Apoptosis %		50% Ethanol Extraction Apoptosis %	
	0µg/ml control	500µg/mL	0µg/ml control	500µg/mL
HCT116	2%	48%	2%	21%



A **B**

Figure 3.2.2.1 Apoptotic effect of *H. triquetrifolium* hexane extract on colon cancer cell line (HCT116) after using Annexin v-cy3 under the florescent microscope. (A) Control. (B) At the concentration 500 µg/ml of *H. triquetrifolium* extract.



A **B**

Figure 3.2.2.2Apoptotic effect of *H. triquetrifolium* water-alcoholic extract on colon cancer cell line (HCT116) after using Annexin v-cy3 under the florescent microscope. (A) Control (B) At the concentration 500 µg/ml of *H. triquetrifolium* extract.

3.2.3 Mitochondrial Cytochrome c Release.

Cytochrome c release from mitochondria is a critical step inthe apoptotic cascade since this activates downstream caspases (71,72).To investigate the release of cytochrome c in colon cancer cell line(HCT-116), lung cancer cell line (A459) and normal muscle cell line (L6), we conducted ELISA kit specific for cytochrome c protein release.

The results of the three extraction methods demonstrate a concentration-dependent increase in the cytochrome c release after treatment with *Hypericum triquetrifolium* on the three cell lines HCT116 (figure 3.2.3.1), A459 (figure 3.2.3.2), and L6 (figure 3.2.3.3). However, there was a significant increase in cytochrome c release by hexane extraction method on the two cancer cell lines comparing with the other methods at non-toxic concentration 250 µg/ml. Whereas for the other

extraction methods, DW and 50% ethanol, there was no difference between them in cytochrome c release level at the 250µg/ml.

According to our apoptosis results, we can conclude that the three different extraction methods have induced apoptosis in the previous cell lines that could be through mitochondrial dependent pathway by releasing cytochrome c.

According to our best knowledge, this is the first reported research that had tested water, ethanol and hexane extracted *H. triquetrifolium* cytoxicity (the survival of the cells by MTT assay) and apoptosis against the previous cell lines. So we conclude that water, ethanol and hexane extracted *H. triquetrifolium* have apoptotic effect on the pervious cell lines through mitochondrial dependent pathway at non-toxic concentration up to 1000µg/ml.

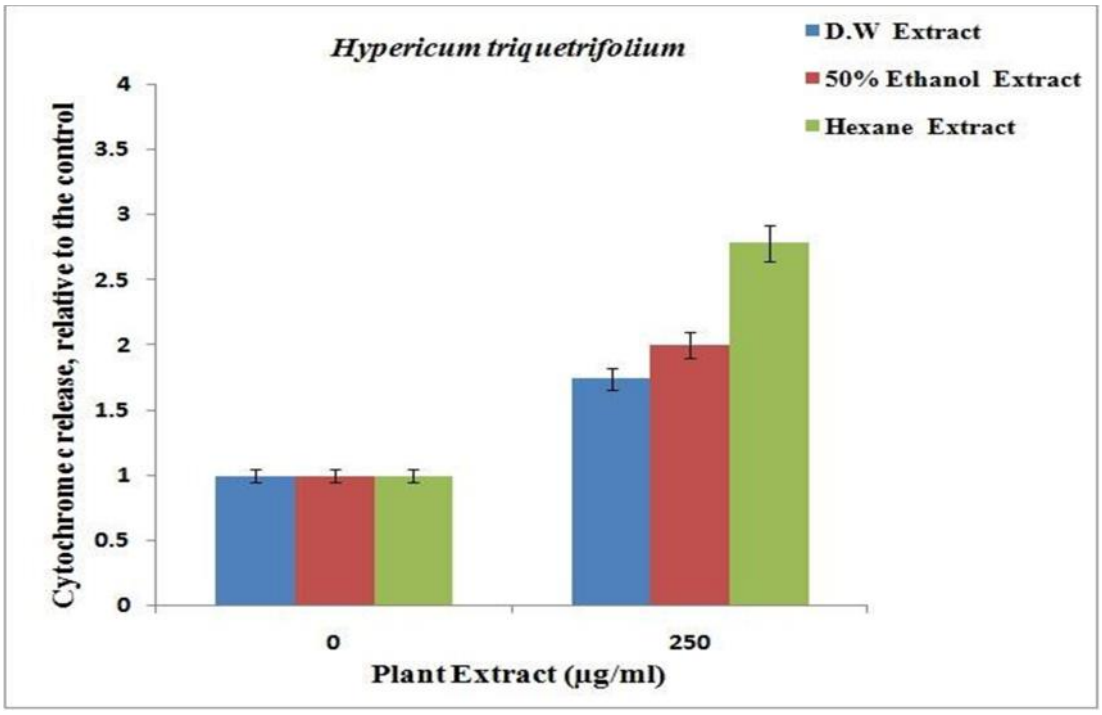


Figure 3.2.3.1:Effect of *H. triquetrifolium* different extracts on cytochrome c release from colon cancer cell line (HCT116) at the concentration (0, 250) µg/ml.

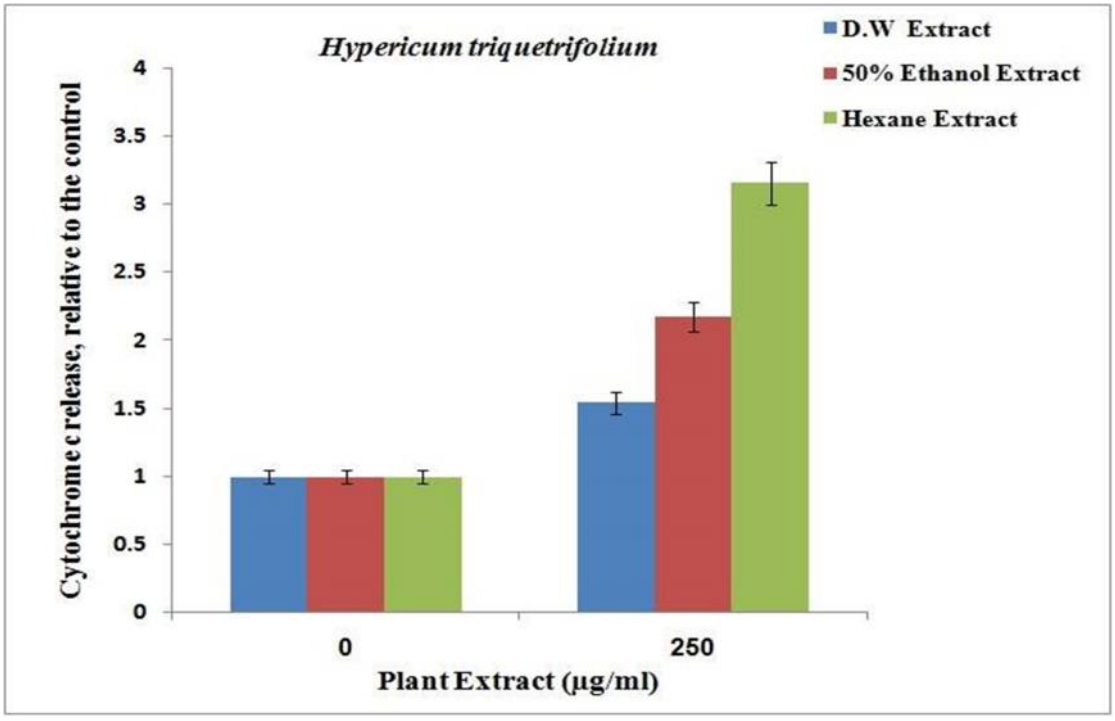


Figure 3.2.3.2 Effect of *H. triquetrifolium* different extracts on cytochrome c release from lung cancer cell line (A459) at the concentration (0, 250) µg/ml.

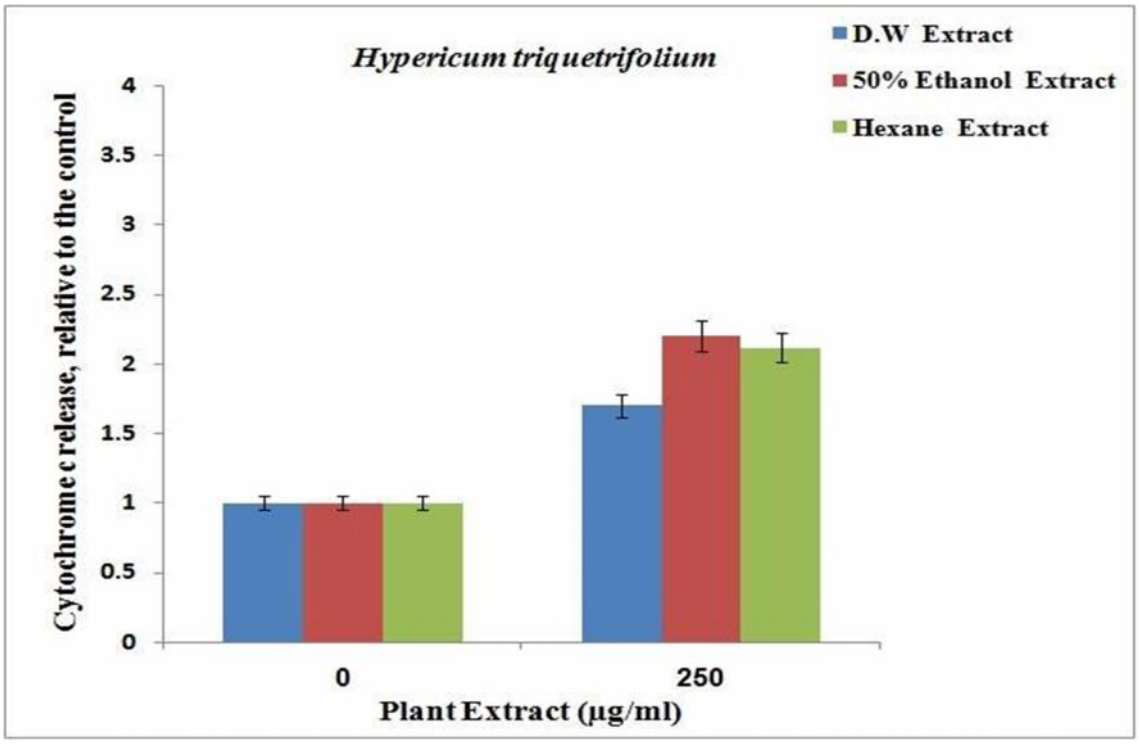


Figure 3.2.3.3Effect of *H. triquetrifolium* different extracts on cytochrome c release from normal muscle cell line (L6) at the concentration (0, 250) µg/ml.

3.3 *Arum palaestinum*

3.3.1Cytotoxic and Apoptotic effects of *Arum palaestinum*

Arum palaestinum is known as a cancer fighting plant among the Arabs up to date (76, 77). Surprisingly, our results show that *A. palaestinum* different extraction methods have no cytotoxic effect at all concentrations tested on the selected cell lines (colon cancer cell line(HCT-116)(figure 3.3.1.1), lung cancer cell line (A459)(figure 3.3.1.2) and normal muscle cell line (L6) (figure 3.3.1.3)) (Table 3.3.1.1). Moreover, the water and water-alcoholic extracts of *Arum Palaestinum* were not neither toxic nor apoptotic inducer when applied on lung, colon and normal muscle cell lines up to 1mg/ml (figure 3.3.1.4). Whereas, the hexane extract was also non-toxic at all concentration tested (0, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/ml) but has an apoptotic effect at non-toxic concentration 500 µg/ml(figure 3.3.1.5). However, according to MTT assay, 7% of cells were dead and the apoptosis was evident 11% in HCT116 cancer cell line (Table 3.3.1.2) through hexane extract. When cells are dead, there is no metabolic activity for the cells; hence in the MTT test there were less dead cells than the apoptosis test. In addition, there were no morphological changes on the cells treated with *A. palaestinum* different extraction methods compared to the control.

Moreover, the results of the water and water-alcoholic extracts of *Arum Palaestinum* did not show a concentration-dependent increase in the cytochrome c after treatment with non-toxic concentration 250µg/ml(figure

3.3.1.6)(figure 3.3.1.7). However, there was a significant increase in cytochrome c release by hexane extraction at the same concentration (figure 3.3.1.8).

A survey of the literature revealed that few studies on the potential anticancer activity of *A. palaestinum* had been undertaken and are mostly ethanobotanical reviews (76, 77).

However, in the supervisor lab (unpublished data), the water-alcoholic extract of *Arum Palaestinum* was not neither toxic nor apoptotic inducer when applied on lung, prostate, and colon cell lines up to 1mg/ml. Moreover, Abu Dahab and Afifi (2007) reported that low concentration (50 µg/ml) of ethanolic *A.palaestinum* extract did not have a cytotoxic effect on breast adenocarcinoma cell line (MCF7) treated for 72 h; but they did not test its effect on apoptosis induction (99). Concomitantly, El-Desouky and colleagues reported that proliferation of breast carcinoma and lymphoblastic leukemia, treated with ethyl acetate fraction of *A.palaestinum* was suppressed at IC50 of about 55 µg/ml. However, they found no effect of the same extract on the growth of hepatocellular carcinoma cells (HepG2) (79). Moreover, Cole and colleagues reported that the water extract of *Arum Palaestinum* combined with three fortifying components (isovanillin, linolenic acid and β-sitosterol) inhibited prostate cancer spheroids IC50 was approximately 3mg/ml and reduced the growth rate of prostate tumors in mice (100).

According to our best knowledge, this is the first reported research that had tested water, water-alcoholic and hexane extracted *A. Palaestinum* cytoxicity (the survival of the cells by MTT assay) or its apoptotic effect (by the annexin v-cy3) simultaneously. So we conclude that water and water-alcoholic extracted *A. palaestinum* are not neither toxic nor apoptotic inducer when applied on lung, colon and muscle cell lines up to 1mg/ml. Whereas, hexane extracted *A. palaestinum* has an apoptotic effect on cell lines that could be through mitochondrial dependent pathway via cytochrome c release at non-toxic concentration 500µg/ml.

Yet we cannot exclude the possibility that *A. palaestinum* might has anticancer properties in a distinct way such as anti-angiogenesis activity.

Hence, more experiments are needed, especially in vivo tests in tumorigenic animal models.

Table 3.3.1.1:The cytostatic and cytotoxic effects of three different extraction ways of *A. palaestinum* on the viability of three cell lines (L6, HCT, A459) at the concentration 1000 µg/ml.

Cell Viability % / Extraction Way	Cell Viability % of L6		Cell Viability % of HCT		Cell Viability % A459	
	Cytostatic	Cytotoxicity	Cytostatic	Cytotoxicity	Cytostatic	Cytotoxicity
DW Extraction	82% ± 1.5	89.5% ± 2.0	98% ± 4.2	91.4% ± 3.8	87% ± 4.64	91% ± 3.8
50% Ethanol Extraction	89% ± 1.7	91.4% ± 3.8	92.5% ± 4.5	91% ± 2.35	88% ± 3.7	92.7% ± 6.1
Hexane Extraction	85% ± 4.0	83% ± 3.3	81% ± 2.1	88% ± 4.0	82% ± 6.0	81% ± 6.0

Table 3.3.1.2:Apoptotic effect of *A. palaestinum* different extracts on colon cancer cell line (0, 500 µg/ml) after using Annexin v-cy3 under the florescent microscope.

Extraction Way / Cell line	Hexane Extraction Apoptosis %		50% Ethanol Extraction Apoptosis %	
	0µg/ml control	500µg/mL	0µg/ml control	500µg/mL
HCT116	2%	13%	2%	2%

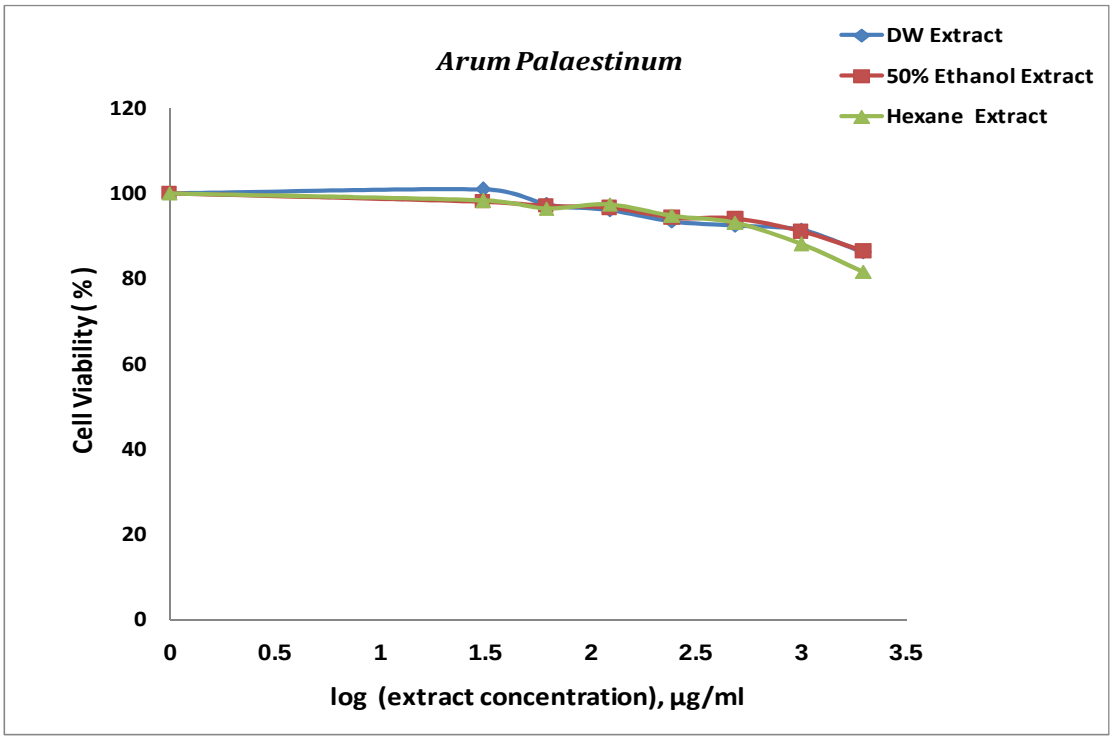


Figure 3.3.1.1:Effect of different concentrations, in logarithmic scale, (0-2000 µg/ml) of *A. palaestinum* different extracts on cell survival of colon cancer cell line (HCT116) obtained by MTT colorimetric assay.

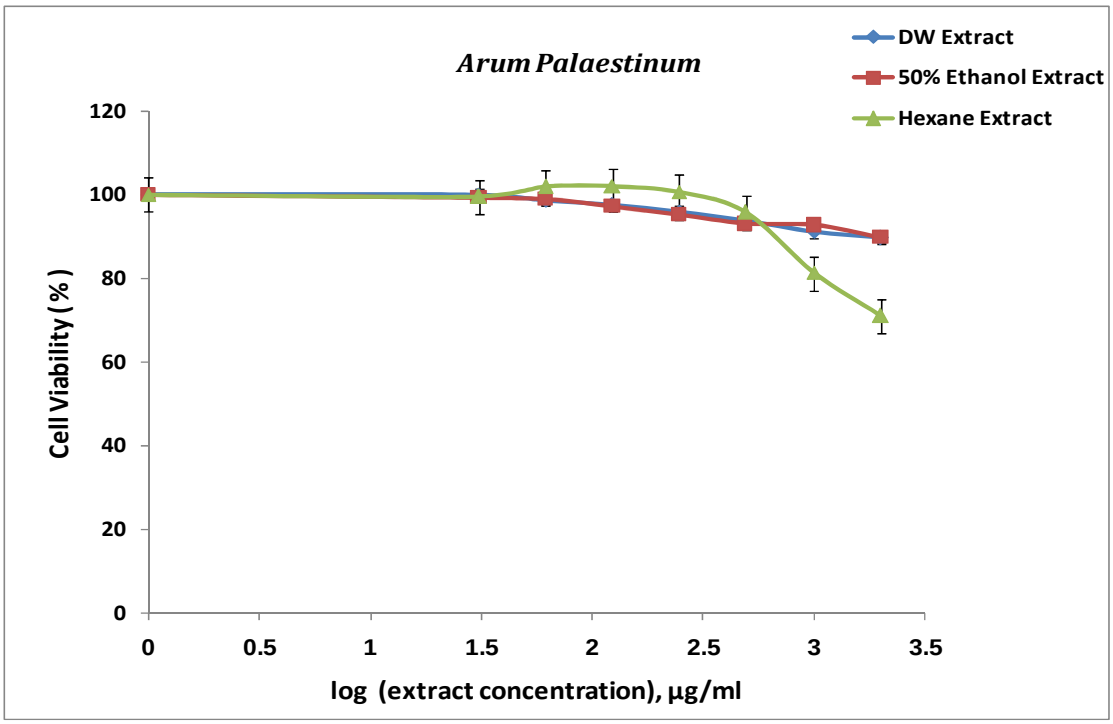


Figure 3.3.1.2:Effect of different concentrations, in logarithmic scale, (0-2000 µg/ml) of *A. palaestinum* different extracts on cell survival of lung cancer cell line (A459) obtained by MTT colorimetric assay.

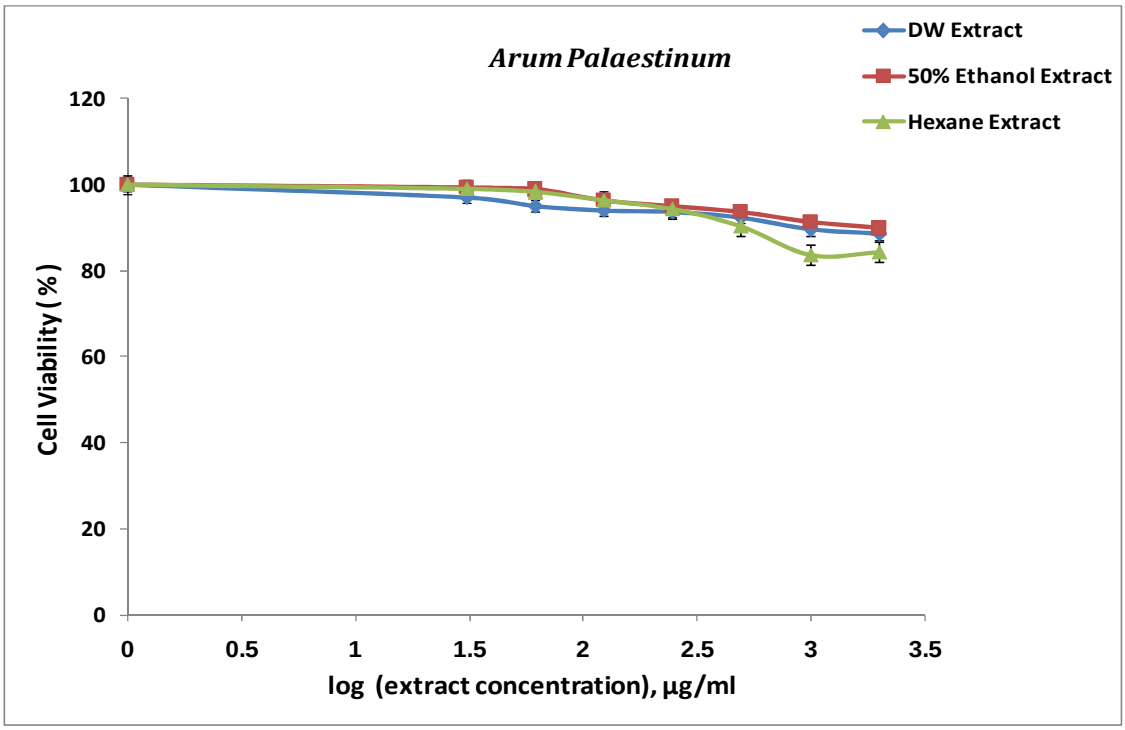
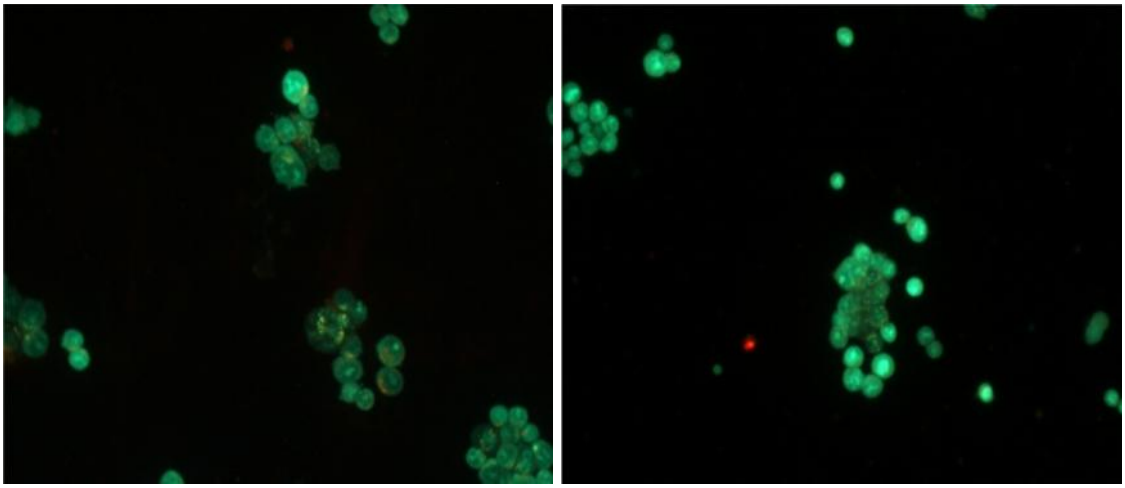
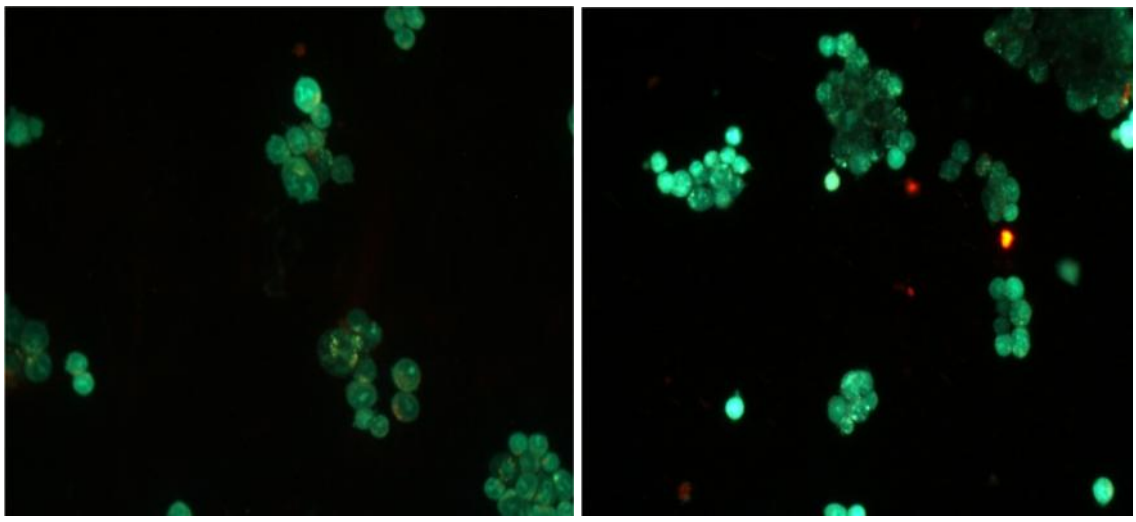


Figure 3.3.1.3:Effect of different concentrations, in logarithmic scale, (0-2000 µg/ml) of *A. palaestinum* different extracts on cell survival of normal muscle cell line (L6) obtained by MTT colorimetric assay.



A B

Figure 3.3.1.4:Apoptotic effect of *A. palaestinum* water-alcoholic extract on colon cancer cell line after using Annexin v-cy3 under the florescent microscope. (A) control. (B) at the concentration 500 µg/mlof *A. palaestinum* extract.



A B

Figure 3.3.1.5:Apoptotic effect of *A. palaestinum* hexane extract on colon cancer cell line after using Annexin v-cy3 under the florescent microscope. (A) control (B) at the concentration 500 µg/mlof*A. palaestinum* extract.

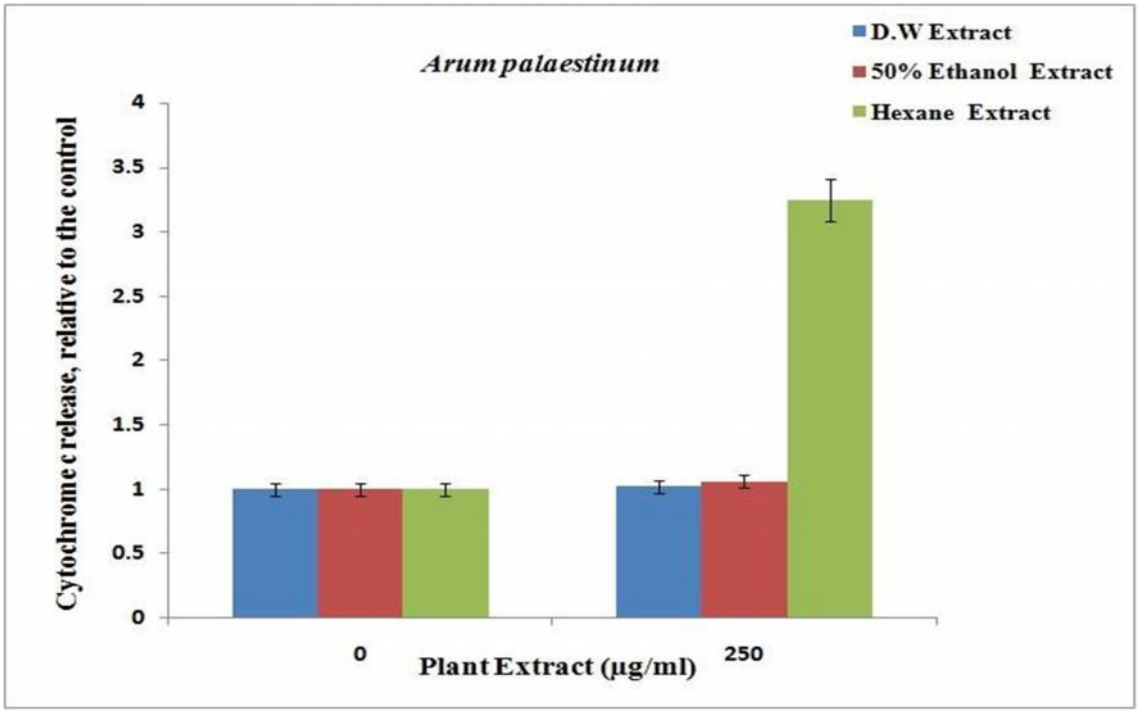


Figure 3.3.1.6:Effect of *A. palaestinum* different extracts on cytochrome c release from colon cancer cell line (HCT116) at the concentration (0, 250) µg/ml.

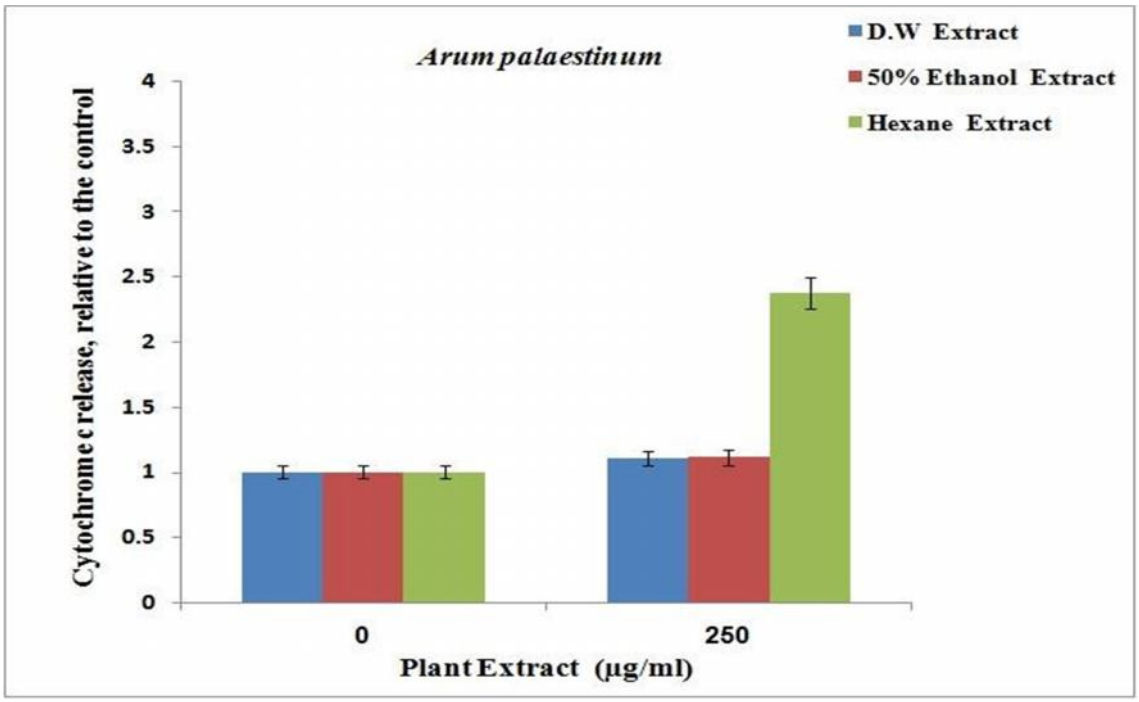


Figure 3.3.1.7:Effect of *A. palaestinum* different extracts on cytochrome c release from lung cancer cell line (A459) at the concentration (0, 250) µg/ml.

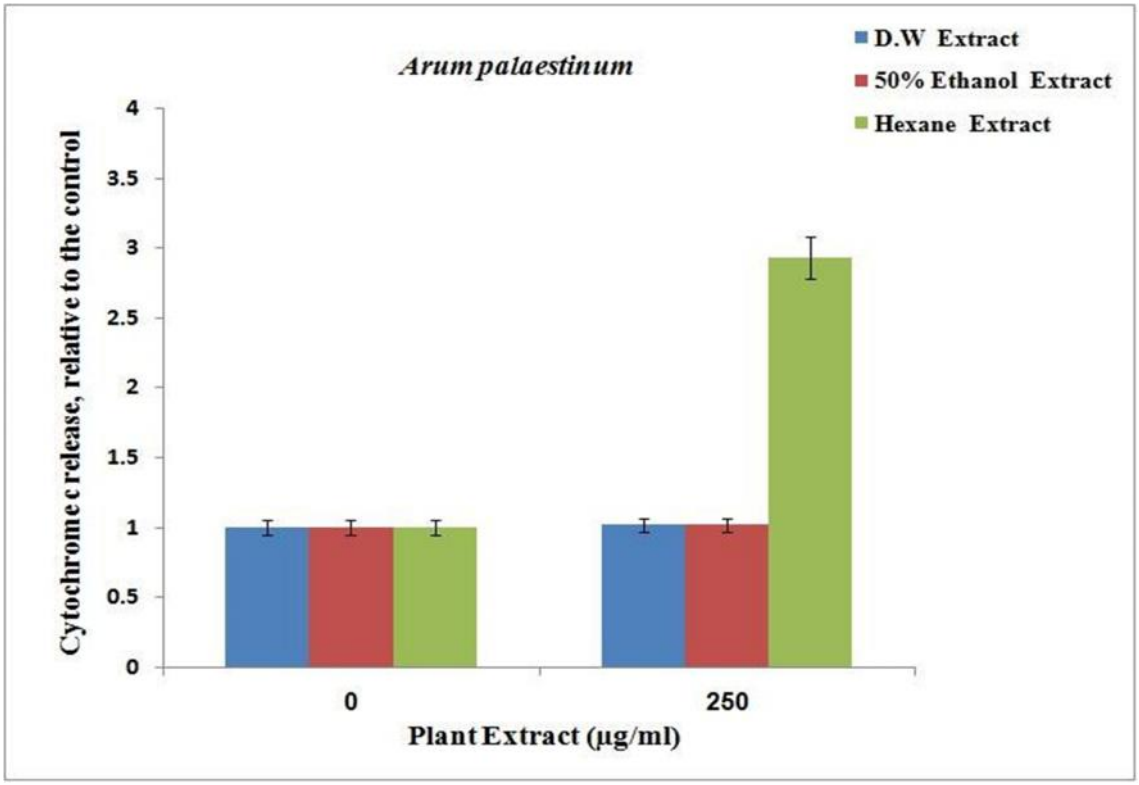


Figure 3.3.1.8:Effect of *A. palaestinum* different extracts on cytochrome c release from normal muscle cell line (L6) at the concentration (0, 250) µg/ml.

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جامعة النجاح الوطنية
كلية الدراسات العليا

التقييم المخبري لنباتي داذي واللوف الفلسطيني على تحفيز الموت المبرمج للخلايا السرطانية

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.
2015

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الملخص

ان استخدام الاعشاب الطبية في علاج السرطان كعلاج تكميلي وبديل اصبح على نحو متزايد خصوصا مع تزايد الأدلة العلمية من ابحاث الطب الحيوي والتجارب السريرية على فعاليتها في علاج السرطان. الاعتماد على الاعشاب الطبية في تصنيع الأدوية المستخدمة في علاج السرطان يعود الى فترة زمنية طويلة ومنها من تم استخدامه في إعداد سريرية مثل العقاقير التقليدية المضادة للسرطان. في الواقع، الاعشاب الطبية هي مصدر اكتشاف الادوية المستخدمة في علاج السرطان وتطوير الأدوية.

استخدام الأعشاب العربية الإسلامية التقليدية في علاج السرطان قد يكون مرشحا واعدا لعلاجات جديدة للسرطان، خاصة ان منتجات هذه الأعشاب الطبيعية عادة ما تكون ذات سمية منخفضة والآثار الجانبية لها قليلة.

هدفت هذه الدراسة الى فحص قدرة نباتين طبيين هما (اللوف الفلسطيني وداذي) على قتل خلايا سرطانية.

من اجل ذلك, تم استخدام نوعين من الخلايا السرطانية هما(القولون و الرئة) و نوع من الخلايا الطبيعية هو(خلايا عضلية), حيث تمت معالجة هذه الخلايا بتراكيز متزايدة (0, 8, 16, 32, 62, 125, 250, 500, 1000 and 2000) مايكروغرام/ ملل من مستخلصات كلا النباتين (الماء المقطر،50%ماء و50% ايثانول، وهكسان).

وتم استخدام صبغة (MTT) لفحص سمية كلا النباتين وكذلك صبغة (Annexin(v-cy3 من أجل فحص الموت المبرمج للخلايا، واستخدام تقنية ELISA لفحص خروج بروتين cytochrome c من الميتوكوندريا.

اظهرت النتائج ان نبات داذي ليس له تاثير سمي على جميع التراكيز المفحوصة لكل المستخلصات ولكن كان لجميع المستخلصات تأثير على تحفيز الموت المبرمج للخلايا على تركيز 500مايكروغرام/ ملل وذلك من خلال مسار المايتكوندريا عن طريق زيادة افراز بروتين cytochrome cالى السيتوبلازم على تركيز 250مايكروغرام/مل. اما بالنسبة لنبات اللوف الفلسطيني, فلم يكون هناك اي تأثير سمي او تأثير على تحفيز الموت المبرمج للخلايا بالنسبة للمستخلص عن طريق (الماء المقطر, 50%ماء و50% ايثانول), اما بالنسبة لمستخلص (الهكسان) فكان له تأثير على تحفيز الموت المبرمج للخلايا على تركيز 500 مايكروغرام/ مل من خلال مسار المايتكوندريا عن طريق زيادة بروتين cytochrome c المفرز الى السيتوبلازم على تركيز 250 مايكروغرام/ملل و لكن دون اي تأثير سمي على جميع التراكيز المفحوصة.

هذه النتائج ترشح نبات داذي كنبتة طبية لعلاج السرطان عن طريق قتل الخلايا بطريقة مبرمجة.