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Screening for Anticancer Activity of Palestinian Plants

By

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I

COMMITTEE DICISION

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DEDICATION

TO

MY DEAR PARENTS, WIFE, SON, BROTHERS, AND SISTERS FOR THEIR SUPPORT AND ENCOURAGEMENT, WITH LOVE AND RESPECT

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ABSTRACT

One hundred and fifteen ethanolic extracts prepared from 96 plant species from Palestine were evaluated for anticancer activity against two prostate cancer cell lines, PC-3 and LNCaP; and one breast cancer cell line, MCF-7, using cell proliferation MTT assay and a 96-microwell plate-reader.

The results demonstrated that the studied plants differ significantly in their activity against test cancer cell lines. The most active plant species against test cancer cell lines include *Cyclamen persicum* Mill, *Lycium europeum*, *Ecballium elaterium* L., *Euphorbia hierosolymitana*, *Capparis spinosa*, *Ononis sicula* Guss., *Anthemis tunictoria* L, *Verbascum sinuatum*, and *Nerium oleander* L. For PC-3 cell line the most active plants include *Cyclamen persicum* Mill, *Lycium europeum* L., *Ecballium elaterium* (L.) Rick, *Euphorbia hierosolymitana* Boiss, *Anthemis tunictoria* L., *Verbascum sinuatum* L. *and Nerium oleander* L. For LNCaP cell line the most active plants include *Cyclamen persicum* Mill, *Verbascum sinuatum* L., and *Capparis spinosa* L. For MCF-7 cell line the most active plant species include *Cyclamen persicum* Mill, *Lycium europeum*L., *Euphorbia hierosolymitana* Boiss, and *Ononis sicula* Guss.

Another important observation was the stimulation of PC-3 cell line by some plants, for instance *Crataegus aronial*, *Ziziphus spina-christi*, *Salvia*

fruiticosa, Retema raetam, and *Parietaria diffusa*. These plant species were used in folkloric medicine for many diseases and disorders. Significantly, stimulation of LNCaP and MCF-7 cell lines was observed also with *Quercus calliprines* and *Chrysanthemum coronarium*, which are used as popular remedies. Therefore, care must be taken with respect to the great number of plant extracts that show stimulation of cancer cells.

The results demonstrated that Ether and Acetone fractions were the most active against the test cell lines. In addition Diethyl Ether dissolved terpeniods and some alkaloids, some of diterpenes and sesquiterpenes compounds act as anticancer drugs.

Test cancer cell lines differed significantly in relation to their susceptibility to different plant extracts used. The most susceptible test cell line was PC-3, whereas the least susceptible one was LNCaP.

CHAPTER ONE

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1 Medicinal plants

A plant is said to be "medicinal" when at least one of its parts contains substances that can be used for therapeutic purpose (Sofowora, 1982; Bruneton, 1995). This definition includes plants used in galenical preparations (e.g., decocations, infusions, etc...), in extraction of surgical dressings, in addition to some food spices and perfumery plants that are used medicinally.

The use of these plants in preventing, or eliminating physical, mental or social diseases is referred to as traditional or folk medicine. This medicine can be described as the combination of knowledge and practice, relying on past experience and observation handed down from generation to generation (Sofowora, 1982). Folk medicine comprises numerous herbal and plant prescriptions for therapeutic purposes. These include healing of wounds, treatment of inflammation and skin ulcers (Karim and Quraan, 1986; Dafni *et al.*, 1994; Ghazanfer, 1994; Tanira *et al.*, 1994), pneumonia and bullet

wounds (Desta, 1993), dermatomucosal, skin and candidal infections (Caceres *et al.*, 1991,1993).

The interest in studying the biological effects of traditional medicinal plants or isolating their active components for treatment of illness, has increased all over the world and comprehensive screening programs have been established (Boulos, 1983; Kottob, 1983; Azzam, 1984). A large proportion of the current research in Ethnobotany remains focused on the American continent where up to 41% of the studies are carried out (Cotton, 1996). In Palestine, the screening of the flora for pharmacological active compounds started in the late sixties (Silva *et al.*, 1981). The abundance of species (>2600) condensed on a very small geographical area (about 25000 Km^2) is a major characteristic of the Palestinian flora. This richness is due to the diversity of habitates created by the soil and climatic conditions, in addition to the lack of medical care, and economics.

The remarkable diversity of environments and habitats stimulates also the process of genetic differentiation and thus the development of new ecotypes finally leading to new species. Indeed, the splitting of some species in ecotypes or chemotypes is another characteristic feature of the Palestinian flora. Above all, passing knowledge from one generation to the next about medicinal plants and their use, is a part of the heritage in this area of the world (Boulos, 1983; Karim & Quraan, 1986).

Many plant species (> 700) have been used in folkloric medicine in Palestine to treat various ailments of man (Palevitch, 1991; Shtayeh & Hamad, 1995; Ali-Shtayeh, *et al.*, 2000). Folk remedies used are prepared as powders poultices, ointments, baths, decoctions, infusions and teas. Decoction is the most popular form of home remedy. Decoctions, infusions and teas are usually prepared just before application and filtered through a cloth or cotton wool. Most plants are stored for use in the dry state, which permits their utilization throughout the year, sometimes fresh plants are used (Sezik *et al.*, 1991). Ninety-four of these plants (Table 2.1) which are used to treat dermatomucosal infections and other ailments, were selected in the present work for antiprostate cancer activity testing. However, some of the selected plants have been tested for biological activities other than antibacterial or anticandidal activities such as antifungul activity (e.g. Amoros *et al.*, 1988; Cacers *et al.*, 1991; Bagchi *et al.*, 1999), hypoglycemic activity (e.g. Yaniv *et al.*, 1987; Gharaibeh *et al.*, 1988; Glombitza *et al.*, 1994), antiulcerogenic, antihelminthic and hepatoprotective (e.g. Akhtar *et al.*, 1989; Naqvi *et al.*, 1991; Sultana *et al.*, 1995; Abreu *et al.*, 1999), analgesic, antipyretic and antirheumatic activities (e.g. Karim and Quraan, 1986; Al-Said *et al.*, 1990; Dafni *et al.*, 1994; Ali *et al.*, 1995), antileishmania and insecticidal activities (e.g. Abreu *et al.*, 1999; Chariandy *et al.*, 1999). To the best of our knowledge the remaining plants have not yet been studied for their anticancer activities.

It is hoped that this study can focus the light on the anticancer activities of the selected plants (Table 2.1).

Plant products, having traditional medicinal values, have always been an interesting concern to the phytochemists and pharmacologists for advanced studies on their chemistry and bioactivity. Chinese traditional medicines have a long history and strong reputation in curing several diseases and are used still today, not only in China but also all over the world (Sarker, 1996). A main fraction of population in developing country remains dependent on ancestorial plant knowledge for health care. This ratio keeps increasing with the state of poverty of these countries. In addition, WHO encourages the inclusion of medicinal plants in programs of developing countries because of the great potential plants represent in combating various diseases (Noumi et al., 1999).

Why peoples want to use medicinal plant more than manufactured medicine?

There is a renewed interest, especially in developed countries, in using plants to treat livestock, pets, and humans because:

- Many people believe that plants are less toxic and safer than manufactured drugs.
- Many people believe that plants are more natural than manufactured drugs.

- Medicinal plants can be made at home and are less expensive than manufactured drugs.
- In developing countries, medicinal plants often are more accessible than manufactured drugs (Jennifer Ketzis, 2000).

1.2 Antiprostate cancer activity and phytochemistry of medicinal plants

Various medicinal plant extracts claimed to be effective as antiprostate disorders agents have been used since biblical times. However, often objective and scientific efficacy has not been shown. Recently, the ability of medicinal plant extracts to control the proliferation of prostate cancer cells (Table. 1.1) were reported (Hryb *et al.*, 1995, Ravenna *et al.*, 1996, Hsieh *et al.*, 1997, Hiremath *et al.*, 1997). Habib *et al.*, (1997) identified a HPLC fraction from *Cernilton pollen* extract and found this fraction to be highly active in inhibiting the growth of DU145 cells, a prostate cancer cell line. The active fraction was later identified as cyclic hydroxamic acid. The 5-α reductase which converts testosterone to the more potent androgen, dehydroxytestosteron (DHT) in the prostate, was identified as the molecular target for many plant extracts (Evans *et al.*, 1995). For example, oenothein B was identified as the active compound of *Epilbium parviflorum*, a plant used in Central Europe for the treatment of prostate disorders, inhibits 5-α reductase (Evans *et al.*, 1995).

gradually grows until puberty, when it begins to expand rapidly, attaining normal adult size, about the size and shape of a chestnut, when a man reaches his early 20s. The prostate is composed of glandular tissue that produces a milky fluid and smooth muscles that contract during sex and squeeze this fluid into the urethra, where it mixes with other fluid and sperm to form semen (Porth, 1994; Medscape, 2000). The prostate also converts testosterone to a more powerful male hormone, dihydrotestosterone, which affects the size of the gland and plays an important role in prostate cancer (Culig *et al.,* 1997; Medscape, 2000).

1.3.2 What is prostate cancer?

Prostate cancer is a malignant tumor that arises in the prostate gland and can eventually spread through the blood and lymph fluid to other organs, including the bones. Fortunately, prostate cancer tends to be slow growing compared to many other cancers (Medscape, 2000).

1.3.3 How serious is prostate cancer?

Prostate cancer is the most common male cancer and is second to lung cancer as a cause of cancer-related deaths in men (Culig *et al.,* 1997; Griffiths *et al.,* 1998; Xiaolin & Rajesh, 1999; Porth, 1994).

Many men with prostate cancer die with it, rather than from it. Because so many prostate tumors are low-grade and slow growing, survival rates are

excellent when prostate cancer is detected in its early stages. When prostate cancer is detected in an early stage, cure rates are as high 98%. If the disease is at a stage known as locally advanced it is more difficult to cure, but survival rates can be prolonged for years in many men. If prostate cancer has spread to distant organs, average survival time is one to three years (Medscape, 2000).

1.4 Screening Methods for Anticancer Activity of Natural Products

1.4.1 Methods for studying cell viability and proliferation in cell populations

The most convenient modern assays for determination of cell viability and cell proliferation have been developed in a microplate format (96-well plates). This miniaturization allows many samples to be analyzed rapidly and simultaneously.

The microplate format also reduced the amount of culture medium and cells required as well as cost of plastic ware. Calorimetric assays allow samples to be measured directly in the microplate reader (Riss & Moravec, 1993). Microplate assay has been developed based on different parameters associated with cell viability and cell proliferation. The most important parameters used are DNA synthesis like [3H]-TdR proliferation assay, and metabolic

1.4.1.1 MTT Assay

1.4.1.1.1 Background information

3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) reduction is one of the most frequently used methods for measuring cell proliferation and neural cytotoxicity. It is widely assumed that MTT is reduced by active mitochondria in living cells (Lui *et al.*, 1997; Riss & Moravec, 1993). Recently, colorimetric assays have become available for analyzing the number of cells by the cleavage of tetrazolium slots added to the culture medium. This technique requires neither washing nor harvesting of cells, and the complete assay from onset of the microculture to data analysis by a microplate reader is performed in the same microtiter plate (Roche, 1999; Promega, 1996).

1.4.1.1.2 MTT assay application

MTT assay is designed to be used for the non-radioactive, spectrophotometric quantification of cell proliferation and viability in cell populations using 96-well plate format. It can be used for: 1- the measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients (Huang *et al.*, 1998; Roch, 1999). 2- the analysis of cytotoxic and cytostatic compounds like anti-cancer drugs and other pharmaceutical compounds (Gergel *et al.*, 1995; Wong & Goeddel, 1994; Roch, 1999). 3- the

assessment of growth inhibitory antibodies and physiological mediators (Fanjul *et al.*, 1996; Roch, 1999).

1.4.1.1.3 MTT assay principle

The assay is based on the cleavage of the tetrazolium salt MTT, in the presence of an electron-coupling reagent, by active mitochondria. The water-insoluble formazan salt produced has to be solubilized in an additional step. Cells, grown in a 96-well tissue culture plate, are incubated with the MTT solution for approximatly 4h. After this incubation period, a water-insoluble formazan dye is formed. After solubilization, the formazan dye is quantitated using microplate reader. The absorbance revealed directly correlates to the cell number (Roch, 1999).

1.4.1.1.4 Advantages of the MTT assay

There are many advantages for MTT assay as followed: 1-safe: no radioactive isotopes are used. 2-accurate: the absorbence revealed, strongly correlates to the cell number. 3-sensitive: low cell number is detected. 4-fast: the use of a multiwell-plate reader allows a larger number of samples to be processed. 5-easy: no washing steps and no additional reagents are required (Riss & Moravec, 1993; Roch, 1999).

1.4.1.1.5 Disadvantages of MTT assay

MTT assay has some disadvantages as followed: 1-requires volatile organic solvent to solubilize the formazan product. 2-plates can't be read and returned to incubator for further color development (Promega, 1996).

1.4.2 Extraction techniques

Antimicrobial activity of plant is usually assessed after extracting plant material with organic and aqueous solvents, in order to separate the chemical constituents into groups of different polarities (Nadir *et al.*, 1985).

Many factors may affect the extractability and hence the biological activity of the chemical constituents of the plants. The pH of the extracting medium is one of these factors. For this reason, when working on medicinal plants it is recommended to apply various methods of extraction to ensure the recovery of the active components (Nadir *et al.*, 1985).

Decoction is one of the traditional extraction techniques. It is prepared by placing the plant drug in cold water, bringing it to boil for 15 minutes or longer (up to 1 hour), and then allowing the mixture to stand for a further 15 minutes, the extract (aqueous or organic) are decanted or filtered as and when required. This type of extraction may result in the alteration of many active constituents (e.g., some glycosides are decomposed during boiling).

Another technique of extraction is infusion, which is carried out by pouring boiling water or organic solvent on a specific quantity of plant material and allowing the mixture to stand for 10-15minutes or more (Sofowora, 1982).

1.4.3 Methods of separation

Different chemical methods have been used for separation, determination and identification of ingredients occurring in the plant including distillation, filtration, crystallization, extraction, chromatographic methods and spectroscopic methods (Henry *et al.*, 1978).

1.4.3.1 Chromatography

Accounts of the early history of chromatography have been given by Weil, (1950); Williams, (1951); Farradane, (1951); and Zechmeister, (1951). Russian botanist Tsweet, (1910) was the first to be aware of the great advanages of chromatography. He described in details the separation of pigments and colorless substances by filtration through columns, followed by development with pure solvents (Lederer, 1957).

Chromatography is a method by which the chromotographed substance moves in a system of two phases, one of which is stationary and the other is mobile (Pattison, 1978).

1.4.3.1.1 Thin layer Chromatography (TLC)

The first report on it was introduced by Isamailov and schrasber in 1938 (Abdel-Latif, 1994). TLC-separation is the result of combination of separation mechanism most often adsorption, partition and ion exchange. TLC method utilizes a calculated factor as the basis for quantitative analysis, this is called a retardation factor (R_f) which has a specific value for a specific solute using a specific mobile and stationary phase (Abdel-Latif, 1994).

1.4.3.1.2 Column Chromatography

Column Chromatography processes are very useful techniques for separation large amounts of samples. The mobile phase may be liquid or gas. The separation may be due to adsorption that depends on the interactions between the mobile phase and stationary phase. A continued passage of the solvent aids the settling of the packing particles (Snyder & Kirkland, 1971).

1.5 Objectives of the present study

This *in vitro* study was aimed at:

1. Identifing and selecting some traditional medicinal plants that are claimed to be effective in the treatment of prostate disorders.

2. Identifing and selecting the most active fractions of active plants.

3. Determining whether their use in folkloric medicine to treat these diseases is justified.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO

MATERIALS & METHODS

2.1 Anticancer activity of plant extracts

2.1.1 Plant material

2.1.1.1 Collection

Plant material (aerial parts, leaves, roots, seeds, and whole plants) of 96 plant species belong to 43 botanical families, commonly used in Palestinian folk medicine, were collected from various locations in the northern part of the country (Table 2.1). The aerial parts of the mature plants were collected in the period between April and July 2000. The collected plant material was either used fresh, or dried in the shade, and then ground to a powdered material using an appropriate seed mill. All plants were identified by Prof. M. S.Ali- Shtayeh in the Department of Biological sciences at An-Najah University, and specimens of the plants were preserved there.

Table 2.1 Selected plants used for anticancer susceptibility testing.

Species/ Family (Voucher Specimen No.)	Common name	Arabic name	Parts used*	Popular uses	Ref. For folk popular uses
Achillea fragrantissima (Forssk) Sch. Bip. (Asteraceae, Compositae) W36**	Yarrow	قيسوم	AP,FL	Treatment of diabetes, digestive problem problems, arthritis, fever reduction, severe cough, antidiuretic, stomach ailments, tumors and infections, antispasmodics, arthritis, fever and general weakness, heart pains, kidney stones, edema, delayed menstruation, skin diseases, rheumatism, arthritis, gout and other forms of inflammation.	3,7, 57, 79, 32, 58, 33, 70, 16.
Alcea setosa (Boiss.) alef. (Malvaceae) W23	Holly hock	ورد الجمل	FL		
Allium erdelii Zucc. (Liliaceae) W76	Wild Garlic	ثوم شفاف	WP		
Allium sativum L. (Liliaceae) W39	Garlic	ثوم	LF	Skin and circulatory system (heart and blood vessels), antihelmintics.	7, 38.
Ammi majus L. (Umbelliferae, Apiaceae) W120	Bishop's weed	خله	FL, LF		
Anchusa aegyptaca (L.) DC. (Boraginaceae) W22	Egyptian Alkanet	حمحم مصري	WP		
Anthemis tunictoria (L.) (Compositae) W63	Chamomile	كموميل اصفر	FL,,RT		
Arum dioscoridis Sibth. & Sm. (Araceae) W15	Spotted Arum	لوف مبرقش	LF	Cancer	7
Asparagus aphyllus L. (Liliaceae) W83	Asparagus	هيلون	AP		
Asphodelin Lutea (L.) Reichenb. (Liliaceae) W6	Jacob's rod	عتعاط	WP	Antispasmodic, diuretic, nutritive, skin disorders.	57, 7
Calycotome villosa(Poir.) (Papilionaceae) W84	Thorny broom	قنديل	AP		
Capparis spinosa (L.) (Capparidaceae) W68	Caper bush	قبار	FR,FL	Antihepatotoxic, hepatitis, gastronomic, antihelmintic and for diabetes, treat earache, coughs, diuretics, stimulant, vasoconstrictive, arteriosclerosis and for chills, reproduction enhancement, depurative, resolvent cataplasms for skin inflammation, against painful menstruation, hearing problems, general pain, neuralgia, male and female infertility, rheumatism,	2, 7, 57, 17, 22, 25, 32, 33, 35, 67, 79, 10, 14.
Cardaria draba (L.) Desv. (Cruciferae) W17	Hoary Pepperwort	جنيبره	AP		

Table 2.1/ continues

Ceratonia siliqua (L.) (Caesalpiniaceae) W2	Locust tree	خروب	S	Treating warts, diarrhoea, diabetes, syphilis and venereal diseases, epilepsy, clear the voice, astringent, antihelmintic, abdominal pains,	57, 17, 35, 45, 79, 52.
Chrysanthemum coronarium L. (Chenopodiaceae) W72	Corn marigold	بسباس	FL		
Cicer arietenum L. (Leguminosae) W77	Check bees	حمص	AP		
Cichorium pumilum Jacq. (Compositea) W12	Dwarfchicory	هندباء	AP	Antipyretic,antirheumatics, carminative, digestive.	50
Citrus limon L. (Rutaceae) W31	Lime	ليمون	FL	Worm infection, to treat sore throats, mastitis, against eye redness, gastric hyperacidity, against bronchial affections, antitussive and for common cold and cough urinary system and stones.	7, 66, 20, 14, 61, 27.
Clematis cirrhosa L. (Rananculaceae) W1	Clematis	غاشية	AP	Treat the reproductive system, useful for impotency. Male impotence,	57, 58,42, 48, 73, 76.
Companula rapunculus(L.) (Companulaceae) W7	Bell-Flower	ورد الجمل	AP		
Conium maculathum L. (Compositae, Asteraceae) W40	Poison hemlock	شوكران	LF, FL		
Convolvulus arvensis L. (Convolvulaceae) W97	Bind weed	مدادة	AP	Treat recent wounds, wound healing and laxative.	12, 52.
Coridothymus capitatus (L.) Reichb. (Labiatae)W14	Thyme	زعيمة	AP	Anti-inflammatory and antimicrobial activity, for eye infection, headache, disphoretic, stomach ache, carminative, whoopin cough, antihelmintic, antispasmodic, kidney disorders, antipyretic, emmenagogue and vermifuge , abdominal pains, heart disorders, dropsy, paralysis, blindness, respiratory, digestive and urinary systems, inflamation.	57, 7, 44, 14.
Crataegus aronia L. Bosc. Ex DC (Rosaceae) W19	Hawthorn	زعرور	LF	Cardiac sedative, hypotensive,Rheumatism, diabetes, digestive system, urinary system and stones.	7, 45, 47.

Table 2.1/ continues

Cyclamen persicum (Mill.) (Primulaceae) W37	Cyclamen	صابون الراعي	B, AP	Antirheumatic, headache cardiac sedative, treatment of goiter, anthelmintic, laxative, diarrhea and abdominal pains, eye infections, edema, bone fracture, nerve infections, female infertility, lowlibido, open wounds, abscesses, eczema and skin burns, toothache.	13, 7, 57, 65, 54.
Daucus carota L.ssp.maximus.(Desf.)Ball (Umbelliferae, Apiaceae) W100	Wilde carrot	جزر بري	LF, FL, RT	It used as aphrodisiac	29.
Ecballium elaterium (L.) Rick. (Cucurbitaceae) W28	Squirting cucumbe	قثاء الحمير	AP	Cathartic, Jaundice, constipation, hemorrhoids, eye infections, depression, fears, skin diseases, and hepatitis	7, 57, 12.
Echinops adenocaulos Boiss. (Compositae, Asteraceae) W75	Globe Thistle	أرث	AP	Antiinflammatory activity	62.
Erodium malacoides (L.) L'Her. (Geraniaceae) W42	Stock's bill	ابرة العجوز	WP		
Eruca sativa Mill. (Cruciferae) W92	Gaden Rockets	جرجير	WP	Skin diseases	7.
Eryngium creticum (Lam.) (Apiaceae,Umbiliferae) W47	Snake root	قرصعنه	LF,YB	For diabetes, fluid retention, diuretic, renal stones, skin diseases, calefacient, helminthiases and bronchitis, treat scorpion bites, cataracts, low libido, toothaches, ulcers and intestinal parasites, open wounds and cuts, gum tonic, anti-inflammatry.	45, 57, 79, 52, 34, 49.
Euphorbuia hierosolymitana Boiss. (Euphorbiaceae) W50	Spurge	حلبلبون	WP	Constipation and abdominal pains, excessive libido, wounds and warts,	32, 57.
Foeniculum vulgare (L.) Mill. (Umbelliferae) W87	Fennel	شومر	AP	Diarrhoea, antiemetic, antispasmodic, against gastric hyperacidity, gastronomic, antiinflammatory for digestive tract, flavouring agent, bronchodilator, antitussive lactagogue, galactagogic, analgesics, treat skin diseases, corminative, used in infusions and tinctures, antiseptic, diuretic, expectorant, abdominal colic, coughs, as toothbrush, liver complaint, stomach ache, flatulence, colic, pancreas complaint, dyspnoea, tonic, failing eyesight, headache and weakness, heart disease,	7, 57, 17, 22, 13, 45, 35, 37, 32, 67, 14, 29.
Gagea chloranth (Bieb.) Schult. Fil (Liliaceae) W93	Gagea	زعيتمان			
Gundelia tournefortii L. (Compositae, Asteraceae) W35	Gundelia	عكوب	LF		
Inula viscosa (L.) Ait (compositae) W11	Inula	عرق الطيون	AP	Treatment of diabetes, antihelminitic, expectorant, diuretic, for lung and bronchial disorders, anti-inflammatory, reconstituant. Hemorrhoids, eye infections, fever, headaches, bone fractures, mascle spasms, general tonic, local paralysis, mucus in the respiratory tract, rheumatism, toothache skin diseases.	7, 57, 17, 45, 79, 71, 1, 19, 36, 51, 81, 55, 12.

Table 2.1/ continues

Juglans regia L. (Juglandaceae) W13	Walnut	جوز	LF, FR	Treat eczema, nervous problem, as food, for syphilis, anthelmintic astringent, stomachic, nerve tonic, treat scrofula, rickets, gastro-enteritis, vermifuge, as ahypoglycaemic agent, antidote poison, tonic, dental hygien, depurative, galactofuge, rubefacient, antiscrophulous, antiseptic, skin diseases, antiparasitics and repellents.	7, 17, 22, 35, 45, 67, 80, 12, 38.
Lactuca serriola (L.) (Compositae) W24	Wild lattuce	خيص	AP	Vulnerary, sedative, snake bites, diuretic, laxative.	67, 13, 14.
Lactuca tuberosa Jacq. (Compositae) W101	Lattuce	خس بري	AP		
Lamium moschatum Mill. (Labiatae) W86	Dead nettle	جليلة	WP		
Lawsonia inermis (L.) (Lythraceae) W58	Henna	حنا	LF	Enlargement of liver and spleen, incalculosis, injaundice, in leprosy, skin diseases, burns, colds. Anti-inflammatory activity, cytotoxic activity, hair and scarp problems, treat hair dandruff and split ends, remedy for splite nails, for birth control, fever, local anaesthetic, mouth ulcers, antifungal, used in dermatology in leprosy and leucoderma, gums and skin disorders, antipyretic, analgesic.	6, 35, 45, 27, 56, 5.
Linum pubescens Banks & Sol. (Linnaceae) W94	Pink flax	كتان زهري	AP,FL	Skin disorders and prostate disorders, urine intermittence.	7.
Lupinus albus L. (Papilionaceae) W85	Lupines	ترمس مر	S	Anthelmintic, diuretic, skin diseases, antiparasitics and repellents.	7, 12, 38.
Lupinus pilosus Murr. (Papilionaceae) W88	Lupine	ترمس بري	AP, S		
Lycium europeum L. (Solanaceae) W8	Box thorn	عوسج	WP	Treat skin infections, anesthetic for toothache pain, antiseptic, eye wash, treat cataract, analgesic, antistomachache, analgesic for foot pains, abdominal pains in children, eye infections and cataracts, skin irritation, toothache and gum problems.	24, 32, 57, 14.
Majorana syriaca (L.) Rafin. (Labiatae) W82	Thyme	زعتر بري	AP	Respiratory system	7.
Mandragora autumnalis Bertol. (Solanaceae) W61	Mandrake	تفاح مجن	FR	Resolvent for whitlows, pimples and phlegmons	14.

Table 2.1/ continues

Mentha viridis L. (Labiateae) W114	Mint	نعنع	AP	To treat diarrhea, digestive and to relieve menstrual pain, antispasmodic, scorpion-bite, jaundice.	14, 29.
Micromeria fruticosa (L.) Druce. (labiatae) W5		زعتر بلاط	AP	Anti-inflammatory, for eye infections and headache, abdominal pains and diarrhea, contamination eye, heart disorders, high blood pressure, weariness, exhaustion, colds, coughs, runny noses, wounds, respiratory system.	7, 57, 69. 18.
Micromeria nervosa (labiatae) W60		زعتر ناعم	LF	Anti-inflammatory effects, for infections and headache.	8.
Nerium oleander (L.) (Apocynaceae) W110	Oleander	دفله	FL, LF	Antidote, antibacterial, antileprotic, anticancer, cardiotonic and CNS depressant, pregnanes and triterpenes, treat dog bites, Jaundice, weak heart, internal bleeding, bone fractures, delayed menstruation, Eczema and skin irritation,	57, 41, 53.
Notobasis syriaca (L.) Cass. (Compositae, Asteraceae) W38	Syrian thistle	خرفيش الكبير	YB		
Ononis sicula Guss. (Leguminosae) W78	Spiny Restharrow	شبرق (وسم)	WP		
Papaver rhoeas (L.) (Papaveraceae) W89	Common poppy	خشخاش	AP	Poisonous, pectoral,expectorant, CNS and musculotropic depressant, cough, eye infections, sedative, measles, children's fever, antitussive, soporific, emollient.	17, 22, 45, 25.
Parietaria diffusa (Mert. & Koch.) (Urticaceae) W48		عشبة الدم	AP	To stop bleeding from fresh skin wounds, vulnerary, diuretic and depurative, vermifuge, antitussive, sedative incases of intestinal colic, hemorrhoid lentitive, antiecchymotic, resolvent for skin inflammation.	8, 25, 57.
Paronychia argentea (Lam.) (Caryophyllaceae) W3	Silvery Whittle-wart	الماسة	AP	Treatment of diabetes, blindness, heart pains, kidney stones, edema,	7, 79, 57.
Persea gratissima Gaertn (Lauraceae) W108		ابو كادو	S		
Petroselinum sativum Hoffm. (Umbelliferae) W56	Parsley	بقدونس	WP	Gastronomic use, digestive, hypotensive,renal lithiasis, carminative, diuretic, emmenagogue, Urination, intermittence and prostate disorders.	7, 14, 12.
Phagnalon rupester (L.) DC. (Compositae) W107	African Fleabane	قديح	AP	To make deliberate burns, to treat asthma, anesthetic for toothache, to treat headache, to induce burns and as tinder, urinary system and stones.	7, 57, 8, 32, 58, 77, 28,

Table 2.1/ continues

Phlomis viscosa Poir. (Labiatae) W25	Jerusalem sage	ركاب الجمل	YB		
Phragmites australis (Cav.) Trin. (Labiatae, Lamiaceae)W116	Reed	قصاب	FL		
Pinus halepensis Mill. (Pinaceae) W102	Aleppo Pine	صنوبر	LF	Antirheumatic fever, expectorant, diuretic, antiseptic, for wounds antidiabetic, for bronchitis, tuberculosis, skin abces.	13, 17, 45,52.
Pistacia Lentiscus (L.) (Anacardiaceae) W4	Mastic, Lentisk	سريس	LF	For stomachal pains, migrain, analgesic, sedative in gastralgia, facilitate child birth, for fever, protective covering for wounds, breeth freshener, treat chest pain, expectorant, skin infections, haircare, for diarrhea in children, could be masticated to sweeten breath, stimulant, diuretic, swelling, for gastro-intestinal disorders, anti-inflammatory, aid to minstruation, magic, eardiac stimulant, astringent, fever, kidney stones, muscle paralysis, sore throat, mucus in the respiratory tract, Eczema,	35, 22, 7, 57, 78, 63, 52, 43.
Portulaca oleracea L. (Portulacaceae)W113	Purslane	بقلة	AP		
pyrus syriaca Boiss (Rosaceae) W53	Pear	اجاص بري	LF, FL		
Quercus calliprinos L. (Fagaceae) W26	Kermes Oak	بلوط	R	Urination decrease, skin disorders, as astringent, homeostatic agent, ulcers, heart pains, coughs, digestive system	35, 32, 7, 57.
Reseda alba L. (Resedaceae) W21	Mignonette	حصادي	WP	Insect repellant, soothing inflamed eye and sour throat, anti-inflammation, treat inflamed eyes, antirheumatic, treat infertility, treat paralysis, analgesic, treat stomach –ache back ache, gale abortive, toxic, skin diseases, antipuritic, abdominal pains, arm and leg paralysis, female infertility, rheumatism,	
Retema raetam (Fossk.) Webb. (Papilionaceae) W32	Ratame	رتم	LF		9, 17, 24, 32, 42, 57, 45, 79,
Rhus coriaria L. (Anacariaceae) W106	Sumac	سماق	AP	Wounds, burns, bronchiis, against excessive sweeting of the feet, Astringent, anti-dysentery, stops bleeding, spice, treat gastric ulcer, for mouth ulcers. Treat abdominal pain, swollen legs and poor circulation, tooth and gum aches,	13, 45, 80, 57, 14, 52.
Ricinus communis L. (Euphorbiaceae) W57	Castor Comunis	خروع	AP	For intestinal obstruction due to constipation, feverish, headache, horn cancer. Skin diseases	7, 61, 52, 4.
Rosa centifolia L. (Rosaceae) W98	Rose	ورد جوري	FL		
Rosmarinus officinalis L. (Labiatae) W112	Rosemary	حصى لبان	AP	For common cold and purgative, diuretic and cough, antiseptics for the circumcision wound, relaxation, gastronomic, antispasmodic and spice, urinary system.	52, 14, 12, 7.
Rubia tenuifolia D'urv. (Rubiaceae) W52	Wild Madder	فوه	AP	Diuretic activity.	71.

Table 2.1/ continues

Ruscus aculeatas L. (Liliaceae) W33	Butcher's broom	عجرم	RT	Diuretic, stop bleeding, depurative, anti-arthritic, vasoconstrictive. Kidney stones, edema,	13,22, 57, 30, 31, 46, 60.
Ruta chalepensis L. (Rutaceae) W103	Rue	فيجن	AP,FL,	Anti-rheumatic, against abdominal colic, for snake bites, aphrodisiac for headaches, wounds, anti-spasmodic, diuretic, sedative, analgesic, anti-inflammatory, diarrhea, dysentery, colic, stomach pains, constipation, emetic, laxative gastritis, enterocolitis, snake bites, head lice, Abdominal pains, earaches, strained eyes, fever and headaches, poor blood circulation, Kidney stones, local paralysis, nervous tension general pain insanity, coughs and asthma, rheumatism, skin disorders, perinatal toxicology, antipyretic, analgesic and CNS depressant activities.	7,57, 11, 35, 45, 21, 74, 75, 3, 83, 68.
Saccharum ravennae (L.) Murray (Labiatae, Lamiaceae) W99	Wild cane	قصاب	FL		
Salvia dominica L. (Salvadoraceae) W49	Sage	خويخه	FL		
Salvia fruiticosa L. (Labiatae) W30	White sage	ميرميه	LF	Against bronchial affections, headache, antitussive, for cystitis, digestive, hepatoprotectant, hypotensive, inrheumatic arthritis, as dentifrice, treatmant of diabetes, Anti-inflammatory gargle, antiseptic, anti-haemorrhoids pains, ulcer pains, colds and coughs,	58, 7, 57, 79,23, 14.
Salvia hierosolymitana Boiss. (Labiatae) W55	Jerusalem Sage	لسينه	LF		
Sarcopoterium spinosum (L.) Sp. (Rosaceae) W10	Shruppy bamet	بولان	AP	Treatment of diabetes, diuretic, useful in renal calculi, anti-inflammatory, for haemorrhoids, abdominal pains and indigestion, poor blood circulation, edema, external inflammation, toothache.	7, 57, 79, 45, 32,
Satureja thymbra (L.) (Labiatae) W91		ندغ البساتين	AP, FL	Fungicide, constipation and abdominal pains, heart pains, swollen legs, poor blood circulation, edema, swollen legs, stress, paralysis, weariness, exhaustion, dizziness, mucus in respiratory tract, rheumatism, open wounds, toothache.	69, 57. 40.
Scabiosa prolifera L. (Dipsacaceae) W73	Morning bride	ثليجه	AP		
Scolymus maculatus L. (Compositae, Asteraceae) W74	Spotted Golden Thistle	سناريه	YB		
Silene vulgaris (Moench) Garcke (Caryophyllaceae) W16	Rattlebox	فقيع	AP		
Sinapis arvensis L. (Scrophulariaceae) W20	Mustard	خردل	WP		
Sonchus oleraceus L. (Solanaceae) W45	Sow thistle	علك خيل	WP		
Spinacia olecarcea		سبانخ	WP		

Table 2.1/ continues

Styrax officinalis L. (Styracaceae) W44	Snow bell	عبهر	YB		
Trigonella foenumgraecum L. (Papilionaceae) W18	Fenugreek seed	حلبه	AP	Stomach disorders, dysentery, to treat boils, abscesses and carbuncles, antispasmodic, galactagogue and malaria, hypertension, kidney disorders,stones, circumcision, for nephritis, diabetes, abdominal pains and food poisoning, open wounds,	7, 29, 14, 57.
Urtica pilulifera L. (Urticaceae) W41	Nettle	قراص داعري الساق	WP, S	Anti-inflammatory, for cystitis, digestive tract, antiasthmatic, against hair loss antiodema. Hemorrhaging, edema, weariness, exhaustion, pains in muscle and leg, male impotence, arthritis, open wounds, infected wounds, pain around wounds, rheumatism and arthritis.	7, 14, 57.
Urtica urens (Urticaceae) W9	Nettle	قراص رباعي الساق	WP		
Varthemia iphionoides Boiss&Blanche (Compositae) W34	Common varthemia	كتيله	AP	Stomach ache, eye ailments, edema,	32,57.
Verbascum sinuatum (L.) (Scrophulariaceae) W64	Mullein	عورور	LF, FL, YB, RT	Used for neuralgic pain, gastric disturbance and bronchitis, emollient, anti-inflammatory, soothing inflamed eye, anti-rheumatism for ophthalmic infections.	17, 45.
Vicia faba L.. (Papilionaceae) W90	Broad Bean	فول	AP	For hypertension, heart failure, renal failure, liver cirrhosis, increase diuresis, natriuresis and otorrhoea and prostate disorders.	27, 7.
Viscum cruciatum sieber et. Boss. (Linaceae) W105	Mistletoe	هدال	LF	Tumor inhibition, anti-spasmodic, anti-hypertensive, diuretic, Cytotoxic against larynx cancer cells.	7, 45, 64.
Ziziphus spina-christi (L.) (Rhamnaceae) W29	Syrian christ thorn	سدر	LF	Treat blisters, bruises, chest pains, dandruff, fractures, headache, mouth and gum problems, laxative, pectoral, nutritive, to cure toothache, astringent, anti-diarrhoetics, fermifuges, anti-inflammatory (eye wash) analgesic, pectoral, anti-rheumatic, purgative, stomach pain antihelmantic, back ache, arthritis, gums, joints, skin disorders, abdominal pains, constipation, intestinal parasites, rheumatism, open wounds, boldness.	17, 57, 45, 39,82.

* AP, aerial parts; FL, flowers; WP, whole plants; RT, roots; LF, leaves; B, bulb; YB, young branches; S, seeds.

[1] Abu Zarga *et al.*, 1998; [2]Ageel *et al.*, 1986; [3]Ageel *et al.*, 1989; [4]Ali, 1999; [5] Ali & Grever, 1998; [6]Ali *et al.*, 1995; [7] Ali-Shtayeh *et al.*, 2000; [8] Ali-Shtayeh *et al.*, 1997; [9]Ali-Shtayeh *et al.*, 1998; [10] Al-Said *et al.*, 1988; [11] Al-Said *et al.*, 1990; [12] Alkofahi et al., 1997; [13] Al-Wareh *et al.*, 1993; [14]Amico & Sorce, 1997; [15]Amoros *et al.*, 1988; [16]Barel & Yashphe, 1991; [17] Bellakhdar *et al.*, 1991; [18] Bellino &Marceno, 1981; [19]Benayache *et al.*, 1991; [20]Bhat & Jacobs, 1995; [21] Caceres *et al.*, 1990; [22] Chieji, 1984; [23] Conigueral *et al.*, 1989; [24] Dafni & Yanive, 1994; [25] Defeo *et al.*, 1991; [26] Disi *et al.*, 1998; [27] Dutta & Nath, 1998; [28] El-Damy *et al.*, 1994; [29] El-Kamali & Khalid, 1998; [30] Elsholy *et al.*, 1975; [31] Facino *et al.*, 1995; [32] Friedman *et al.*, 1986; [33] Gadgoli & mishra, 1999; [34]Garcia *et al.*, 1999; [35] Ghazanfar, 1994; [36] Grande *et al.*, 1992; [37] Gribanovski-sassu *et al.*, 1969; [38]Guarrera, 1999; [39] Halaska *et al.*, 1998; [40] Haykel & Omar 1988; [41] Huq *et al.*, 1998; [42] Hussain, 1995; [43]Hussain, 1997; [44]Kandil *et al.*, 1994; [45] Karim &Quraan, 1986; [46] Karting *et al.*, 1991; [47] Kinghorn & Balandrin, 1993; [48] Kizu *et al.*, 1995; [49] Lisciani *et al.*, 1984; [50] Manadhar, 1991; [51] Manez *et al.*, 1999; [52]Merzouki & Ed-Derfoufi, 1997; [53]Mostaqul Huq *et al.*, 1999; [54] Murata &Takahashi, 1984; [55] Okzuz, 1976; [56] Ong, & Norzalina, 1999; [57] Palevitch *et al.*, 1984; [58] Palevitch & Yaniv, 1991; [59] Qureshi *et al.*, 1991; [60] Rauwald & Grunwid, 1991; [61]Reddy et al., 1998; [62]Rimbau *et al.*, 1999; [63] Rios *et al.*, 1987; [64]Saenz *et al.*, 1997; [65] Sakai *et al.*, 1992; [66]Seaforth *et al.*, 1998; [67] Schauenberg, 1990; [68]Shah *et al.*, 1991; [69] Shimoni *et al.*, 1993; [70] Shoji, *et al.*, 1994; [71]Silva & Abraham, 1981; [72] Suleiman *et al.*, 1988; [73] Thapliyal & Bahuguna, 1993; [74] Ulubelen et *al.*, 1994; [75] Ulubelen *et al.*, 1988; [76] Uniyal & sato, 1992; [77] Viollon & Chaumant, 1994; [78] Wyllie *et al.*, 1990; [79]Yanive *et al.*, 1987; [80] Yesilada *et al.*, 1993; [81] Yoshida *et al.*, 1995; [82] Yuan *et al.*, 1987; [83] Zeichen de Sa *et al.*, 2000.

** Plants were collected and identified under the supervision of Prof. M. S. Ali-Shtayeh by Walid Khaleeliah, Suheil Abu-Ghdeib, Reem Yaghmour, Rabee Zayed, Rana Jamous, Abdel Rahman Salameh, Kamal Kamel, and Tayseer Khalid, An-Najah National University, Nablus

2.1.1.2 Extraction

100g of both dry powdered plant and some fresh plants were infused in 70% ethanol until complete exhaustion (usually 1:5 w/v ratio) for 72h at room temperature with periodic shaking. The extract was then filtered twice through Whatman filter paper No.1, and the filtrates were evaporated under reduced pressure and dried using a rotary evaporator at 60°C. Dried extracts were stored in labeled sterile bottles at –20 °C (Ali-Shtayeh et al., 1997; Ali-Shtayeh & Abu Ghdeib, 1998; Kandil *et al.*, 1994).

2.1.1.3 Preparation of stock solution

Extracts stock solution was prepared by dissolving 10mg from the above powdered extracts in 1ml of 100% dimethyl sulfoxide (DMSO) and kept in labeled eppendorf tubes at –20°C for further use.

2.1.1.4 Preparation of medicinal plant library (MPL)

250µl, U-shape and transmissible 96 microwell-plates were prepared for the test as follows:

1. Each well was filled with 180µl of 10% DMSO.
2. 20µl from stock solution (10 mg/ml) was added into 180µl of 10% DMSO in order to obtain the first drug concentration of 1mg/ml.

3. 20µl of this drug was added into another well in order to obtain the second drug concentration of 100µg/ml (Figure 2.1).

4. The 96 microwell-plate were filled with different drugs.

5. The last column was filled with 10% DMSO only without any drug as a control (Figure 2.1).

6. Each plate was covered, labeled, and kept at -20C° for further study.

MPL-1	1	2	3	4	5	6	7	8	9	10	11	12 **	
A	W1*	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	DMSO	10µl from stock into 180µl 10%DMSO
B	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	DMSO	1:10 dilution from A
C	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22	DMSO	10µl from stock into 180µl 10%DMSO
D	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22	DMSO	1:10 dilution from C
E	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	DMSO	10µl l from stock into 180µl 10%DMSO
F	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	DMSO	1:10 dilution from E
G	W34	W35	W36	W37	W38	W39	W40	W41	W42	W43	W44	DMSO	10ul from stock into 180µl 10%DMSO
H	W34	W35	W36	W37	W38	W39	W40	W41	W42	W43	W44	DMSO	1:10 dilution from G

Figure 2.1 Medicinal plant library (MPL)

*Extract number, ** Control column. 10% DMSO

2.1.2 Anti proliferation assay

2.1.2.1 Cell lines

The following human cell lines were used in the present study: prostate adenocarcinoma PC-3, prostate adenocarcinoma LNCaP and breast

adenocarcinoma MCF-7 (Table 2.2). These cell lines were obtained from American Type Culture Collection (ATCC, Rockville, Md.USA).

Table 2.2 Cell lines used in this study with their media and supplements.

Cell line	Cell type	Media	Supplements	ATCC No.	References
PC-3	Prostate (And -)	Minimum Essential Medium With Earle's Salts. With L-Glutamine.	- 10% Fetal Calf Serum (FCS). - 1% Penicillin-Streptomycin (10000 IU/ML -10000 UG/ML).	CRL-1435	Bahk et al., 1998; Halicka et al., 1997; Eilon et al., 2000
LNCaP	Prostate (And +)	RPMI-1640 Medium With L-Glutamine.	- 10% Fetal Calf Serum (FCS). - 1.5 g/L Sodium bicarbonate. - 1mM Sodium pyruvate, 90%. - 10 mM HEPES. - 4.5 g/L Glucose. - 1% Penicillin-Streptomycin (10000 IU/ML -10000 UG/ML).	CRL-1740	Bahk et al., 1998; Halicka et al., 1997; Eilon et al., 2000; Onozawa et al., 1998
MCF-7	Breast (ER+)	RPMI-1640 Medium With L-Glutamine.	- 10% Fetal Calf Serum (FCS). - 1% Penicillin-Streptomycin (10000 IU/ML -10000 UG/ML). - Insulin 1 UI/ml.	HTB-22	Halicka et al., 1997; Eilon et al., 2000

2.1.2.2 Cells Culture

The three types of cell lines were maintained at 37 °C in a humidified incubator containing 5% CO2 in the air (Eilon *et al.*, 2000; Halicka *et al.*, 1997; Kelner *et al.*, 1998; Bahk *et al.*, 1998). Cells were seeded in 75 cm^2, gamma-sterilized, tissue culture-treated and screw cap with venting position, tissue-culture flasks (TPP, Europe/Switzerland), with 10 ml media for each cell line as in (Table 2.2).

2.1.2.3 Cells harvesting and counting

After 7 days of incubation (cells will be confluent in the flask) the media were aspirated and the cells were harvested with 2ml of 0.05% Trypsin / 0.02% Ethylene Diamine Tetra acetic Acid (EDETA) solution (Onozawa *et al.*, 1998; Castaneda &Kinne, 1999). 10 ml of media was added to the cells immediately after harvesting. 100µl from the cells were stained with 100µl of (0.4%) trypan blue solution and then counted using hemocytometer under an inverted microscope. MCF-7 and PC-3 cells were diluted with media (Table 2.2) to give a concentration of $3x10^4$ cells / 200µl. $4x10^4$ cell / 200µl were used for LNCaP cells in MTT assay.

2.1.2.4 MTT assay

2.1.2.4.1 Preparation of MTT dye

75mg of 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (sigma) were dissolved in 50 ml of RPMI-1640 medium without L-Glutamin, without phenolred (Sigma), and then kept in 50 ml covered and labeled tubes at -20°C for further study (Strom et al., 1999, Xiaoline & Rajesh, 1999).

2.1.2.4.2 Preparation of 96 microwell- plate for MTT assay

LNCaP cells were seeded at $4x10^4$ cell/200µl, but PC-3 and MCF-7 were seeded at $3x10^4$ cells/200µl (Appendix B). The three types of cells were seeded in 96 microwell- plates with $0.31cm^2$ growth area, flat bottom, gamma-sterilized, tissue culture-treated and transmissible (TPP, Europe/Switzerland). The cells were allowed to attach in a 5% CO2 incubator at 37° C for 24 h. After that, 10µl from each plant extract (Figure 2.1) were added to each well using a multi-channel pipette. After 24h incubation for each plate, viable cells were quantitated as follows.

2.1.2.4.3 Quantitation of viable cells

Viable cells were quantitated by 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (MERCK, Germany). In brief 50 µl of MTT solution (1.5mg/ml) was added to each well and left for 3 h incubation, then100 µl of absolute acetone-ethanol solution (1:1) were added to dissolve violet crystals (Srivastava *et al.*, 1998).

Viability was quantitated by measuring *A570* , using a 96 microwell-plate reader with a reference wave length of 650 nm. The percentage of cell survival was determind as (mean *A650* of treated wells/ *A650* of untreated control wells) x100% (Srivastava *et al.*, 1998).

2.2 Anticancer activity of plant extracts fractions

2.2.1 Fractionation Methods

2.2.1.1 Column chromatography

The ethanolic extract of each plant was dissolved in a minimum amount of chloroform-ethanol [1:1] (1g/5ml) and then subjected to column chromatography using silica gel (pore diameter of 60A°, 230-400mesh). The following solvents were eluted as follows [hexane, diethyl ether, acetone, acetonitrile, 80% acetonitrile + 20% ethanol, 60% acetonitrile + 40% ethanol, 40% acetonitrile + 60%ethanol, 20% acetonitrile + 80% ethanol, absolute ethanol, 70% ethanol and deionized water] for each extract (four fractions for each solvent were collected). The fractions were collected and the solvents were evaporated using a rotary evaporator at 55°C and a freeze drier. The final powdered material was stored in labeled sterile bottles for further use.

2.2.2 Preparation of stock solution

Stock solution fractions was prepared by dissolving 10mg from the above mentioned powdered fractions in 1ml of 100% dimethyl sulfoxide (DMSO) in order to obtain a final concentration of 10 mg/ml and then kept in labeled eppendorf tubes at –20° C for further use.

2.2.2.1 Preparation of medicinal plant library (MPL)

250µl, U-shape and transmissible 96 microwell-plates were prepared for the test as with whole extract mentioned before. Each plate was covered, labeled, and then kept at -20°C for further use (Figure 2.1).

2.2.3 Antiproliferation assay

Antiproliferation assay for the fractions prepared above were carried out as described previously.

2.3 Statistical analysis

The data were analyzed and the treatment were compared using analysis of variance (ANOVA) obtained by Duncan's multiple-range test ($P < 0.05$).

CHAPTER THREE

RESULTS

CHAPTER THREE

RESULTS

3.1 In vitro cell toxicity of the ethanolic extracts of Palestinian plants

The results of cytotoxic activity *in vitro* testing of 115 ethanolic extracts of 96 Palestinian plants (Table 2.1) against two prostate cancer cell lines and one breast cancer cell line are illustrated in Appendix A. The study has demonstrated that nine of the studied plants 9/96 (10%) (Appendix A) are potentially important sources of anti-cancer agents > 40% inhibition.

3.1.1 Cytotoxic activity of ethanolic extracts against hormone refractory prostate cancer cell line (PC-3)

The inhibitory effect against the cell line varied (10% - 95% inhibition) significantly between plants (Appendix A). Extracts of *Cyclamen persicum, Lycium europeum, Ecballium elaterium, Euphorbia hierosolymitana, Anthemis tunictoria, Verbascum sinuatum, and Nerium oleander* were the most active extracts (>40% inhibition) (Table 3.1& Figure 3.1). These extracts (Table 3.1) differed significantly in their activity ($F = 33.281$, $DF = 10$, $p < 0.01$). Extracts of *Cyclamen persicum, Euphorbia hierosolymitana* and *Verbascum sinuatum* were more active (> 70% inhibition). Other extracts showed inhibitory effect of less than 40% inhibition (Appendix A).

At the low concentration (5μg/ml) only *Cyclamen persicum* and *Capparis spinosa* extracts gave anticancer activity of >40% inhibition (Appendix A).

Another important observation was the stimulation of PC-3 cell line growth by some plant extracts (Appendix A). These extracts, with their respective stimulation, are obtained from *Parieteria diffusa* (68%); *Conium maculathum* (40%); *Retema raetam* (60%); *Salvia fruiticosa* (42%); *Ziziphus spina-christi* (70%); and *Crataegus aronial* (62%).

3.1.2 Cytotoxic activity of ethanolic extracts against hormone sensitive prostate cancer cell line (LNCaP)

The inhibitory effect against the cell line varied (10% - 91% inhibition) significantly between plants (Appendix A). Extracts of *Cyclamen persicum, Verbascum sinuatum,* and *Capparis spinosa* were the most active extracts (>40% inhibition) (Table 3.1& Figure 3.1). These extracts (Table 3.1) differed significantly in their activity ($F = 14.182$, $DF = 10$, $p < 0.01$). With the extract of *Cyclamen persicum* corms gave the highest activity (> 90% inhibition). The other extracts showed lower activity with less than 40% inhibition (Appendix A).

Extracts of *Cyclamen persicum* corms were more active (> 90% inhibition) compaired with the aerial parts extracts (Between 40% and 70%inhibition) (Table 3.1& Figure 3.1).

At low concentration (5µg/ml) only *Cyclamen persicum* and *Capparis spinosa* extracts gave anticancer activity of > 40% inhibition (Appendix A).

Another important observation was the stimulation of LNCaP cell line growth by *Chrysanthemum cornarium* extract (Appendix A).

3.1.3 Cytotoxic activity of ethanolic extracts against hormone sensitive breast cancer cell line (MCF-7)

The inhibitory effect against the cell line varied (10% - 96% inhibition) significantly between plants (Appendix A). Extracts of *Cyclamen persicum, Lycium europeum, Euphorbia hierosolymitana,* and *Ononis sicula,* gave anticancer activity of >40% inhibition (Table 3.1& Figure 3.1). These extracts (Table 3.1) differed significantly in their activity ($F = 9.820$, $DF = 10$, $p < 0.01$). Extracts of *Cyclamen persicum* and *Ononis sicula* were the most active (> 70% inhibition). Other extracts showed inhibition effect less than 40% inhibition (Appendix A).

At low concentration (5µg/ml) only *Cyclamen persicum* extract gave anticancer activity of >40% inhibition (Appendix A).

Another important observation was the stimulation of MCF-7 cell line growth by *Quercus calliprines* extract (Appendix A).

3.2 *In vitro* cell toxicity of active ethanolic plant extracts and their fractions

The results of cytotoxic activity *in vitro* testing of 9 active plant extracts and their fractions (Table 3.1) against two prostate cancer cell lines (PC-3 and LNCaP) and one breast cancer cell line (MCF-7) are presented in Tables 3.1 - 7, and Figures 3.1 – 7.

Table 3.1 Cytotoxic activity* of ethanolic extracts** of Palestinian plants in vitro

Scientific Name	Parts used***	Mean cell inhibition ± SD.					
		PC-3		MCF-7		LNCaP	
		AVE.	SD	AVE.	SD	AVE.	SD
Lycium europeum	AP	42.67efghij	5.51	56.33 bcdefg	18	28 defghijk	1
Ecballium elaterium	AP	52.67efghi	5.51	39.67defghijk	12.7	27.7defghijk	12.4
Cyclamen persicum	RT	94.67a****	3.51	95.67a	2.31	90.3 a	3.06
Euphorbia hierosolymitana	WP	76 bcd	9.85	45.67cdefghijk	5.51	31 cdefghijk	11.3
Cyclamen persicum	AP	69 bcd	7.81	63 bcde	10.4	23.3defghijk	7.51
Anthemis tunictoria	RT	44.67 efghij	2.31	29.67 efghijk	11.9	35 cdefghijk	11
Verbascum sinuatum	FL (dry)	44 efghij	1	33 efghijk	3.61	26 defghijk	10
Capparis spinosa	FL	27.67 jk	3.51	28.67 k	11.6	46.3 cdefg	7.57
Ononis sicula	WP	37.33 fghijk	6.43	72 bcd	14.7	31 cdefghijk	14.5
Nerium oleander	LF	46.67efghij	9.29	35 bcdefgh	5.57	34 cdefghijk	1.15
Verbascum sinuatum	FL(fresh)	77.5 bcd	6.5	39.67 defghijk	17.5	63.7 b	11

* mean of three replicate micro-well. ** concentration= 50 microgram / ml. *** AP, aerial parts; FL, flowers; WP, whole plants; RT, roots; LF, leaves. ****Values in the same column followed by the same letter were not significantly different based on Duncan's multiple-range test ($p<0.05$)

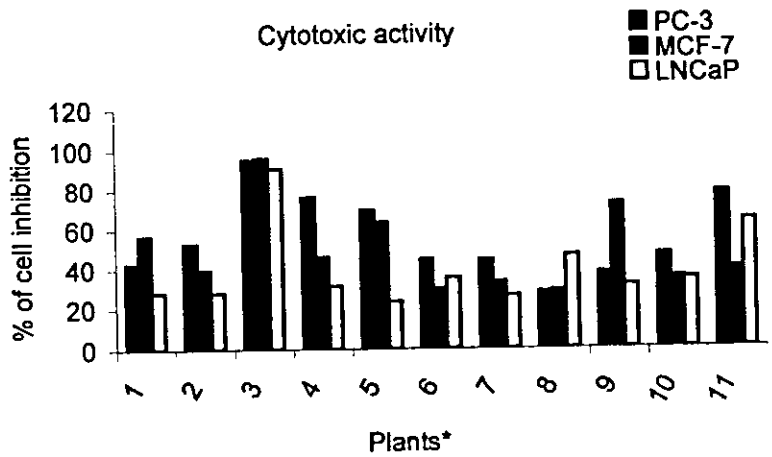

Figure 3.1 Anticancer activity of ethanolic extracts of selected Palestinian plants.
*1.*Lycium europeum, 2.Ecballium elaterium, 3.Cyclamen persicum* (corms), *4.Euphorbia hierosolymitana, 5.Cyclamen persicum* (aerial parts), *6.Anthemis tunictoria, 7.Verbascum sinuatum* (dry), *8.Capparis spinosa, 9.Ononis sicula, 10.Nerium oleander, 11.Verbascum sinuatum* (fresh).

3.2.1 *In vitro* cell toxicity of *Cyclamen persicum* (Corms) against PC3, LNCaP, and MCF-7 cell lines

Cyclamen persicum showed anticancer activity against the test cell lines with an inhibition of > 90% (Table 3.1). The three cell lines did not show any comparable result ($F = 2.679$, $DF = 2$, $p > 0.05$).

3.2.1.1 *In vitro* cell toxicity of fractions of *Cyclamen persicum* (Corms) against PC-3, LNCaP, & MCF-7 cell lines

The inhibitory effect against the three cell lines varied (10% to 99% inhibitions) significantly between *Cyclamen persicum* fractions. Diethyl ether and 70% ethanol fractions were the most active (> 80%inhibition). The other

solvent (used in the test) fractions showed inhibition effect less than 40% (Table 3.2 & Figure 3.2).

Table 3.2 In *vitro* cytotoxic activity of fractions of *Cyclamen persicum* against PC-3, LNCaP, and MCF-7 cell lines

Mean cell inhibition ± SD							
Fraction No.	Cancer cell line	PC-3		MCF-7		LNCaP	
	Name of the solvent	AVE	SD	AVE	SD	AVE	SD
F1	n-hexane	-11	33.23	1.5	14.85	-0.5	13.44
F2	n-hexane	-8.5	27.58	8	1.414	-3	5.657
F3	n-hexane	-9	14.14	-16	10.61	2	1.414
F4	n-hexane	-4	45.25	-15	2.828	-60	36.77
F5	n-hexane	-19	28.99	0	16.97	-24	14.85
F6	Diethyl Ether	-16	13.44	-7.5	0.707	-14	24.04
F7	Diethyl Ether	-7	7.071	2.5	2.121	7	21.21
F8	Diethyl Ether	33	11.31	6.5	12.02	39	35.36
F9	Diethyl Ether	98	11.31	93	1.414	89.5	3.536
F10	Acetone	14.5	34.65	-3	2.828	-10	11.31
F11	Acetone	23	46.67	-4.5	4.95	-1	2.828
F12	Acetone	-9.5	16.26	-11	7.071	-14	28.99
F13	Acetone	8	8.485	-7	15.56	-6.5	13.44
F14	Acetonitrile	-22	43.84	-12	9.899	4.5	2.121
F15	Acetonitrile	-3.5	24.75	2.5	6.364	5.5	2.121
F16	Acetonitrile	6.5	10.61	-7	5.657	-6	19.8
F17	70% ethanol	99	7.778	94.5	0.707	86.5	0.707
F18	70% ethanol	94.5	0.707	93.5	2.121	78	8.485
F19	70% ethanol	95	1.414	91	8.485	88.5	0.707
F20	70% ethanol	93	0	94.5	2.121	89	12.73

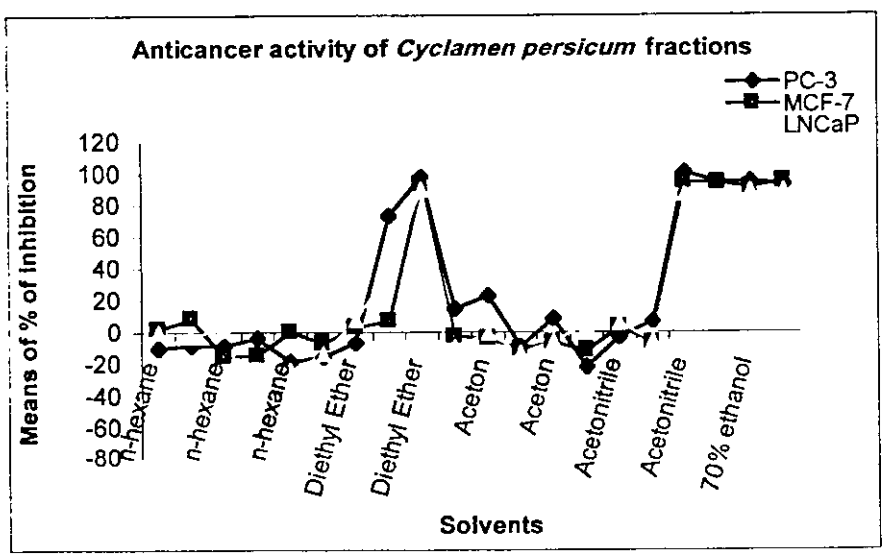

Figure 3.2 *In vitro* cytotoxic activity of fractions of *Cyclamen persicum* against PC-3, LNCaP, and MCF-7 cell lines

3.2.2 *In vitro* cell toxicity of *Lycium europeum* against PC-3, LNCaP and MCF-7 cell lines

Lycium europeum showed anticancer activity against the test cell lines with a mean percent of inhibition > 20 % (Table 3.1). The three cell lines differed significantly in their inhibition ($F = 5.081$, $DF = 2$, $p < 0.05$). The most affected cell lines were PC-3 and MCF-7 with percent inhibition of > 40% (Table 3.1).

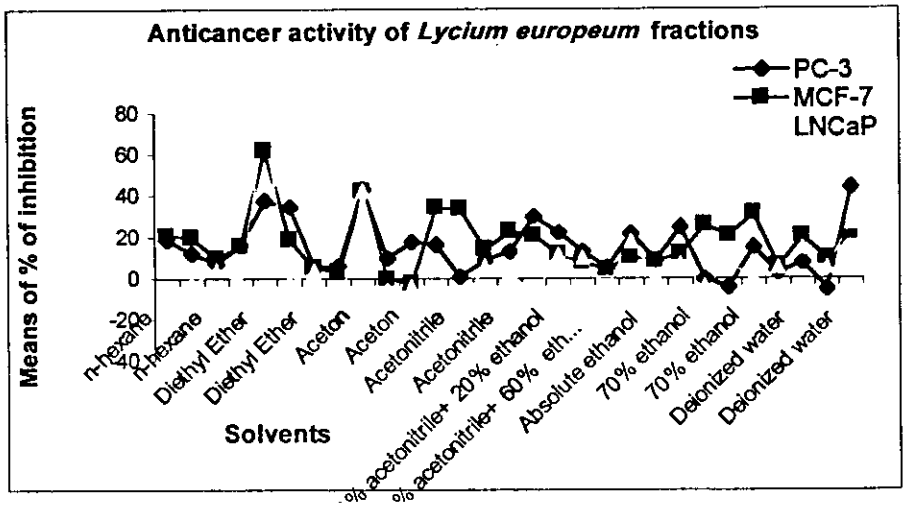

Figure 3.3 *In vitro* cytotoxic activity of fractions of *Lycium europeum* against PC-3, LNCaP, and MCF-7 cell lines

3.2.2.1 *In vitro* cell toxicity of fractions of *Lycium europeum* against PC-3, LNCaP and MCF-7 cell lines

The inhibitory effect against the three cell lines varied (10% -74% inhibition) significantly between *Lycium europeum* fractions, with acetone

and diethyl ether fractions were the most active (> 40% inhibition). The other fractions showed inhibition effect less than 40% (Table 3.3 & Figure 3.3).

Diethyl ether fraction was the most active fraction (> 62% inhibition) against LNCaP and MCF-7 cell lines, but it was less active against PC-3 cell line (> 38% inhibition). The acetone fraction showed comparable activities (> 42% inhibition) against the three cell lines (Table 3.3 & Figure 3.3).

Table 3.3 In vitro cytotoxic activity of fractions of *Lycium europeum* against PC-3, LNCaP, and MCF-7 cell lines

Mean cell inhibition ± SD							
Fraction No.	Cancer cell line	PC-3		MCF-7		LNCaP	
	Name of the solvent	AVE.	SD	AVE	SD	AVE.	SD
F1	n-hexane	18.5	10.61	20.5	27.58	-19	24.75
F2	n-hexane	12	8.485	19.5	24.75	-3	5.657
F3	n-hexane	7	12.73	9.5	10.61	4	2.828
F4	n-hexane	16	5.657	15.5	12.02	-2	14.14
F5	Diethyl Ether	37.5	2.121	61.5	13.44	73.4	27.79
F6	Diethyl Ether	34.5	3.536	18.5	3.536	-2	39.6
F7	Diethyl Ether	5.5	14.85	5	5.657	3.5	2.121
F8	Diethyl Ether	6	15.56	3	7.071	-19	7.778
F9	Acetone	43	4.243	56	12.4	42.5	16.26
F10	Acetone	9.5	6.364	-0.5	3.536	-6	14.14
F11	Acetone	17.5	0.707	-3	2.828	-4.5	10.61
F12	Acetone	16.5	2.121	20	11.01	-15	24.04
F13	Acetonitrile	0.5	3.536	14	10.12	-15	26.16
F14	Acetonitrile	8.5	0.707	14	14.14	6	15.56
F15	Acetonitrile	13	7.071	22.5	24.75	3	18.38
F16	Acetonitrile	30	1.414	20.5	9.192	-9	21.21
F17	80% acetonitrile+20% ethanol	22	1.414	11.5	6.364	11	21.21
F18	60% acetonitrile+40% ethanol	13	2.828	7.5	2.121	9.5	16.26
F19	40% acetonitrile+60% ethanol	5	21.21	4	0	-5	14.14
F20	20% acetonitrile+80% ethanol	21.5	0.707	10	5.657	18	9.899
F21	Absolute ethanol	8.5	0.707	8	2.828	1	5.657
F22	Absolute ethanol	24.5	10.61	11.5	24.75	6.5	26.16
F23	70% ethanol	-0.5	3.536	25.5	19.09	-4	26.87
F24	70% ethanol	-4.5	7.778	20.5	2.121	6.5	26.16
F25	70% ethanol	15	4.243	31	32.53	0.5	26.16
F26	70% ethanol	2.5	21.92	5.5	0.707	5	22.63
F27	Deionized water	7.5	16.26	20	26.87	14.5	10.61
F28	Deionized water	-5.5	21.92	9.5	17.68	5	16.97
F29	Deionized water	33	18.38	21.5	6.364	26.5	10.61

Table 3.4 *In vitro* cytotoxic activity of fractions of *Verbascum sinuatum* against PC-3, LNCaP, and MCF-7 cell lines

Fraction No.	Mean cell inhibition ± SD						
	Cancer cells	PC-3		MCF-7		LNCaP	
	Name of the solvent	AVE	SD	AVE	SD	AVE	SD
F1	n-hexane	-0.5	19.09	14	12.73	26	2.828
F2	n-hexane	2	1.414	14.5	14.85	1.5	20.51
F3	n-hexane	7.5	17.68	12.5	10.61	11	18.38
F4	n-hexane	1	15.56	6	14.14	29	11.31
F5	Diethyl Ether	15	17.3	41.5	4.95	76	28.28
F6	Diethyl Ether	12	16.25	12	4.243	12.5	16.26
F7	Diethyl Ether	11	14.32	11.5	9.192	12	18
F8	Diethyl Ether	7	14.5	9.5	20.51	8.5	0.707
F9	Acetone	86.5	10.61	58	11.31	90.5	16.26
F10	Acetone	8	16.06	21	24.04	18	28.28
F11	Acetone	6	15.36	14	16.97	-1.5	14.85
F12	Acetone	-19	5.657	17.5	17.68	12.5	10.61
F13	Acetonitrile	-15	0.707	6	11.31	10.5	17.68
F14	Acetonitrile	4.5	16.26	10	14.14	4.5	21.92
F15	Acetonitrile	-3	9.899	10.5	28.99	11	5.657
F16	Acetonitrile	3	16.06	6.5	3.536	-0.5	10.61
F17	80% acetonitrile+20% ethanol	86.5	10.61	75	5.657	57.5	14.85
F18	60% acetonitrile+40% ethanol	89	8.485	95.5	6.364	87	4.243
F19	40% acetonitrile+60% ethanol	97.5	3.536	99	2.121	88.5	4.95
F20	20% acetonitrile+80% ethanol	18.5	17.58	17	4.243	29	35.36
F21	Absolute ethanol	33	15.25	35	4.243	49	11.31
F22	Absolute ethanol	6.5	6.364	29.5	9.192	32.5	7.778
F23	70% ethanol	3.5	21.82	12	2.828	26.5	2.121
F24	70% ethanol	-14	0.707	18.5	12.02	11	12.73
F25	70% ethanol	3.5	17.68	20.5	20.51	6.5	21.92
F26	70% ethanol	6	11.31	5	2.828	3	22.63
F27	Deionized water	6	20.41	8	2.828	-7.5	24.75
F28	Deionized water	19	4.243	8	9.899	3.5	24.75
F29	Deionized water	11	8.485	12.5	2.121	12	22.53

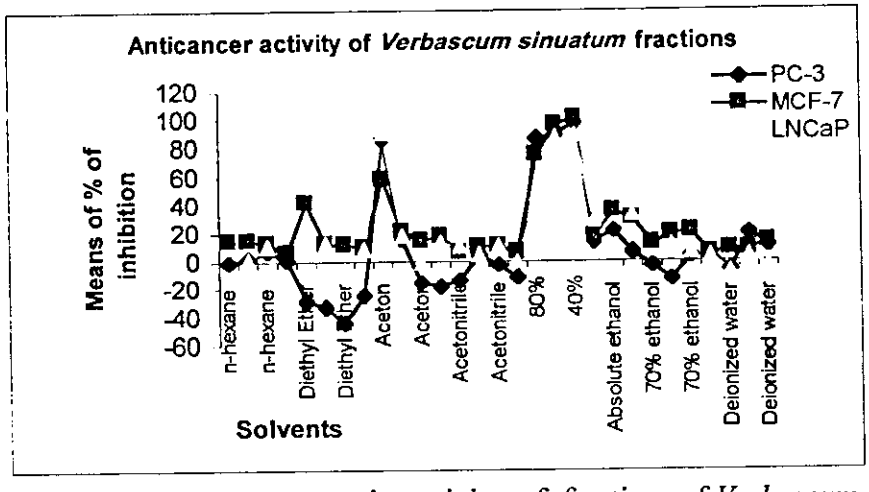

Figure 3.4 *In vitro* cytotoxic activity of fractions of *Verbascum sinuatum* against PC-3, LNCaP, and MCF-7 cell lines

3.2.4 *In vitro* cell toxicity of *Nerium oleander* against PC3, LNCaP and MCF-7 cell lines

Nerium oleander showed anticancer activity against the test cell lines with a mean percent inhibition of > 30% (Table 3.1). The three cell lines did not differ significantly in their inhibition ($F = 3.649$, $DF = 2$, $p > 0.09$). The most affected cell line was PC-3 with a mean percent inhibition of 46.7% (Table 3.1).

3.2.4.1 *In vitro* cell toxicity of fractions of *Nerium oleander* against PC-3, LNCaP and MCF-7 cell lines

The inhibitory effect against the three cell lines ranged (10% - 75% inhibition) significantly between *Nerium oleander* fractions. Diethyl ether,

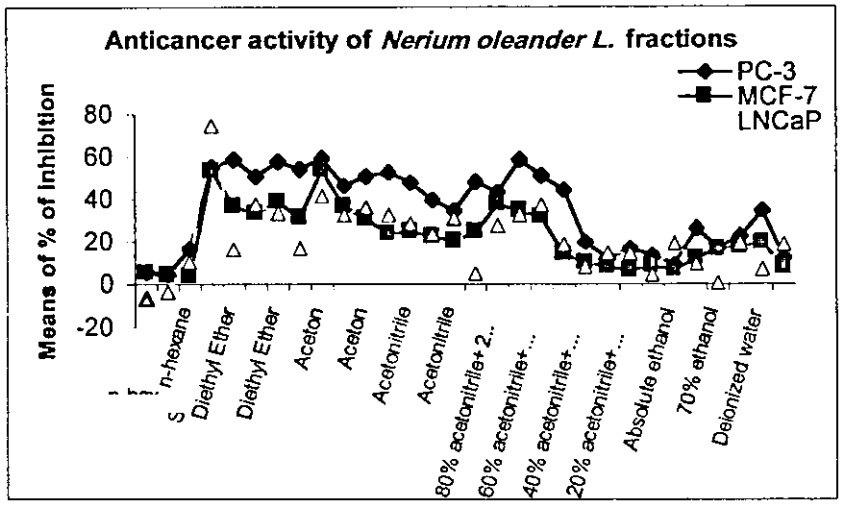

Figure 3.5 *In vitro* cytotoxic activity of fractions of *Nerium oleander* against PC-3, LNCaP, and MCF-7 cell line.

acetone, acetonitrile and acetonitrile + ethanol fractions gave > 40% inhibition, wile the other fractions showed inhibition effect less than 40% (Table 3.5 & Figure 3.5).

Diethyl ether and acetone fractions gave comparable activity (> 50% inhibition) against the three cell lines. Acetonitrile and Acetonitrile + ethanol fractions were active against PC-3 cell line but they were less active against MCF-7 and LNCaP cell lines (< 35% inhibition). (Table 3.5 & Figure 3.5).

Table 3.5 *In vitro* cytotoxic activity of fractions of *Nerium oleander* against PC-3, LNCaP, and MCF-7 cell lines

Mean cell inhibition ± SD							
Fraction No.	Cancer cell line	PC-3		MCF-7		LNCaP	
	Solvents name	AVE.	SD	AVE	SD	AVE.	SD
F1	n-hexane	5.5	6.364	5	4.243	-7	8.485
F2	n-hexane	4.5	2.121	4	1.414	-3.5	2.121
F3	n-hexane	16.7	14.64	3.5	7.778	10.5	13.44
F4	Diethyl Ether	54.8	6.01	53	1.414	55	4.95
F5	Diethyl Ether	58.5	13.44	36.5	6.364	16.5	3.536
F6	Diethyl Ether	50.5	3.536	33	1.414	37.5	7.778
F7	Diethyl Ether	57.5	10.61	38.5	9.192	33.5	2.121
F8	Diethyl Ether	54	1.414	31	2.828	17	5.657
F9	Acetone	59	4.243	53.5	0.707	50	9.192
F10	Acetone	46	2.828	36.5	0.707	32.5	3.536
F11	Acetone	50.5	6.364	30.5	6.364	36	2.828
F12	Acetone	52.5	3.536	23.5	7.778	32.5	9.192
F13	Acetonitrile	47.5	6.364	24.5	3.536	28.5	12.02
F14	Acetonitrile	39.5	2.121	22	4.243	23.5	2.121
F15	Acetonitrile	34.5	0.707	20	7.071	31	8.485
F16	Acetonitrile	48	1.414	24.5	4.95	5	2.828
F17	80% acetonitrile+20% ethanol	43	1.414	37.5	4.95	27.5	14.85
F18	80% acetonitrile+20% ethanol	58.5	10.61	34.5	4.95	32.5	9.192
F19	60% acetonitrile+40% ethanol	51	2.828	31.5	6.364	37	4.243
F20	60% acetonitrile+40% ethanol	44	2.828	14	2.828	18.5	7.778
F21	40% acetonitrile+60% ethanol	19.5	7.778	10	2.828	8	8.485
F22	40% acetonitrile+60% ethanol	12	11.31	8	0	14.5	2.121
F23	20% acetonitrile+80% ethanol	16.5	16.26	6	2.828	14.5	3.536
F24	20% acetonitrile+80% ethanol	13.5	12.02	8.5	2.121	4.5	7.778
F25	Absolute ethanol	9	1.414	6.5	3.536	19	2.828
F26	Absolute ethanol	26	5.657	12	8.485	9.5	7.778
F27	70% ethanol	17	4.243	16.5	3.536	0.5	2.121
F28	70% ethanol	22.5	3.536	17.5	13.44	19	16.97
F29	Deionized water	34.5	14.85	19	15.56	7	16.97
F30	Deionized water	12	11.31	7.5	7.778	18.5	0.707

3.2.5 *In vitro* cell toxicity of *Ecballium elaterium* against PC-3, LNCaP and MCF-7 cell lines

Ecballium elaterium revealed weak anticancer activity against the test cell lines with a mean percent inhibition of > 20% (Table 3.1). The three cell lines differed significantly in their inhibition ($F = 4.078$, $DF = 2$, $p < 0.076$). The most affected cell line was PC-3 with percent inhibition of 52.67 % (Table 3.1).

3.2.5.1 *In vitro* cell toxicity of fractions of *Ecballium elaterium* against PC-3, LNCaP and MCF-7 cell lines

The inhibitory effect against the three cell lines varied (10% - 70% inhibition) significantly between *Ecballium elaterium* fractions. Diethyl ether

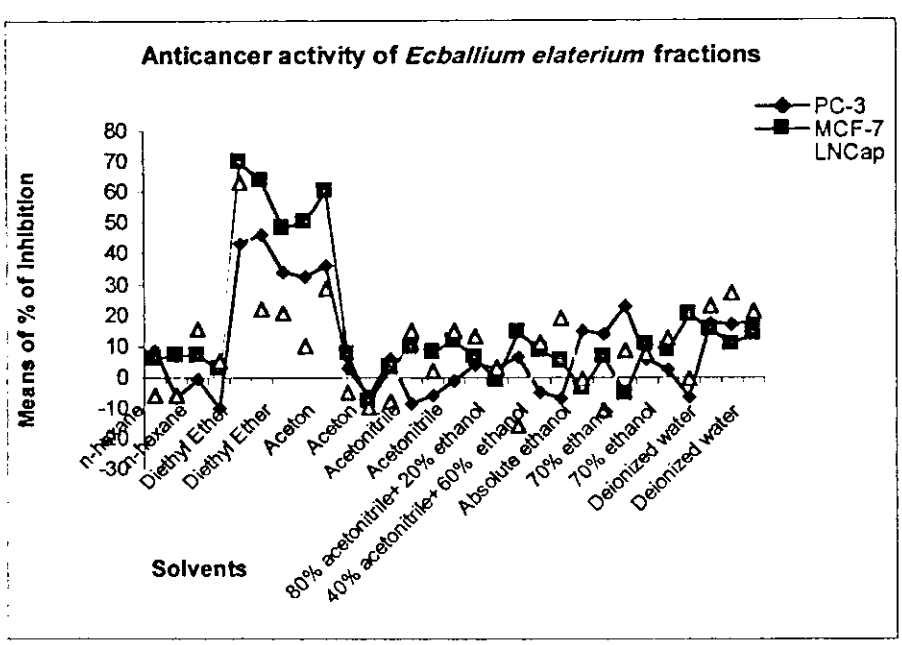

Figure 3.6 In vitro cell toxicity of fractions of *E. elaterium* against PC3, LNCaP and MCF-7 cell lines

was the most active fraction. Other fractions showed inhibition effect less than 40% (Table 3.6 & Figure 3.6).

Diethyl ether was the most active fraction (> 63% inhibition) against LNCaP and MCF-7 cell lines, but it was less active (> 43% inhibition) against PC-3 cell line.

Table 3.6 *In vitro* cytotoxic activity of fractions of *Ecballium elaterium* against PC-3, LNCaP, and MCF-7 cell lines

Fraction No.	Mean cell inhibition ± SD						
	Cancer cells	PC-3		MCF-7		LNCaP	
	Solvents names	AVE.	SD	AVE	SD	AVE.	SD
F1	n-hexane	9.5	0.707	6	8.485	-6	5.657
F2	n-hexane	-6.5	2.121	7	2.828	-6	9.899
F3	n-hexane	-0.5	3.536	7	11.31	15.5	9.192
F4	n-hexane	-10	1.414	2.5	6.364	5	19.8
F5	Diethyl Ether	43	16.97	59.5	10.51	63	26.87
F6	Diethyl Ether	46	1.414	53.5	13.33	22	4.243
F7	Diethyl Ether	34	2.828	38	21.11	20.5	0.707
F8	Diethyl Ether	32.5	13.44	40	21.01	10	2.828
F9	Diethyl Ether	36	7.071	50	15.46	28.5	4.95
F10	Acetone	3	2.828	7.5	6.364	-5	24.04
F11	Acetone	-6	0	-8	2.828	-10	8.485
F12	Acetone	6	1.414	3	5.657	-8	12.73
F13	Acetonitrile	-8.5	3.536	9.5	4.95	5	15.25
F14	Acetonitrile	-6	1.414	8	2.828	2	14.14
F15	Acetonitrile	-1	4.243	11.5	10.61	15	4.243
F16	Acetonitrile	4	2.828	6.5	17.68	13	8.485
F17	80% acetonitrile+20% ethanol	3	2.828	-1	7.071	3	7.071
F18	60% acetonitrile+40% ethanol	6.5	3.536	14.5	17.68	-16	7.071
F19	40% acetonitrile+60% ethanol	-5	4.243	8.5	21.92	11	1.414
F20	20% acetonitrile+80% ethanol	-7	2.828	5	16.97	19	0
F21	Absolute ethanol	15	2.828	-4	12.73	-0.5	0.707
F22	Absolute ethanol	14	1.414	6.5	18.23	-11	0
F23	70% ethanol	23	2.828	-5.5	7.778	8.5	19.09
F24	70% ethanol	6	4.243	10.5	7.778	7.5	10.61
F25	70% ethanol	2.5	2.121	8.5	13.44	12.5	17.68
F26	70% ethanol	-6.5	2.121	20	7.071	-0.5	3.536
F27	Deionized water	17.5	2.121	15.5	9.192	23	1.414
F28	Deionized water	17	2.828	10.5	17.68	27	4.243
F29	Deionized water	18	1.414	14	15.56	21	8.485

3.2.6 *In vitro* cell toxicity of *Ononis sicula* against PC3, LNCaP and MCF-7 cell lines

Ononis sicula showed anticancer activity against the test cell lines with a mean percent of inhibition > 30% (Table 3.1). The three cell lines differed significantly in their inhibition ($F = 9.342$, $DF = 2$, $p < 0.015$). The most affected cell line was MCF-7 with an inhibition of 72 % (Table 3.1).

3.2.6.1 *In vitro* cell toxicity of fractions of *Ononis sicula* against PC3, LNCaP and MCF-7 cell lines

The inhibitory effect of *Ononis sicula* fractions against the three cell lines differed (10% - 95% inhibition) significantly. Diethyl ether and acetone were the active fractions. Other fractions showed inhibition effect less than 40% (Table 3.6 & Figure 3.6).

The acetone fraction was the most active fraction (> 76% inhibition) against the three cell lines. It was more active (> 94% inhibition) against PC-3 and MCF-7cell lines than LNCaP (> 75% inhibition) cell line.

Diethyl ether fraction was the second most active fraction (>40% inhibition) against LNCaP and MCF-7cell lines, but it was less active (<10% inhibition) against PC-3,cell line (Table 3.7 & Figure 3.7).

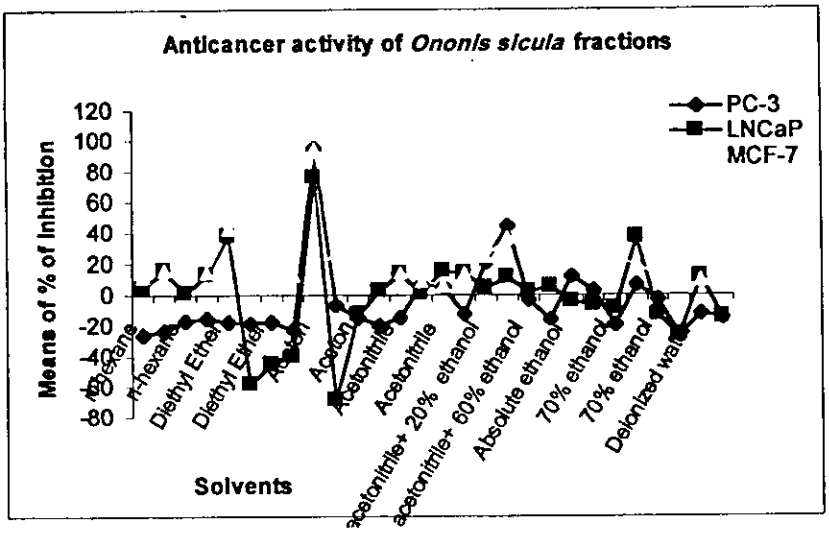

Figure 3.7 *In vitro* cell toxicity of fractions of *Ononis sicula* against PC3, LNCaP and MCF-7 cell lines

Table 3.7 *In vitro* cytotoxic activity of fractions of *Ononis sicula* against PC-3, LNCaP, and MCF-7 cell lines

Mean cell inhibition ± SD							
Fraction No.	Cancer cell line	PC-3		MCF-7		LNCaP	
	Name of the solvent	AVE.	SD	AVE	SD	AVE	SD
F1	n-hexane	-26	22.63	11.5	6.364	4	4.243
F2	n-hexane	-24	21.82	16	4.243	15.5	16.26
F3	n-hexane	-17	15.25	14	5.657	1	2.828
F4	n-hexane	-16	16.26	14	5.657	12.5	6.364
F5	Diethyl Ether	10	16.87	42.5	19.09	41	12.73
F6	Diethyl Ether	-19	9.899	29.5	12.02	-59	23.33
F7	Diethyl Ether	-18	4.243	18.5	3.536	-45	19.09
F8	Diethyl Ether	-23	7.778	12.5	6.364	-40	1.414
F9	Acetone	94.5	3.536	94	1.414	75.5	13.44
F10	Acetone	-6.5	10.61	27	7.071	-69	18.18
F11	Acetone	-15	16.97	24.5	6.364	-13	15.56
F12	Acetone	-20	21.21	14.5	0.707	2	22.53
F13	Acetonitrile	-15	36.77	14	7.071	13.5	24.75
F14	Acetonitrile	6.5	21.92	8.5	3.536	0.5	3.536
F15	Acetonitrile	5	22.63	5.5	3.536	15	5.657
F16	Acetonitrile	-13	10.61	13.5	4.95	13.5	9.192
F17	80% acetonitrile+20% ethanol	21	15.36	24.5	7.778	4	12.73
F18	60% acetonitrile+40% ethanol	34.5	10.61	36.5	16.26	11	14.14
F19	40% acetonitrile+60% ethanol	-3.5	9.192	28.5	4.95	1.5	13.44
F20	20% acetonitrile+80% ethanol	-16	21.21	18	15.56	4.5	7.778
F21	Absolute ethanol	12	5.657	21	4.243	-4.5	2.121
F22	Absolute ethanol	2.5	4.95	14.5	9.192	-7	22.63
F23	70% ethanol	-20	0.707	22.5	16.26	-9	4.243
F24	70% ethanol	6	16.77	19	14.14	36.5	16.26
F25	70% ethanol	-3.5	18.79	11.5	3.536	-14	2.121
F26	70% ethanol	-27	21.72	4.5	3.536	-27	6.364
F27	Deionized water	-13	14.75	9	8.485	11	15.36
F28	Deionized water	-15	15.46	14.5	13.44	-15	16.97

3.2.7 *In vitro* cell toxicity of *Euphorbia hierosolymitana* against PC3, LNCaP and MCF-7 cell lines

Euphorbia hierosolymitana showed anticancer activity against the test cell lines with a mean percent inhibition of > 30% (Table 3.1). The three cell lines differed significantly in their inhibition ($F = 18.638$, $DF = 2$, $p < 0.01$). The most affected cell lines were PC-3 and MCF-7 with percent inhibition of > 40 % (Table 3.1).

3.2.7.1 *In vitro* cell toxicity of fractions of *Euphorbia hierosolymitana* against PC3, LNCaP and MCF-7 cell lines

The inhibitory effect of *Euphorbia hierosolymitana* against the three cell lines differed (10% - 91% inhibition) significantly. Diethyl ether was the most active fraction. Other fractions achieved inhibition effect less than 40% (Table 3.8 & Figure 3.8).

Diethyl ether was the most active fraction (> 63% inhibition) against LNCaP and PC-3 prostate cancer cell lines, but it was less active (> 43% inhibition) against MCF-7 breast cancer cell line (Table 3.8 & Figure 3.8).

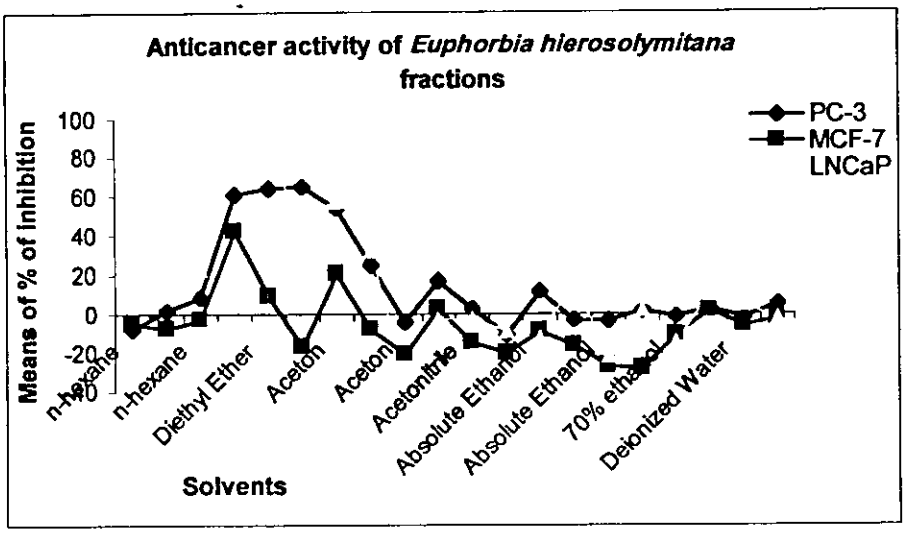

Figure 3.8 *In vitro* cytotoxic activity of fractions of *Euphorbia hierosolymitana* against PC-3, LNCaP, and MCF-7 cell line.

Table 3.8 *In vitro* cytotoxic activity of fractions of *Euphorbia hierosolymitana* against PC-3, LNCaP, and MCF-7 cell line.

Mean cell inhibition ± SD							
Fraction No.	Cancer cell line	PC-3		MCF-7		LNCaP	
	Name of the solvent	AVE.	SD	AVE	SDEV	AVE.	SDEV
F1	n-hexane	-7.5	14.85	-5.5	10.61	16	15.56
F2	n-hexane	1.5	9.192	-7.5	20.51	17.5	34.65
F3	n-hexane	8.5	6.364	-3	9.899	22.5	23.33
F4	Diethyl Ether	61	8.485	42	16.87	91.5	0.707
F5	Diethyl Ether	64	1.414	9	18.38	77	1.414
F6	Diethyl Ether	65	15.46	7	12.63	52.5	19.09
F7	Diethyl Ether	54	16.97	20.5	13.23	57	1.414
F8	Acetone	25	21.11	-8	4.243	32	28.28
F9	Acetone	14	13.94	-21	2.828	-13	17.38
F10	Acetonitrile	17	5.657	2.5	0.707	-3	11.31
F11	Acetonitrile	2.5	4.95	-15	4.243	-2.5	20.51
F12	Acetonitrile	-11	11.11	-21	23.33	-9	15.36
F13	Absolute Ethanol	11.5	13.44	-9	15.56	-13	17.48
F14	Absolute Ethanol	-3	19.8	-17	14.85	-12	19.7
F15	Absolute Ethanol	-3.5	18.99	-27	25.46	-2	10.81
F16	70% ethanol	1.5	10.41	-29	9.192	2	12.33
F17	70% ethanol	-1.5	20.51	-11	14.14	-15	16.97
F18	70% ethanol	2.5	10.61	1.5	12.02	1	16.87
F19	Deionized Water	-2	18.38	-6	7.071	4.5	10.61
F20	Deionized Water	5	2.828	-2.5	2.121	-2.5	2.121

3.2.8 *In vitro* cell toxicity of *Anthemis tunictoria* against PC3, LNCaP and MCF-7 cell lines

Anthemis tunictoria showed anticancer activity against the test cell lines with a mean percent inhibition of > 30% (Table 3.1). The three cell lines did not differ significantly in their inhibition ($F = 1.927$, $DF = 2$, $p > 0.225$). The most influenced cell line was PC-3 with a percent of inhibition = 44% (Table 3.1).

3.2.9 *In vitro* cell toxicity of *Capparis spinosa* against PC3, LNCaP and MCF-7 cell lines

Capparis spinosa showed anticancer activity against the test cell lines with a mean percent inhibition of > 20% (Table 3.1). The three cell lines differed significantly in their inhibition ($F = 4.864$, $DF = 2$, $p < 0.0555$). The most affected cell line was LNCaP with a mean percent inhibition of 46.3% (Table 3.1).

CHAPTER FOUR

DISCUSSION

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DISCUSSION

4.1 Anticancer activity of the selected medicinal plants

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents (Alonso Paz *et al.*, 1995). The first step towards achieving this goal is the screening of plants used in a popular medicine. It is becoming increasingly clearer that extracts of natural products are an important available and excellent source for drug development (Hsieh *et al.*, 1997).

The development of several plant-derived anti-cancer drugs such as vincristine, comptothecin and taxol abroad has spurred researchers interest in identifying a suitable indigenous plant with anti-cancer activity (Anantharaman, 1995). The present study has demonstrated that nine of the studied plants 9/96 (10%) (Appendix A) are potentially important sources of anti-cancer agents. The use of these plants in traditional medicine for treating various diseases as cancer and prostate disorders (Ali-Shtayeh *et al.*, 2000; Palevitch *et al.*, 1984) is probably justified. Anticancer activity of medicinal plants, e.g., *Cernilton pollen, Urtica dioica, Serenoa repens, Striga orobanchioides,* and *Epilobium parviflorum* extracts, against prostate cancer cell lines have also been reported by other workers (Plosker *et al.*, 1996,

Habib *et al.*, 1997, Hryb *et al.*, 1995, Hiremath *et al.*, 1997, & Lesuisse *et al.*, 1996).

4.2 Anticancer activity of selected extracts against PC-3, LNCaP and MCF-7 cell lines

Anticancer activity of medicinal plants extracts, against PC-3, LNCaP, and MCF-7 cancer cell lines have also been reported by other workers (Ren & Tang, 1999; Lu & Serrero, 1999; Alkofahi *et al.*, 1996; Onozawa *et al.*, 1998). In the present study, among 96 locally available plant species tested *in vitro* against the test cell lines, the extracts of *Cyclamen persicum, Lycium europeum, Ecballium elaterium, Euphorbia hierosolymitana, Anthemis tunictoria, Verbascum sinuatum, Capparis spinosa, Ononis sicula,* and *Nerium oleander* were most active (40-95% inhibition). The results are therefore consistent with those of Alkofahi *et al.* (1997) who showed that from 43 ethanolic crude extracts corresponding to 29 different plant species, 5 % of the extracts have anticancer activity including MCF-7 cell line. Also, Moraes *et al.* (1997) found that among 72 ethanolic extracts correspond to 32 different plant species, 14% of the extracts have antitumor activity.

Another important observation was the stimulation of PC-3 cell line by some plants. For instance *Crataegus aronia, Ziziphus spina-christi,*

Salvia fruticosa, Retema raetam, and *Parietaria diffusa,* which are used in folkloric medicine against many diseases (Ali-Shtayeh *et al.*, 2000), were significantly found to stimulate PC-3 cell line proliferation. On the other hand *Quercus calliprines* and *Chrysanthemum coronarium*, which are also used as popular remedies (Ali-Shtayeh *et al.*, 2000) were significantly found to stimulate cancer cells of LNCaP and MCF-7 cell lines. The results are therefore consistent with those Moraes *et al.* (1997) who found that among 72 ethanolic extracts correspond to 32 different plant species, 5% of the extracts have tumor stimulation. Therefore, care must be taken with respect to the large number of plant extracts that show stimulation of cancer cells.

Anticancer activity results of plants used in Palestinian folkloric medicine against cancer (Ali-Shtayeh *et al.*, 2000) revealed that none of these plants can be considered active. This may be due to many reasons: lack of anticancer compounds in these plants, loss of active ingredients during extract preparation, or inproper extraction system.

The active extracts showed that cytotoxic activity on the tested three cell lines by the decreased rate of cell proliferation, reduced clonogenicity, increased proportion of cells in G_1 phase of the cycle, induction of apoptosis and down regulation of *bcl-2* expression (Dorota Halicka *et al.*, 1997).

4.2.1 Anticancer activity of selected extracts against PC-3 cell line

In the present study the inhibitory effect against these cell line varied (30- 95 % inhibition) significantly between active (Table 3.1). Extracts of *Cyclamen persicum, Euphorbia hierosolymitana,* and *Verbascum sinuatum* were the most active (>70 % inhibition).

In addition the study showed that PC-3 and LNCaP prostate cancer cell lines were more susceptible to extracts of *Verbascum sinuatum* than MCF-7 breast cancer cell line. The results also showed that PC-3, hormone refractory cell line were more susceptible to extracts of *Euphorbia hierosolymitana* than MCF-7 and LNCaP hormone sensitive cell lines.

4.2.2 Anticancer activity of selected extracts against LNCaP cell line

In the study the inhibitory effect against these cell line varied (23-90% inhibition) significantly between active extracts (Table 3.1). Extracts of *Cyclamen persicum, Capparis spinosa,* and *Verbascum sinuatum* were the most active (>50 % inhibition).

The results also showed that LNCaP cell line was more susceptible to extracts of *Capparis spinosa* than MCF-7 and LNCaP cell lines.

4.2.3 Anticancer activity of selected extracts against MCF-7 cell line

From the results the inhibitory effect against these cell line rainged (30-95 % inhibition) significantly between active extracts (Table 3.1). Extracts of *Cyclamen persicum, Ononis sicula,* and *Lycium europeum* were the most active (>55 % inhibition).

The results showed that MCF-7 breast cancer cell line were more susceptible to extracts of *Ononis sicula* than PC-3 and LNCaP prostate cancer cell lines.

The study demonstrates that plants are an important source of anticancer compounds, and that they may provide a renewable source of useful cancer drugs that can be inhibits cancer cells. Farther studies are therefore needed on these plants in the search for new and more potent anticancer substances from natural sources.

4.3 Anticancer activity of fractions of active plant extracts

The results showed that Diethyl Ether fraction for *Cyclamen persicum, Euphorbia hierosolymitana, Ecballium elaterium* and *Nerium oleander* were the most active. Diethyl Ether (nonprotogenic and weak polar solvent) dissolves terpeniods (monoterpens, sesquiterpenes, diterpenes, sesterterpenes, and triterpenes). Some of diterpenes and sesquiterpenes compounds act as

anticancer drugs; while monoterpens and sesquiterpenes are volatile (small compounds) and mostly evaporated during extraction and evaporation steps, sesquiterpenes may contains α, β-unsaturated carbonyle which is know to be anticancer moiety. Furthermore, Diethyl ether may also dissolve some alkaloids. Alkaloidal salts and quaternary alkaloids are not soluble in ether (Connolly & Hill, 1991).

The study also showed that acetone fraction of *Ononis sicula, Verbascum sinuatum* and *Lycium europeum* were the most active. Acetone is much more polar than ether, any moisture occurs during fractionation steps may further enhance polarity of acetone (acetone is completely miscible with water) few compounds turned to be very polar; these compounds appeared and showed activity following extraction with 70% ethanol (Dev, 1986).

Hexane fractions do not showed any activity, it is highly non-polar solvent which dissolved fats and waxes; these compounds mostly inactive against cancer.

4.4 Conclusions and recommendations

1. Further studies are needed to test the biological activities of wild plants and explore their potential as sources of anticancer agents and for other benefits to human health.

2. Further studies are needed to find out active constituents of many plants.

3. For achieving better results, plant extracts would be more efficient if prepared using infusion extraction technique with suitable solvents.

4. The establishment of Palestinian research institute on medicinal plants and a horticultural station to grow and preserve endangered indigenous species of medicinal plants is recommended.

5. The extracts of *Cyclamen persicum, Lycium europeum, Ecballium elaterium, Euphorbia hierosolymitana, Anthemis tunictoria, Verbascum sinuatum, and Nerium oleander* were most active against PC-3 cell line.

6. The extracts of *Cyclamen persicum, Lycium europeum, Euphorbia hierosolymitana,* and *Ononis sicula,* were most active against MCF-7 cell line.

7. Extracts of *Cyclamen persicum, Verbascum sinuatum,* and *Capparis spinosa* were most active against LNCaP cell line.

8. Further work is therefore needed on these plants to identify and study their active ingredients.

9. The most susceptible cell line was PC-3, whereas the least susceptible was LNCaP cells.

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APPENDICES

APPENDIX A

SCREENING RESULTS

Ser.No.[a]	Scientific Name	Parts used[b]	% Of Inhibition													
			PC-3					MCF-7					LNCaP			
			10X*				1X**	10X				1X	10X				1X
			#1	#2	AVE.	SD.		#1	#2	AVE.	SD.		#1	#2	AVE.	SD.	
W 1	*Clematis cirrhosal*	AP	-1	16	7.5	12.02	-1	30	-1	14.5	21.92	13	3	6	4.5	2.1213	-9
W2	*Ceratonia siliqua*	FR	-11	20	4.5	21.92	1	13	5	9	5.657	6	31	12	21.5	13.435	26
W3	*Paronychia argentea*	AP	36	32	34	2.828	7	24	33	28.5	6.364	1	-1	-13	-7	8.4853	4
W4	*Pistacia lentiscus*	LF	-6	28	11	24.04	18	17	-5	6	15.56	12	-4	-5	-4.5	0.7071	8
W5	*Micromeria fruticosa*	AP	1	-24	-12	17.68	6	-16	-18	-17	1.414	-4	-36	-32	-34	2.8284	30
W6	*Asphodelin lutea*	WP	-33	-17	-25	11.31	-17	31	-7	12	26.87	-33	24	21	22.5	2.1213	7
W7	*Companula rapanculus*	AP	-6	18	6	16.97	-2	18	-1	8.5	13.44	1	-23	-10	-16.5	9.1924	12
W8	*Lycium europeum*	AP	40	48	42.7	5.623	12	43	69	56	18.38	12	29	27	28	1.4142	-7
W9	*Urtica urens*	WP (F)[c]	-21	29	4	35.36	-7	1	8	4.5	4.95	8	-5	3	-1	5.6569	-21
W10	*Sarcopoterium spinosum*	AP	-14	-24	-19	7.071	25	18	32	25	9.899	-26	-12	-21	-16.5	6.364	-39
W11	*Inula viscosa*	AP (F)	32	5	18.5	19.09	15	45	11	28	24.04	3	-24	-20	-22	2.8284	-21
W12	*Cichorium pumilum*	AP (F)	28	35	31.5	4.95	-11	23	19	21	2.828	21	17	19	18	1.4142	26
W13	*Juglans regia L.*	AP (F)	-37	-17	-27	14.14	4	17	15	16	1.414	7	7	-5	1	8.4853	23
W14	*Coridothymus capitatus*	AP	-29	13	-8	29.7	8	3	-15	-6	12.73	-6	-15	-11	-13	2.8284	15
W15	*Arum palaestinum*	LF	-2	35	16.5	26.16	18	19	-3	8	15.56	11	15	25	20	7.0711	14
W16	*Silene vuglars*	AP	-50	-25	-38	17.65	14	13	19	16	4.243	0	14	13	13.5	0.7071	15
W17	*Cardaria draba*	AP	-6	26	10	22.63	3	3	22	12.5	13.44	-4	16	18	17	1.4142	22
W18	*Trigonella foenumgraecum*	AP (F)	-26	11	-7.5	26.16	14	18	2	10	11.31	31	-2	5	1.5	4.9497	-9
W19	*Crataegus aronial*	LF	-50	-73	-62	16.26	-24	3	-6	-1.5	6.364	23	-18	-6	-12	8.4853	8
W20	*Sinapis arvensis*	WP	12	4	8	5.657	10	18	-1	8.5	13.44	14	4	9	6.5	3.5355	-1
W21	*Reseda alba*	WP	8	14	11	4.243	29	14	20	17	4.243	-5	2	8	5	4.2426	18
W22	*Anchusa egyptaca*	WP	19	3	11	11.31	22	10	22	16	8.485	0	-1	5	2	4.2426	-5
W23	*Alcea setosa*	FL	45	27	36	12.73	-22	14	13	13.5	0.707	5	29	20	24.5	6.364	34
W24	*Lactuca serriola*	AP	-15	10	-2.5	17.68	-30	30	-13	8.5	30.41	10	9	12	10.5	2.1213	30

W25	*Phlomis viscosa*	YB	-11	18	3.5	20.51	13	26	-13	6.5	27.58	12	33	35	34	1.4142	19
W26	*Quercus calliprines*	RT	-16	-1	-8.5	10.61	-63	-50	-45	-48	3.536	7	-22	-33	-27.5	7.7782	8
W28	*Ecballium elaterium*	AP	49	57	52.7	5.467	-6	31	49	39.7	12.73	-5	19	37	27.7	12.43	24
W29	*Ziziphus spina-christi*	LF	-85	-54	-70	21.92	-13	-19	-44	-32	17.68	7	-4	-15	-9.5	7.7782	-21
W30	*Salvia fruiticosa*	LF	-50	-34	-42	11.31	-36	2	-9	-3.5	7.778	7	-23	-9	-16	9.8995	1
W31	*Citrus limon*	FL	-32	-10	-21	15.56	-17	11	-31	-10	29.7	19	-1	-5	-3	2.8284	-11
W32	*Retema raetam*	YB	-64	-56	-60	5.657	-11	15	14	14.5	0.707	-2	-16	-5	-10.5	7.7782	-23
W33	*Rescus aculeatas*	RT	31	39	35	5.657	8	18	5	11.5	9.192	-1	10	4	7	4.2426	1
W34	*Varthemia iphionoides*	AP	-12	1	-5.5	9.192	-9	26	8	17	12.73	1	-9	-28	-18.5	13.435	-6
W35	*Gundelia tournefortii*	LF	9	7	8	1.414	-7	-28	2	-13	21.21	-4	6	13	9.5	4.9497	2
W36	*Achilea biebersteinii*	WP	40	30	35	7.071	-1	16	15	15.5	0.707	-17	-2	14	6	11.314	13
W37	*Cyclamen persicum*	B	97	92	94.7	3.536	45	94	97	95.7	2.121	68	88	93	90.3	3.5355	51
W38	*Notobasi syriaca*	YB	8	4	6	2.828	-8	17	10	13.5	4.95	5	14	-27	-6.5	28.991	-5
W39	*Allium sativum*	LF	38	20	29	12.73	3	22	11	16.5	7.778	10	34	28	31	4.2426	-22
W40	*Conium maculathum*	LF	-42	-37	-40	3.536	-33	27	6	16.5	14.85	7	8	6	7	1.4142	-4
W41	*Urtica pilulifera*	WP	-23	10	-6.5	23.33	-30	8	-3	2.5	7.778	9	-34	-30	-32	2.8284	-13
W42	*Erodium malacoides*	WP	-10	19	4.5	20.51	-35	11	-6	2.5	12.02	2	-34	-6	-20	19.799	-4
W43	*Capparis spinosa*	FR	4	-3	0.5	4.95	-7	5	3	4	1.414	8	5	21	13	11.314	-10
W44	*Styrax officinalis*	YB	-18	-33	-26	10.61	-3	5	-11	-3	11.31	5	9	5	7	2.8284	-5
W45	*Sonchus oleraceus*	WP	11	-7	2	12.73	1	-9	23	7	22.63	-34	-24	-9	-16.5	10.607	3
W46	*Spinacia olecurcea*	WP	-28	16	-6	31.11	-16	7	-2	2.5	6.364	13	22	7	14.5	10.607	-10
W47	*Eryngium creticum*	YB	-12	25	6.5	26.16	-12	13	-19	-3	22.63	1	34	13	23.5	14.849	-3
W48	*Parieteria diffusa*	WP	-84	-52	-68	22.63	-27	-34	-32	-33	1.414	4	9	-10	-0.5	13.435	27
W49	*Salvia dominica*	FL	-35	-19	-27	11.31	-11	9	-30	-11	27.58	-4	-26	-30	-28	2.8284	30
W50	*Euphorbuia hierosolymitana*	WP	82	69	76	9.192	19	50	42	45.7	5.657	1	23	39	31	11.314	34
W52	*Rubia tenuifolia.*	AP	28	37	32.5	6.364	19	6	4	5	1.414	21	32	21	26.5	7.7782	21
W53	*Pyrus syriaca*	FL	15	18	16.5	2.121	29	15	-27	-6	29.7	17	24	5	14.5	13.435	17
W54	*Cyclamen persicum*	AP	64	75	69	7.778	29	70	55	63	10.61	28	29	19	23.3	7.0711	27
W55	*Salvia hierosolymitana*	LF	4	6	5	1.414	1	20	14	17	4.243	13	9	20	14.5	7.7782	20
W56	*Petroselinum sativum*	WP	25	27	26	1.414	23	45	30	37.5	10.61	30	31	37	34	4.2426	35
W57	*Ricinus communis*	AP	16	9	12.5	4.95	27	31	1	16	21.21	34	-4	-3	-3.5	0.7071	23
W58	*Lawsonia inermis*	LF	21	25	23	2.828	21	44	35	39.5	6.364	6	10	9	9.5	0.7071	26
W59	*Anthemis tinctoria L.*	FL	21	21	21	0	23	28	21	24.5	4.95	1	13	19	16	4.2426	30

W60	*Micromeria nervosa*	AP	20	19	19.5	0.707	18	7	8	7.5	0.707	-1	-30	-6	-18	16.971	30
W61	*Mandragora autumnalis*	FR	37	24	30.5	9.192	22	16	17	16.5	0.707	7	7	22	14.5	10.607	-1
W62	*Anthemis tunictoria*	LF	37	33	35	2.828	21	2	-2	0	2.828	-12	32	47	39.5	10.607	14
W63	*Anthemis tunictoria*	RT	43	46	44.7	2.121	22	38	22	29.7	11.9	1	43	27	35.5	11.314	21
W64	*Verbascum sinuatum*	FL (F)	45	43	44	1.414	30	36	31	33	3.536	-1	33	19	26	9.8995	24
W65	*Verbascum sinuatum*	LF (F)	38	29	33.5	6.364	27	10	27	18.5	12.02	29	20	50	35	21.213	39
W66	*Verbascum sinuatum*	AP	17	14	15.5	2.121	34	31	3	17	19.8	19	23	40	31.5	12.021	37
W67	*Verbascum sinuatum*	RT (F)	15	31	23	11.31	11	30	13	21.5	12.02	30	30	26	28	2.8284	35
W68	*Capparis spinosa*	FL (F)	25	30	27.7	3.536	42	36	21	28.7	10.61	30	52	41	46.3	7.7782	42
W72	*Chrysanthemum coronarium*	FL	41	6	23.5	24.75	28	24	18	21	4.243	4	-67	-35	-51	22.63	7
W73	*Scabiosa prolifera*	AP	28	11	19.5	12.02	24	-24	-9	-17	10.61	-4	30	31	30.5	0.7071	10
W74	*Scolymus maculatus*	YB	30	15	22.5	10.61	16	18	-10	4	19.8	16	32	11	21.5	14.849	19
W75	*Echinops adenocaulos*	AP	30	32	31	1.414	16	20	-5	7.5	17.68	7	-1	25	12	18.385	26
W76	*Allium erdelii*	WP	29	45	37	11.31	38	26	4	15	15.56	14	20	18	19	1.4142	39
W77	*Cicer arietenum*	AP	28	31	29.5	2.121	32	27	2	14.5	17.68	29	45	10	27.5	24.749	30
W78	*Ononis sicula*	WP	32	41	37.3	6.364	31	62	82	72	14.14	5	41	21	31	14.142	31
W79	*Urtica urens*	WP	40	35	37.5	3.536	8	33	38	35.5	3.536	35	29	21	25	5.6569	33
W80	*Daucus carota*	LF	7	9	8	1.414	12	37	11	24	18.38	27	6	9	7.5	2.1213	37
W81	*Inula viscosa*	LF	22	31	26.5	6.364	28	45	30	37.5	10.61	26	13	14	13.5	0.7071	30
W82	*Majorana syriaca*	AP	1	-1	0	1.414	-3	-28	5	-12	23.33	12	-14	12	-1	18.385	22
W83	*Asparagus aphyllus*	AP	-23	-12	-18	7.778	-31	24	31	27.5	4.95	12	-3	-2	-2.5	0.7071	13
W84	*Calycotome villosa*	AP	5	9	7	2.828	-13	46	28	37	12.73	-24	-7	-8	-7.5	0.7071	25
W85	*Lupinus albus*	SE	14	15	14.5	0.707	-9	7	18	12.5	7.778	16	19	20	19.5	0.7071	17
W86	*Lamium moschatum*	WP	1	-18	-8.5	13.44	-5	29	28	28.5	0.707	-21	9	8	8.5	0.7071	10
W87	*Foeniculum vulgare L.*	AP	37	34	35.5	2.121	19	24	28	26	2.828	5	37	39	38	1.4142	32
W88	*Lupinus pilosus*	AP	-5	-24	-15	13.44	-12	21	22	21.5	0.707	9	2	-9	-3.5	7.7782	15
W89	*Papaver rheos*	AP	-1	-21	-11	14.14	1	18	17	17.5	0.707	18	9	-19	-5	19.799	25
W90	*Vicia faba*	AP	-13	-6	-9.5	4.95	-2	20	23	21.5	2.121	1	23	-14	4.5	26.163	2
W91	*Satureja thymbra*	AP	3	-9	-3	8.485	-22	20	12	16	5.657	4	-16	-28	-22	8.4853	16
W92	*Eruca sativa*	WP	8	-30	-11	26.87	-3	22	27	24.5	3.536	11	9	20	14.5	7.7782	19
W93	*Gagea chloranth*	RT	-3	3	0	4.243	-16	5	20	12.5	10.61	16	-5	5	0	7.0711	14
W94	*Linum pubescens*	AP	-17	-21	-19	2.828	-26	33	12	22.5	14.85	-2	0	-3	-1.5	2.1213	-1
W95	*Juglans regia*	FR	-10	-5	-7.5	3.536	-15	17	12	14.5	3.536	10	33	-6	13.5	27.577	30

W96	*Daucus carota*	RT	-1	-3	-2	1.414	2	10	7	8.5	2.121	11	20	14	17	4.2426	16
W97	*Convolvulus arvensis*	AP	3	5	4	1.414	-8	-20	11	-4.5	21.92	11	26	24	25	1.4142	30
W98	*Rosa centifolia*	FL (F)	-4	-11	-7.5	4.95	-4	16	15	15.5	0.707	8	8	-23	-7.5	21.92	26
W99	*Saccharum ravennae*	WP	-4	-17	-11	9.192	5	9	19	14	7.071	11	4	-23	-9.5	19.092	16
W100	*Daucus carota*	FL	-21	3	-9	16.97	-4	21	25	23	2.828	10	30	-3	14.5	23.334	22
W101	*Lactuca tuberosa*	AP	-4	-2	-3	1.414	16	33	32	32.5	0.707	23	9	-9	0	12.728	17
W102	*Pinus halepensis*	LF	5	11	8	4.243	-2	38	38	38	0	14	12	-6	3	12.728	19
W103	*Ruta calapensis*	AP	30	33	31.5	2.121	-7	27	32	29.5	3.536	14	27	29	28	1.4142	16
W104	*Trigonella foenumgraecum*	AP	-6	9	1.5	10.61	13	33	22	27.5	7.778	16	-4	-14	-9	7.0711	6
W105	*Viscum cruciatum*	LF	-20	-14	-17	4.243	-35	5	14	9.5	6.364	-3	-19	-31	-25	8.	-14
W106	*Rhus coriaria L.*	AP	-7	4	-1.5	7.778	-6	16	-20	-2	25.46	4	2	9	5.5	4.9497	5
W107	*Phagnalon rupester*	AP	8	-6	1	9.899	-2	-26	13	-6.5	27.58	2	10	17	13.5	4.9497	1
W108	*Persea gratissima*	SE	15	1	8	9.899	3	17	-13	2	21.21	-4	40	41	40.5	0.7071	9
W109	*Urtica pilulifera*	SE	1	1	1	0	-3	25	12	18.5	9.192	1	18	-4	7	15.556	19
W110	*Nerium oleander L.*	FL	40	53	46.7	9.192	20	37	29	35	5.657	16	35	33	34.3	1.4142	32
W111	*Conium maculathum*	FL	22	12	17	7.071	-15	27	27	27	0	10	37	42	39.5	3.5355	37
W112	*Rosmarinus officinalis L.*	AP	17	12	14.5	3.536	10	18	16	17	1.414	16	-19	18	-0.5	26.163	5
W113	*Portulaca oleracea*	AP	1	3	2	1.414	12	-1	19	9	14.14	12	12	-15	-1.5	19.09	1
W114	*Mentha viridis*	AP	5	9	7	2.828	4	-6	12	3	12.73	-8	-7	-27	-17	14.142	5
W115	*Nerium oleander L.*	LF	57	44	50.5	9.192	26	41	24	32.5	12.02	1	11	35	23	16.971	25
W116	*Phragmites australis*	FL	-16	-2	-9	9.899	-36	-1	8	3.5	6.364	4	1	21	11	14.142	-25
W117	*Verbascum sinuatum*	FL (F)	73	82	77.5	6.364	7	51	29	39.7	15.56	16	71	56	63.7	10.607	3
W118	*Verbascum sinuatum*	AP (F)	52	22	37	21.21	-10	21	38	29.5	12.02	10	8	17	12.5	6.364	16
W120	*Ammi majus*	FL	8	19	13.5	7.778	-1	27	20	23.5	4.95	16	10	17	13.5	4.9497	22
W121	*Ammi majus*	LF	33	23	28	7.071	0	11	23	17	8.485	18	19	7	13	8.4853	-14

*First concentration = 50µg/ml; ** Second concentration = 5µg/ml. [a] walid number ; [b] AP, aerial parts; FL, flowers; WP, whole plants; RT, roots; LF, leaves; B, bulb; SE, seeds; FR, fruits; YB, yang branches. [c] Fresh parts and the others were dry parts.

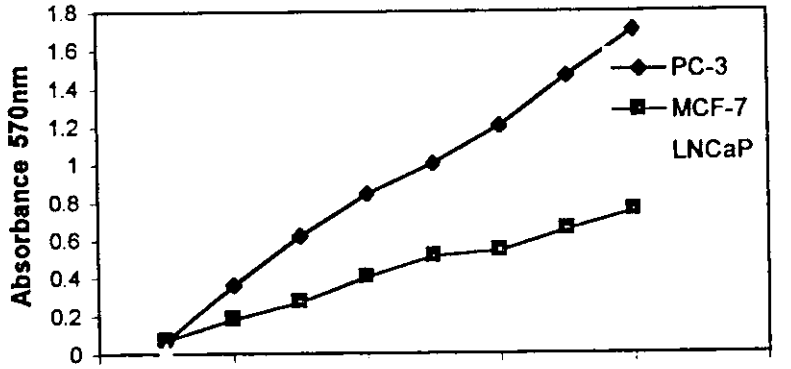

Figure B.1. Effect of cell number on absorbance 570nm measured using MTT assay.

Each point represents the mean ± SD of four replicates. There was a linear response between cell number and absorbance at 570nm. For PC-3 and MCF-7 cell lines $3x\ 10^4$ cells/well were used, but $4x\ 10^4$ cells/well was used for LNCaP cell line.

APPENDIX C

ANOVA Tables

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	K*-1	Between groups SS	MSTR	F= MSTR/MSE	<= 1
Within Groups (Error)	N**-K	Within groups SS	MSE		
Total	N-1	Total SS			

* K= number of experimental groups.
** N= total number of data in the experiment.

Table C.1 In vitro cell toxicity of *L. europeum* against PC3, LNCaP and MCF-7 cell lines and their fractions

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	1204.6667	602.3333	5.0806	.0512
Within Groups (Error)	6	711.3333	118.5556		
Total	8	1916.0000			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.2 In vitro cell toxicity of *E. elaterium* against PC3, LNCaP and MCF-7 cell lines and their fractions

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	938.0000	469.0000	4.0783	0.0761
Within Groups (Error)	6	690.0000	115.0000		
Total	8	1628.0000			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.3 In vitro cell toxicity of *C. persicum* (Root) against PC-3, LNCaP, & MCF-7 cell lines

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	48.2222	24.1111	2.6790	0.1474
Within Groups (Error)	6	54.0000	9.0000		
Total	8	102.2222			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.4 In vitro cell toxicity of *E. hierosolymitana* against PC3, LNCaP and MCF-7 cell lines and their fractions

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	3160.2222	1580.1111	18.6383	0.0027
Within Groups (Error)	6	508.6667	84.7778		
Total	8	3668.8889			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.5 In vitro cell toxicity of *C. persicum* (Ariel Parts) against PC-3, LNCaP, & MCF-7 cell lines

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	3694.8889	1847.4444	24.4875	0.0013
Within Groups (Error)	6	452.6667	75.4444		
Total	8	4147.5556			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.9 In vitro cell toxicity of *O. sicula* against PC3, LNCaP and MCF-7 cell lines and their fractions

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	2922.8889	1461.4444	9.3416	0.0144
Within Groups (Error)	6	938.6667	156.4444		
Total	8	3861.5556			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.10 In vitro cell toxicity of *N. oleander* against PC3, LNCaP and MCF-7 cell lines and their fractions

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	288.6667	144.3333	3.6489	0.0919
Within Groups (Error)	6	237.3333	39.5556		
Total	8	526.0000			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.11 In vitro cell toxicity of *V. sinuatum* (Fresh Flours) against PC3, LNCaP and MCF-7 cell lines and their fractions

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	2216.0000	1108.0000	7.1026	0.0262
Within Groups (Error)	6	936.0000	156.0000		
Total	8	3152.0000			

K = 3 cell lines.
N = 3 x 3 = 9

Table C.12 Cytotoxic activity of ethanolic extracts against hormone refractory prostate cancer cell line (PC-3)

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	12697.212	1269.7212	33.2810	0.0000
Within Groups (Error)	6	839.3333	38.1515		
Total	8	13536.545			

K = 11 plant extracts.
N = 11 x 3 = 33

Table C.13 Cytotoxic activity of ethanolic extracts against hormone sensitive breast cancer cell line (MCF-7)

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	13143.2121	1314.3212	9.8195	0.0000
Within Groups (Error)	6	2944.6667	133.8485		
Total	8	16087.878			

K = 11 plant extracts.
N = 11 x 3 = 33

Table C.14 Cytotoxic activity of ethanolic extracts against hormone sensitive prostate cancer cell line (LNCaP)

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	2	12355.8788	1235.5879	14.1824	0.0000
Within Groups (Error)	6	1916.6667	87.1212		
Total	8	14272.5455			

K = 11 plant extracts.
N = 11 x 3 = 33

بسم الله الرحمن الرحيم

دراسة تأثير نباتات فلسطين على نشاط الخلايا السرطانية

إعداد

وليد محمود حسان خليليه

بإشراف

أ.د. محمد سليم اشتيه

لقد تمت دراسة تأثير المستخلصات الكحولية لست وتسعون من نباتات فلسطين ضد نوعين من خلايا سرطان البروستاتة وهي: (PC-3 and LNCaP) و نوع واحد من خلايا سرطان الثدي وهي(MCF-7).

وقد استخدم في هذه الدراسة اختبار خاص بقياس حساسية الخلايا السرطانية للمواد السامة وهو: (MTT assay). كما تم عمليات تجزئة للمستخلصات التي أعطت فعالية مضادة للخلايا السرطانية المستخدمة في هذه الدراسة باستخدام طريقة العمود المجزئ (Column Chromatography) مع عدد من المذيبات وهي:الهكسان(n-hexane) و الأيثر(Diethyl Ether) و الأسيتون (Acetone) و الأسيتونايتريل (Acetonitrile) و الكحول النقي (Absolute Ethanol) و الكحول المخفف (70% ethanol) و الماء المقطر .

وأظهرت الدراسة وجود اختلافات معنوية بين النباتات بالنسبة لتأثيراتها المضادة للخلايا السرطانية المستخدمة في التجربة. فكانت النباتات التالية وهي: صابون الراعي (*Cyclamen persicum*) و العوسج (*Lycium europeum*) وقثاء الحمير(*Ecballium elaterium*)

والحليلبون (*Euphorbia hierosolymitana*) والعورور (*Verbascum sinuatum*) والدفلة (*Nerium oleander*) والأقحوان الأصفر (*Anthemis tunictoria*) هي الأعلى فعالية ضمن النباتات المدروسة ضد الخلايا السرطانية من نوع (PC -3). كما وأظهرت الدراسة أن النباتات التالية وهي: صابون الراعي (*Cyclamen persicum*) والقبار (*Capparis spinosa*) والعورور (*Verbascum sinuatum*) هي الأعلى فعالية ضمن النباتات المدروسة ضد الخلايا السرطانية من نوع (LNCaP). أما بالنسبة للخلايا السرطانية من نوع (MCF-7) فكانت النباتات التالية وهي: صابون الراعي (*Cyclamen persicum*) والشبرق (*Ononis sicula.*) و العوسج (*Lycium europeum*) والحليلبون (*Euphorbia hierosolymitana*) هي الأعلى فعالية ضمن النباتات المدروسة ضد هذا النوع من الخلايا السرطانية.

وأظهرت الدراسة أن عدد من النباتات المستخدمة في هذه الدراسة قد حثت الخلايا السرطانية الثلاث المستخدمة في الدراسة وحفزتها على الانقسام بدلا من قتلها أو إيقافه نشاطها وهي: الزعرور (*Crataegus aronial*) والسدو (*Ziziphus spina-christi*) و الميرمية (*Salvia fruiticosa*) و الرتم (*Retema raetam*) وعشبة الدم (*Parietaria diffusa*) مع الخلايا السرطانية من نوع (PC-3) . أما البلوط (*Quercus calliprines*) و البابونج (*Chrysanthemum coronarium*) فكانت الأكثر فعالية في حث الخلايا السرطانية من نوع (MCF-7) و (LNCaP) على التوالي.

و بعد أن تم إجراء عملية التجزئة (Fractionation) للمستخلصات الكحولية التالية: مستخلص صابون الراعي و العوسج و العورور و الحليلبون و قثاء الحمير و الدفلة و الشبرق تبين أن المواد الفعالة لكل من الحليلبون و صابون الراعي و قثاء الحمير و الدفلة استخلصت في الأيثر بينما المواد الفعالة لكل من الشبرق و العوسج و العورور استخلصت في الأسيتون الأكثر قطبية" من الأيثر.