A Study on the Effect of Some Plant Extracts on Certain Malignant Cell Lines in Vitro

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بسم الله الرحمن الرحيم

(والذين والزيتون وطور سين وخفاف ألبه الأمين)

لاقنا 2 قانا الإنسان فخ أحسن ترميم (الله).

(فنبضناه بالزمن وهو سقيم وآيتنا عليه شجرة من يقطن

(المصابات)

حصان الله العلي

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عن أنس بن مالك رضي الله عنه قال: إن خياطًا دعا مرسول الله صلى الله عليه وسلم لطعام صنعه، قال أنس: فذهب مع مرسول الله صلى الله عليه وسلم فأخبره أن ينفع الدباء من حوالي القصعة، قال: فلما أزل أحب الدباء من يومين.

الدباء هو القرع اليقظيني
DEDICATION

Dedicated to my father and my mother
To my wife
To my son Ahmad
To my daughters Shahd, Tasneem and Malak.
To all my family members
DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institutes, except where due acknowledgment has been made in the text.

Signature
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ABSTRACT

In Palestine like in other countries of the world, cancer is one of the most serious health problems that affect the duration and quality of the individuals’ life. Enormous efforts are invested to cope with this problem, but unfortunately limited success has ever been achieved with most of the therapeutic strategies. These efforts are usually complicated with the need for well experienced surgeons, lack of specificity and high cost, as well as being usually accompanied with a wide range of side effects.

As the conventional therapeutic strategies fail to fulfill the major requirements for a successful cancer therapy, the use of naturally developed anticancer agents has evolved as an alternative safe, low-cost and convenient one. Therefore, the use of plant extracts with potential anticancer therapeutic effects might be particularly significant, especially in Palestine, which is rich in thousands of plant species known for their medical uses. Moreover, the lack of expertise, the scares economical resources and the complicated political situation in Palestine don’t allow the application of sophisticated surgical, chemo- and radio-therapies to cure cancer.

Therefore, the current study, investigates the effect of crude water extracts from Bottle gourd (Lagenaria siceraria), Fig (Ficus carica) and Nettle (Urtica pilulifera) on cell lines derived from different human tissue origins (Hep3b: Hepatocellular carcinoma; Hela: cervical epithelial cancer; and PC-3: prostate cancer).

The results showed a concentration-dependent reduction in the final number of cancer cells in consequence to treatment with the aforementioned crude extracts. Two kinds of anticancer effects were evaluated and found to contribute to this reduction: the antiproliferation effect (decreased number of metabolically active cells) and cytotoxicity (decreased number of live cells). The three plants examined possess both of the effects with various degrees. Urtica pilulifera possess the strongest and most profound effects on the three cell lines, mainly by induction of cell death. On the other hand Lagenaria siceraria probably affects the three cell lines by a combination of cytotoxicity
and antiproliferation almost to a similar degree. *Ficus carica* most probably reduces the final number of metabolically active cells mainly by its antiproliferative effect.

Both *Ficus carica* and *Lagenaria siceraria* are edible plants that were chosen on the bases of being mentioned in the holy Quran. Therefore, although their effect is lower than that of *Urtica pilulifera*, their amount in the diet or as a treatment can be safely scaled up when ingested in their native form. On the other hand, despite its possible toxicity *Urtica pilulifera* is frequently orally used as a medication in many conditions by traditional medicine.

Further studies are needed to assess the active ingredients of *Ficus carica*, *Lagenaria siceraria* and *Urtica pilulifera*, involved in the antiproliferative or cytotoxic effects of these plants. These studies must involve the establishment of in vivo animal models and the application of more efficient extraction and fractionation techniques.
دراسة حول تأثير بعض المستخلصات النباتية على عدد من الخلايا السرطانية

المستخلص

يعتبر مرض السرطان من أخطر التحديات الصحية التي تواجه العالم في عصرنا الحديث، فعلى مدى العقود السابقة شكل هذا المرض بأنواعه المختلفة "السبيب الثاني للوفاة". و على الرغم من التقدم العلمي الكبير في أساليب العلاج إلا أن نسبة النجاح كانت محدودة، هذا بالإضافة للأعراض الجانبية الناجمة من هذه العلاجات وكذلك ارتفاع ثمنها ونقص الخبرات خصوصاً في فلسطين و كثير من دول العالم. هذه الأسباب دعت الباحثين للعودة إلى استعمال المواد المستخلصة طبيعياً من الكائنات الحية وخاصة النباتات والطحالب. وحيث أن فلسطين تشتهر بالنوع الحيوي النباتي فكان من الضروري أن يتم دراسة تأثير بعض هذه النباتات على مرض السرطان.

في هذا الإطار تم اختيار ثلاث نباتات معروفة في فلسطين وهي، النين ، والبليطين، والقريص. و لقد تم الاختيار بناءً على أن الأول والثاني ذكرا في القرآن الكريم كنباتين مبارزان أما القريص فإنه من أشهر النباتات الطبية والتي تستخدم شعبياً لعلاج العديد من الأمراض ( الروماتيزم، الحصى وأمراض الكلى).

ولدراسة أثر هذه النباتات على الخلايا السرطانية تم تحضير مستخلصات مائية من النباتات الثلاث كل على حدة، كما تم إضافتها على ثلاث أنواع من الخلايا السرطانية (كبد، ربوستات، عنق الرحم) ضمن الوسط الغذائي لهذه الخلايا. وقد تم قياس أثر هذه المستخلصات على معدل انقسام الخلايا، ونسبة موتها (السمية)، وقد تم تأكيد هذه النتائج بدراسة تغييراتها الشكلية.

و لقد دلت النتائج على أن نبات القريص هو الأشد أثرًا، سواء بخفض معدل الانقسام أو بزيادة نسبة السمية، مما يدل على أن الأثر الأرجح له هو قتل الخلايا. أما بالنسبة للنين والبليطين فقد أظهرما أثرًا جيدًا من حيث خفض معدل انقسام الخلايا أضاف إلى ذلك أن سمية النين كانت الأقل و بنسبة معينة البليطين مما يدعم أن أثرهما على الخلايا لم يكن بقل مлина خلايا فقط.

مزيداً من الدراسات حول أثر هذه النباتات سواءً معملياً أو على حيوانات التجارب ضرورية لتتأكد أثر هذه النباتات على السرطان.

تمثل هذه النتائج دافع قوي للتركيز على تناول النين والبليطين بشكل جيد للحماية والوقاية من السرطان.

"وَاللَّهُ خَيرٌ حَافِظًا وَهُوَ أَرْحَمُ الرَّاحِمُينَ"
Acknowledgment

Praise be to Allah, the lord of the worlds, and peace and blessings of Allah be upon the noblest of the Prophets and Messengers, our Prophet Muhammad.

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<td>ATCC</td>
<td>American Type Culture Collection</td>
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<tr>
<td>ADA</td>
<td>Adenosine deaminase activity</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Saline.</td>
</tr>
<tr>
<td>BPH</td>
<td>Benign Prostate Hyperplasia</td>
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<tr>
<td>DMEM</td>
<td>Dulbecco's Modified Eagle's Medium.</td>
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<td>EDTA</td>
<td>Ethylene-Diamine Tetra-acetic Acid.</td>
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<td>ELISA</td>
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<td>EGF</td>
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<td>HCC</td>
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<td>Hela</td>
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<td>HL-60</td>
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<td>HepG2</td>
<td>Human hepatocellular liver carcinoma cell line</td>
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<td>HepA-H</td>
<td>Liver cancer cells.</td>
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<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Buffer</td>
</tr>
<tr>
<td>hpcps</td>
<td>Prostate stromal compartment</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% Inhibitory concentration.</td>
</tr>
<tr>
<td>Lncap</td>
<td>Human, prostate, carcinoma cell line</td>
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<td>PC-3</td>
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<tr>
<td>R</td>
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<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>SE</td>
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INTRODUCTION

I. Overview
Cancer is one of the most serious health problems worldwide, affecting individuals from different sexes, ages, and races. It is a group of diseases, characterized by uncontrolled cellular growth with frequent cancer cells invasion to different body parts and spreading to other organs, a process referred to as Metastasis. Metastasis is the major cause of cancer related mortality (1). In 2005, cancer was the second leading cause of death among both men and women and accounted for 13% of the total 58 million deaths worldwide (1). In 2006, about 10.9 million new cancer cases are expected to be diagnosed worldwide and more than 7.8 million cancer patients may die (1). According to the latest report of cancer registry unit in Gaza strip, 5500 cases have been reported over the period from January, 1995 to December, 2003 (2). In addition, 1026 cancer patients died in 2004 in the Palestinian territories with a mortality rate of 28.2 per 100,000 (2).

Cancer is also a problem of economical dimensions with a very high level of expenses associated to it. For example the National Institute of Health, USA estimates that an overall of $209.9 billion were invested worldwide in 2005, for the sake of cancer research and management (3).

Cancer is a heterogeneous illness which can originate from many different organs of the human body. However, the most frequent cancer types in the world are lung, prostate, stomach, colorectal, and esophagus in men; and breast, lung, stomach, colorectal and cervical in women (1).

Prostate cancer is the most frequently diagnosed and the second leading cause of cancer death among men, with 234460 new cases estimated to occur in USA during 2006, and 27350 American men will die as a result of this disease(3). In Palestine, the mortality rate of prostate cancer was 1.4 per 100,000 during the period from January, 1995 to December, 2002 (2). Despite the fact there are
several cell types in the prostate, nearly all of the prostate cancers are adenocarcinoma, originating in the gland cells (3).

Liver cancer ranks as the sixth most common type of cancer worldwide (3). According to the Palestinian ministry of health liver cancer mortality rate was 1.6 per 100000 over the period from January, 1995 to December, 2003 (2). Many different liver related tumors are identified depending on the type of cells where they originate, from these types about 83% are hepatocellular carcinoma (HCC) that begin in the hepatocytes, the main type of liver cells.

Cervical cancer is the most common cause of cancer death among women in developing countries and the second most common cancer in women worldwide (1). It is caused by a change in the epithelial cells, which line the wall of the cervix, and the most common risk factor for this type of cancer is the human Papillomavirus (HPV) (1).

In the last decades there were great advances in the diagnosis of cancer as well as in the field of molecular oncology. However, the cure rate of most cancers remains low. Several strategies have been used to cure cancer among which the most common are surgery, chemotherapy, radiotherapy, and immunotherapy. Other modern approaches such as hormonal and gene therapy were proposed by researchers to replace conventional cancer therapy, with variable degrees of success (3, 4). All of these therapies have undesired side effects, they are usually not available all the time and they are expensive. For instance, in surgery the immune system is compromised due to the large amount of cortisol released subsequent to the surgery, which increase the probability of cancer relapse (5). Moreover, the current use of chemotherapy is accompanied with difficult side effects. It inhibits bone marrow stem cells proliferation leading to immune suppression (5). Radiotherapy which is widely used in the world is also accompanied by a great deal of side effects. Lymphocytes are most readily affected by radiation resulting in prolonged T-cell suppression (5). Other side effects such as, bone necrosis, lung fibrosis, skin devascularization, ulceration,
nausea, vomiting, and renal damage are also associated with all types of conventional therapies.

As the conventional cancer therapies failed to completely fulfill the criteria for a successful cancer therapy, the use of naturally developed anticancer agents has evolved as an alternative safe, low-cost and convenient one. Nontoxic chemoprevention agents from natural resources were proposed by researchers for this purpose.

Historically, plants with known therapeutic potential have long been used to cure a wide range of diseases. An example for these drugs is Morphine, which is a plant product discovered in 1861 as an analgesic agent. Later, Quinine the active component of Cinchona bark was isolated in 1820 as an effective anti-malaria drug (5, 6). Our Arabic tradition is particularly rich in medical plants that have been used by pioneer Arabic physicians to establish the basis for modern therapies. These were also recommended by our Profit Mohammad ( ﻋﻠﻴﻪ ﻋﻠﻰ ﺍﷲ ﻭﺳﻞ ﻣ) and the Holly Quran. Nigella, Garlic, Onion and Fenugreek are famous examples for these plants that were recently proven to have therapeutic effects on several illnesses.

The use of potentially curative plants might be particularly significant in the Palestinian territories where the plain and mountains are covered with more than 2600 plant species of which more than 700 are known for their uses as medicinal herbs or as botanical pesticides (7).

In this thesis we studied the therapeutic potential of three Arabic and Islamic traditional plants as anticancer agents. These were Fig (Ficus carica) and Bottle gourd (Lagenaria siceraria) that were mentioned in the holy Quran in more than one occasion as paradise and favorite plants and were selected based on that. Nettle (urtica pilulifera) was also tested as a popular traditional plant used for healing and diuretic purposes (8).
II. Objectives

The aim of the current study is to investigate the effect of Bottle gourd (*Lagenaria siceraria*), Fig (*Ficus carica*) and Nettle (*Urtica pilulifera*), extracts on Hep3b from human Hepatocellular carcinoma; Hela: human cervical epithelial cancer; and PC-3- human prostate cancer cells *in vitro* on the basis of Arabic and Islamic traditional medicine.

Specifically this study aims at:

1. Determination of the proliferation activities of each cell line in response to each plant extract treatment.

2. Determination of percent viability of each cell line in response to each plant extracts treatment.

3. Determination of any morphological changes of each cell line in each time performing viability testing assay in parallel.
I. Conventional cancer therapy

Despite the latest advances in medical sciences, and progress in strategies of cancer treatment, cancer currently remains a tragic disease and is one of the major causes of death worldwide. The principal methods of cancer treatment include chemotherapy radiotherapy and surgery.

Chemotherapy is a systemic treatment, to which the whole body is exposed. Among the most successful chemotherapeutic agent are Cisplatin, Mitomycin and Docetaxel. All of these agents enhance serious side effects or long term complication (9, 10 and 11). These side effects include kidney damage, hearing loose, lower blood count, liver damage, nerve damage, and blood vessel damage (12, 13).

External beams of Radiotherapy are associated with unacceptably high levels of local-regional toxicity (13). Particularly, it affects the rapidly dividing cells of mucosa, causing irritative urinary and blood loss (13). Later toxic effects result from damage to the more slowly proliferating cells such as fibroblasts, endothelial, or parachymal stem cells causing chronic fibrosis and vascular damage (13).

Other undesired side effects, such as, immune suppression, bone necrosis, lung fibrosis and skin devascularization are seen with all types of conventional therapies (11, 14).

II. Plants with anticancer effects

Plants are the chief source of natural products that are used in medicine. Even Aspirin, the world best known and most universally used medication, has its natural origins from the glycoside salicin which is found in many species of the plant genera Salix and Populus (15).
The scientific literature is rich in epidemiological studies that support significant differences in the occurrence of cancers between oriental and occidental populations (16). Generally, populations that consume a high level of natural herbal products have a reduced incidence of cancer. An example is the low incidence of colon cancer in Asian countries with high consumption of soybean products (16). Soy beans are the major dietary source of saponins, which have been suggested as possible anticancer agents (17).

There is lately a great interest in screening for plants to be used in cancer prevention and treatment. For this reason, extracts from different plants have been extensively studied. Table (1) summarizes some of these plants with confirmed anticancer activity, while the following sections review some of the studies relevant to this work.

A. Plants mentioned in Islamic literature with therapeutic activity
The use of many types of medicinal plants has been practiced by Arabic and Moslem physicians long before they were shown to possess any therapeutic value by modern research. For example, members of the Ginger family (Zingiberaceae) were mentioned in the Holly Quran in more than occasion and recently shown to protect mice against experimentally-induced mutagenesis and tumorigenesis (18). It also induces apoptosis in various immortalized or malignant cell lines (18). Pomegranate (Punica granatum), is also among the plants mentioned in Quran as a Paradise plant, and many studies highlighted its chemo-preventive potential (19, 20). In addition, Nigella (Nigella sativa), Fenugreek (Trigonella foenum graecum), and Henna (Lawsonia inermis) were also recommended by the Profit Mohammed (P.B.U.H) as medicinal plants. Extracts from these plants were recently shown to possess an anticarcinogenic effect (21). Refer to Table (2) for a summary of these and other studies.
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<th>Latin name</th>
<th>Type of extraction</th>
<th>Proposed active material</th>
<th>Subject</th>
<th>Effect</th>
<th>Ref.</th>
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<tr>
<td>Neem flowers</td>
<td>Azadirachta Indica (Meliaceae)</td>
<td>Methanol</td>
<td>Crude</td>
<td>Rat liver</td>
<td>Chemopreventive</td>
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<td>Turmeric</td>
<td>Curcuma longa (Zingiberaceae)</td>
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<td>Epiallocatechin-3-gallat (EGCG)</td>
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<td>Aqueous</td>
<td>Crude</td>
<td>Adenocarcinoma form colon cancer(HT-29)</td>
<td>Antiproliferative</td>
<td>26</td>
</tr>
<tr>
<td>Cranberry</td>
<td>Vacciniumm acrocarpon</td>
<td>Organic</td>
<td>Polyphenole</td>
<td>9 cancer cell lines Colon, oral, prostate</td>
<td>Antiproliferative</td>
<td>27</td>
</tr>
<tr>
<td>Bamboo</td>
<td>Bamboo</td>
<td>Methanol</td>
<td>Dihydroxypurpurin</td>
<td>Colo320, leukemia CMK-7</td>
<td>Inducing apoptotic cell death</td>
<td>28</td>
</tr>
<tr>
<td>Salomn's seal</td>
<td>Polygonum tinctorium</td>
<td>Organic</td>
<td>Ethyl acetate -and tryptanthrin crude</td>
<td>F344 rats</td>
<td>Chemopreventive</td>
<td>29</td>
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<tr>
<td>Radix</td>
<td>Coptidis rhizome</td>
<td>Aqueous</td>
<td>Crude</td>
<td>F344</td>
<td>Chemopreventive</td>
<td>30</td>
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<tr>
<td>Lemon grass</td>
<td>Cymbopogon citratus stapf</td>
<td>80% ethanol</td>
<td>Crude</td>
<td>F344 rats</td>
<td>Antimutagenic</td>
<td>31</td>
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Table (2): Literature summary of studies that examined the therapeutic potential of Islamic plant extracts

<table>
<thead>
<tr>
<th>Common name</th>
<th>Latin name</th>
<th>Type of extraction</th>
<th>Proposed active material</th>
<th>Subject</th>
<th>Effect</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>Pomegranate</td>
<td>Punica granatum (Punicaceae)</td>
<td>- Aqueous, and organic pericarp</td>
<td>Polyphenols</td>
<td>Human breast cancer cell line</td>
<td>Inhibition of proliferation</td>
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</tr>
<tr>
<td>Pomegranate</td>
<td>Punica granatum (Punicaceae)</td>
<td>Seed oil</td>
<td>Polyphenols</td>
<td>Rat skin tumor</td>
<td>Chemopreventive</td>
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<tr>
<td>Pomegranate</td>
<td>Punica granatum (Punicaceae)</td>
<td>Seed oil</td>
<td>Polyphenols</td>
<td>Mouse mammary organ culture</td>
<td>Chemopreventive</td>
<td>20</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>Punica granatum (Punicaceae)</td>
<td>Seed oil</td>
<td>Linolenic acid</td>
<td>Rat colon cancer</td>
<td>Suppress Azoxymethane induced cancer</td>
<td>33</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>Punica granatum (Punicaceae)</td>
<td>Aqueous or oily compartment</td>
<td>Ellagi, caffeic, luteolin and punic acid</td>
<td>Prostat cancer cell line</td>
<td>Antiproliferative</td>
<td>34</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allium sativum (Liliaceae)</td>
<td>Oil- soluble</td>
<td>Organosulfur compounds</td>
<td>Liver cancer</td>
<td>Chemopreventive</td>
<td>35</td>
</tr>
<tr>
<td>Henna</td>
<td>Lawsonia inermis (Lythraceae)</td>
<td>Chloroform</td>
<td>Tocopherol</td>
<td>Liver carcinoma cell line</td>
<td>Antioxidant</td>
<td>36</td>
</tr>
<tr>
<td>Plant</td>
<td>Scientific Name</td>
<td>Tissue Type</td>
<td>Preparation</td>
<td>Cancer Type</td>
<td>Effect</td>
<td>Value</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------</td>
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<td>-------------</td>
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</tr>
<tr>
<td>Henna</td>
<td><em>Lawsonia inermis</em> (<em>lythraceae</em>)</td>
<td>Aqueous</td>
<td>Crude</td>
<td>Rat Hepatocarcinoma</td>
<td>Reduction of severity</td>
<td>37</td>
</tr>
<tr>
<td>Grape</td>
<td><em>Vitis vinifera</em> (<em>Vitaceae</em>)</td>
<td>Aqueous</td>
<td>Flavanol derivatives</td>
<td>Hepatorenal carcinoma rat</td>
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<td>38</td>
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<tr>
<td>Nigella</td>
<td><em>Nigella sativa</em> (<em>Ranunculaceae</em>)</td>
<td>Aqueous</td>
<td>Crude</td>
<td>Natural Killer cells activity</td>
<td>Augmentation</td>
<td>21</td>
</tr>
</tbody>
</table>
B. Plants studied in this work

1. Bottle gourd (Lagenaria siceraria)

Bottle gourd (Lagenaria siceraria) is one of the cucurbitaceous crops present in Palestine and other parts of the world. It was mentioned in the Holly Quran as mercy and therapy for the Profit Yonis ( ﷲ ﷳ ﷲ ﷳ) . It is classified under the Lagenaria genus of the cucurbitaceae family. The medicinal benefits of Lagenaria siceraria are rarely dealt with in the scientific debate. In one study the Lagenin fraction which was extracted from seeds of the Bottle gourd, was regarded as a novel ribosome–inactivating protein with a ribonucleolytic activity (39).

The Cucurbitaceae family is composed of thirty four genus members and ninety three taxa overall. Many members of these genera have been reported as medicinal plants, and some of these members were considered as anticancer plants. The Cucurbita L, Ecballium A, Lagenaria Ser, Siraitia, Luffa P, Momordica L. and Trichosanthes L, are examples for these genera.

The Momordica genus is composed of four species: Momordica charantia, Momordica balsamina, Momordica dioica and Momordica cochinchesis. The Momordica charantia species (Bitter gourd) contains an array of novel and biologically active phytochemicals, including (triterpenes), proteins and steroids. Triterpenes in (bitter melon) have been clinically demonstrated to possess the ability to inhibit the guanylate cyclase enzyme (40). This enzyme is thought to be linked to the mutagenic signaling of leukemia, and solid tumors (40). Other phytochemicals that have been documented with cytotoxic activity are a group of ribosome-inactivating proteins named alpha- and beta - momorcharin, momordin, and cucurbitacin B. A chemical analog of these proteins was developed (MAP-30) and reported to be able to inhibit prostate tumor growth (41). The phytochemical momordin has a clinically demonstrated cytotoxic activity against Hodgkin’s lymphoma in vivo (42). Several other in vivo studies have demonstrated the cytotoxic and antitumor activity of the entire plant of bitter
melon (43). In one study, a water extract blocked the growth of rat prostate carcinoma (44). Another study reported that a hot water extract of the entire plant inhibited the development of mammary tumors in mice (45). Numerous in vitro studies also demonstrated the anti-cancer activity of bitter melon against numerous cell lines including liver cancer, human leukemia, melanoma and solid sarcomas (46, 47).

Momordica cochinchinensis, also known as (Gac fruit) is a bright-red fruit rich in carotene and lycopene. Similar to Momordica charantia, Momordica cochinchinensis is postulated to contain some antioxidant components in the fruit extract. Recently, Gac fruit was found to contain a higher lycopene concentration than other fruits (48). According to a clinical trial study at the Medical School of Hanoi University, Vietnam, an oil extract of Gac fruit was found to be effective in the treatment of liver cancer (49).

In a recent study the ability of Gac fruit water extract to suppress colon cancer cell line growth in vivo and in vitro was studied. It was found that this extract inhibited the growth of the colon 26-20 adenocarcinoma cell line, transplanted in Balb/c mice, reducing the tumor wet weight by 23.6% (49). Moreover, it was found to inhibit cell proliferation in the (colon 26-20) and (hepG2) cells in a concentration dependent manner (49).

Cucurbita L. genus is one of the most famous members in cucurbitaceae family, it is composed of nine species of which are, Cucurbita pepo L (pumpkin), Cucurbita moschata (crookneck squash ), Cucurbita maxima (winter squash ) and others.

Cucurbita pepo, (pumpkin) is one of the popular plants in Palestine. Its fruits consist of up to 50% fatty acid, carotenoides, proteins, tocopherol and phytosterol (50). Pumpkin seeds have been used for a long time in the traditional medicine in North America and Mexico as an antihelmintic agent and as a supportive treatment of the bladder disorders and urination difficulties (50). The childhood enuresis nocturna and irritable bladder have also been treated
successfully with pumpkin seeds (50). Pumpkin has also been used to eradicate tapeworm (50). Furthermore, Pumpkin seeds are considered an alternative treatment for stage I and II benign prostatic hyperplasia and for irritable bladder (51). Muscatine is a novel ribosome–inactivating protein that has been recently purified from pumpkin seeds, and shown to have a strong inhibitory activity to protein synthesis (52). It has been used for construction of an immunotoxin that can efficiently and selectively kill cultured human melanoma cells (52).

A novel immunotoxin Muscatine (Ng76) was prepared successfully form pumpkin extracts and found to efficiently inhibit the growth of targeted (M21) melanoma cells (53). These results implied that Muscatine could be used as a new potential anticancer agent. Moreover, the pumpkin seeds extract can modulate the immunopathological pathways induced by interferon-γ (50). This finding suggests an immunoregulatory potential of compounds contained in pumpkin seeds.

The anticancer and antiinflamatory activities of squash (Cucurbita maxima) are reported by a study carried out on breast, lung, and central nervous system cancer cell lines (54).

Siraita grosvenorii (Momordica grosvenorii) is the only species in the genus Siraitia form cucurbitaceae family; its fruits have been used for the treatment of pharyngitis, and antitusssive medicine in Japan (55). Extracts from this fruit also where shown to possess an anticancer effect. For example they exhibit a significant inhibitory effect on the two stage mouse-skin carcinogenesis in vivo, induced by 7,12-dimethyl benz[a]anthracene (DMBA) and 12-0-tetradecanoylphorbol –13-acetat (TPA) (56, 57).

Trichosanthes Kirilowii (Maximowii) a species of the Trichosanthes genus from the cucurbitaceae is a popular herbal medicine in China. It has been used to induce midterm abortion and to treat ectopic pregnancies, hydatidiform and trophobalstic moles (58). Maximowii has also been found to posses various pharmacological properties including immunomodulatory, anti- human immunodeficiency virus, and anti tumor activities (58).
Trichosantin is a ribosome inactivating protein produced from the root tubers of \textit{Trichosanthes Kirilowii}. This protein has been regarded as an effective anti-tumor agent that is highly specific to choriocarcinoma cells from trophoplast (59, 60).

The growth inhibitory effect of Maximowii extract on the liver cancer cells (HepA-H) and the cervical cancer (Hela) cells was investigated \textit{in vitro}. The data showed that the Maximowii extract has a strong cytotoxicity and induction of apoptosis of Hela cells (61, 62 and 63).

\textit{Ecballium elaterium} L. Rich, Squirting cucumber is one of the most distributed cucurbitaceous herb in Palestine, locally known as "Qitha al Hamir". In an ethnobotanical study carried out in Palestine it was reported that "Qitha al Hamir" is used as a diuretic, and to treat urine's retention, piles, swollen testicles, and yellow fever (64). Cucurbitacin B a protein separated from this plant was reported as an anti-inflammatory and antitumor agent (65).

2. \textbf{Fig (Ficus carica)}

\textit{Ficus carica} (the common edible fig) is a member of \textit{Ficus} L. genus which belongs to the \textit{Moraceae} family. Many species of this genus such as, \textit{Ficus racemosa}, \textit{Ficus bengalensis}, \textit{Ficus awkeotsang Makino}, \textit{Ficus microcarpa}, \textit{Ficus pumila} and \textit{Ficus carica} are of a medicinal value.

For example ethyl acetate extract of \textit{Ficus racemosa} resulted in significant tumor growth inhibition by 76% in \textit{in-vivo} study (66). An extract from \textit{Ficus racemosa} was used to contra diet the carcinogenic effect of Ferric nitrilotriacetate a well known renal carcinogen (67). When rats were orally treated with this extract, they showed a significant decrease in lipid peroxidation, xanthin oxidation, $H_2O_2$ generation, blood urea nitrogen, DNA synthesis and incidence of tumors (67). These data suggest that \textit{Ficus racemosa} extract is a potent cancer preventive agent.

An aqueous extract of \textit{Ficus pumila} species was reported as an active preventive agent against cervical cancers \textit{in vivo} (68). Pectin esterase, an inhibitor of a
group of cationic polypeptides, was extracted from Jelly fig (*Ficus awkeotsang Makino*). This polypeptide displayed a strong growth inhibition of human leukemia cell lines via induction of apoptosis in a dose and time dependent manner (69).

According to an ethnopharmacological study carried out in the United States of America, *Ficus bengalensis* has also been reported as a medicinal plant used for reduce tumor growth on bone (70). In a different study that aimed to demonstrate the possible antioxidant role of *Ficus bengalensis* against a cholesterol rich diet, it was found that treatment of rabbits with a water extract decreased the serum cholesterol, and triacylglycerol levels (71). In addition, treatment with the same extracts led to decrease in lipid peroxidation (71).

In an ethnopharmacological survey carried out in Palestine, edible fig was reported as one of the most popular plants that are used to cure Warts, kidney stones, respiratory system, asthma and cholesterol (7). A similar study carried out in Pakistan indicated that edible fig fruits are useful in the case of the head blood leprosy, nose bleeding, while the root is tonic and useful in leucoderma and ringworm (72).

An *in vitro* assessment of the antihelmintic effect of Papaya, Fig and Kiwi against gastrointestinal nematodes showed that fig and papaya caused marked damage of the cuticle of *Heligmosomoides polygyrus* (73).

The antidiabetic effect of *Ficus carica* leave extracts has been reported. In one study, the parameters related to oxidative stress in rats were measured and the results confirmed that the antioxidant status is affected in diabetes, and that *Ficus carica* extract tends to normalize it (74).

3. **Roman nettle (Urtica pilulifera)**

*Urtica pilulifera*, a facultative wetland plant, is common in many parts of the world. It is a member of the *urticaceae* family which is considered as an invasive plant. Members of the genus *Urtica*, including *Urtica massaica*, *Urtica parviflora*,
*Urtica pilulifera, Urtica urens,* and *Urtica dioica* are known for the stinging sensation caused by the injection of histamine, acetylcholine, and serotonin into the skin by fine, needle-like projections found on the leaves and stems of the plant (8, 75). Despite this deterrent, many of these nettle species have been used for generations in the preparation of herbal medications (76, 77).

Many plants of the family *Urticaceae* are commonly used in the world for their anti-inflammatory properties, particularly *Urtica leptophylla*. However, *Urtica dioica* is by far the most widely used plant of the family, both culturally and medicinally (78, 79).

*Urtica dioica* is a popular anti-diabetic drug, acting against hyperglycemia (80). To determine this role a water soluble extract of *Urtica dioica* was administered to rats under oral glucose tolerance test. The results showed that rats receiving the extract had 33% lower glucose levels than the control (80). Recently, a number of studies have been conducted to investigate the effect of *Urtica dioica* in the treatment of rheumatoid arthritis as well as the mechanisms through which it acts (81). *Urtica dioica* is also believed to have an antioxidant role against oxidative damage (81, 82). Antioxidants halt the free radical chain reaction, thereby playing a preventive role in cancer, mutations, and accelerated aging. Oxidative damage is usually caused due to the cellular and tissue destructive effects of some chemicals usually formed as byproducts of normal metabolism. These include, superoxide, hydrogen peroxide, hydroxyl radicals, or singled oxygen all of which are common byproducts of normal metabolism, attack cells and tissues. An imbalance between active oxygen and antioxidants may cause stimulation of peroxisomes and polymorphonuclear leukocytes and macrophages, which can result in diseases such as arthritis and accelerated aging (81).

In a study on the antioxidant activity of non cultivated plants, it has been shown that the *Urtica dioica* extract inhibits lipid peroxidation by more than 50% (82). In a similar study, it was reported that an aqueous extract of *Urtica dioica* is the
most effective antioxidant among series of some other medicinal plants (83, 84 and 85). *Urtica dioica* also showed a potential antioxidant effect on ischemic muscle tissue, generated by a turniquet in rats (86). In another study, antioxidant activity of *Urtica dioica* extract was determined to be iron-promoted oxidation of phospholipids, linoleic acid, and deoxyribose (87). Many of the phytochemicals shown to exhibit antioxidant activity are polyphenols, such as caffeic malic acid, common in *Urtica* extract (88).

Extracts of *Urtica dioica* were also shown to have an Antiproliferative effects in vitro. Such effects were determined by testing methanolic aqueous extracts from deride milled stinging nettles root (89). Lymph node carcinoma of the prostate cell (Lncap), and human primary culture of the prostate stromal compartment (hpcps) were cultivated without extract (control) or treated with various extract concentrations at different time intervals. The cell proliferation was determined colorimetrically, and the cytotoxicity was tested by trypan-blue dye exclusion test. The results demonstrated that the methanolic-aqueous extract treated Lncap cells are reduced in proliferation in comparison to the untreated controls, while hpcps remained unchanged.

In an ethno-botanical survey carried out in the west bank, Palestine to evaluate the most popular traditional plants, *Urtica pilulifera* was reported as a popular traditional plant used as aphrodisiac, diuretic, and fresh young leaf are eaten to treat kidney stone Rheumatism and bleeding (64).

Another survey conducted in the west bank and Negev desert, of Palestine concluded that *Urtica pilulifera* is a traditional plant widely used in the treatment of cancer (7). It was suggested that a standard decoction be prepared from 50 g plant leaves in one liter and taken orally, 150 ml, 3-4 times/day until the condition improves (7).

**III. Cancer cell lines**

A cell line refers to cells derived from a single cell type that have been adapted to grow continuously in the laboratory and are used in research (90).
Prostatic cancer cell line PC-3, cervical epithelial cancer cells (Hela) and Hepatocellular carcinoma cell line (Hep3b) were used in the study.

A. Hepatocellular carcinoma (Hep3B) cell line
The Hep3b cell line is a hepatocellular carcinoma derived from 8 years human male juvenile (91). Analysis of the cell culture fluid form Hep3b revealed that 17 of the major human plasma proteins are synthesized and secreted by this cell line. Also, Hep3b produces the two major polypeptides of Hepatitis B virus surface antigen. This cell line was used to generate an animal model for metastatic hepatocellular carcinoma by injection into athymic mouse (101).

The Hep3b cell line was used to determine the cytotoxic effects of 6-xanthone compounds from the peel of fruits of *Garcinia mangostana* on hepatocellular carcinomas using the MTT proliferation assay (102).

A Similar study examined the effect of *Ginkgo biloba* extract on cell proliferation and cytotoxicity in human hepatocellular carcinoma cells using this cell line in addition to HepG2 cell line (103). In this study cell proliferation and cytotoxicity were determine by the (MTS) assay.

B. Cervical epithelial cell line (Hela)
The Hela cell line was derived from a cervical epithelial cancer from a 31 years old female, and it has been reported to contain Human Papillomavirus18 (HPV-18) (91). Hela cells have been used in many studies which aimed to investigate the anticancer role of artificial and natural products.

The antiproliferative effect of *Chlorophytum comosum* root extract was tested in vitro against Hela, HL-60, and U937 cells (97). The extract was found to have antiproliferation effect, and to induce apoptosis in these human cell lines (97).

The cytotoxic effect of the anticancer drug Rhodamine and Herbal extracts of Glycerhizan Radix, Rhei Rhizoma and Zingibers Rhizoma on Hela cells was also suggested (98). Hela cells were used to indicate that the combination of
anticancer drugs with some herbal extracts contributes to the enhancement of clinical outcomes in cancer thereby (98).

In a cytotoxicological study of Iranian conifers on Hela cells, a colorimetric assay (MTT) was used, and the findings showed that *Juniperus Sabina* extract posses an inhibitory effect against Hela cells by reducing the cell viability to less than 50%(99). Another similar study investigated the suppressant effects of *Vandellia cordifolia* on human cervical cancer Hela cell line. The result indicated that this plant extract suppressed Hela cell line proliferation by cell cycle progression inhibition at the G1 to S phase transition (100).

C. Prostate cell line (PC-3)
The Prostate cell line (PC-3) was initiated from a grade-IV bone metastasis Prostate adenocarcinoma from a 62 years-old Caucasian male (91). The establishment, characterization, and tumorigenicity of (PC-3) cell line were reported (92). The cultured cells showed anchorage-independent growth in both monolayers and in soft agar suspension and can produce subcutaneous tumors in nude mice.

Electron microscopic studies revealed many features common to neoplastic cells of epithelial origin including numerous microvilli, junctional complexes, abnormal nuclei and nucleoli, abnormal mitochondria, annulate lamellae, and lipoidal bodies. In general, the functional and morphological characteristics of PC-3 are those of a poorly-differentiated adenocarcinoma (92).

The PC-3 cell line has been used *in vivo* and *in vitro* in many studies in the screening for new anticancer agents. PC-3 cells were used in a recent study to test the cytotoxic and antiproliferative effects of the crude extract of Thai medicinal plants for cancer treatment (93). A similar study evaluated the effects of plant hormones on PC-3 cell line *in vitro* reported that PC-3 cells exhibited differential sensitivities to three hormones, and PC-3 proliferation was significantly inhibited (94). The human prostate carcinoma PC-3 was also used to determine the chemosensetivity of prostate cancer to genistein isoflavon...
(metabolites of soy) and ß-lapachone (plant product) \textit{in vitro} using trypan-blue and MTS bioassay (95). The type of cell death, apoptosis or necrosis in response to treatment of PC-3 cells was determined using different assays such as annexin V-FITC and PI/TUNEL apoptosis assays (96).
## MATERIALS AND METHODS

### I. Materials

#### Reagents and disposables

<table>
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<tr>
<th>Biological Industries</th>
<th>Biological Industries</th>
<th>Biological Industries</th>
</tr>
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<tr>
<td><em>Biet Haemic, Israel</em></td>
<td>• DMEM powdered medium</td>
<td>• 0.05% trypsin-EDTA solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cell Proliferation kit (XXT kit)</td>
</tr>
<tr>
<td>Sartorius. UK</td>
<td>• Sartolab RF/BT Vacuum Filtration Units (0.2µm filter)</td>
<td>• Minisart 0.2µm syringe filter.</td>
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<tr>
<td>Greiner bio-one.</td>
<td>• Cell culture flasks.</td>
<td>• PS Plate six-well.</td>
</tr>
<tr>
<td>Germany</td>
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<td>• PS Plate-96-well.</td>
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#### Buffers and solutions

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<th>DMEM medium</th>
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<td></td>
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</tr>
<tr>
<td></td>
<td>• 25mM HEPES (pH 7.4),</td>
</tr>
<tr>
<td></td>
<td>• Penicillin (180 units/ml)</td>
</tr>
<tr>
<td></td>
<td>• Streptomycin (100µg/ml)</td>
</tr>
<tr>
<td></td>
<td>• Amphotericin-B (0.2 µg/ml)</td>
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<table>
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<tr>
<th>HEPES (Buffer)</th>
<th>HEPES (Buffer)</th>
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<tr>
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<td>• 280 mM NaCl, 1.5 mM Na2HPo4 and 25mM HEPES</td>
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</table>
II. Methods

A. Plant collection

Three plants were studied in this thesis: *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. They were collected at different times, localities and conditions.

1. Roman nettle (*Urtica pilulifera*)

*Urtica pilulifera* locally named “Qurrais” (قريص) or “Huraiq” (خريق) belong to the family *Urticaceae* which is a member of the *Urticales* order (Figure 1). It is an annual plant that grows to 1 meter high by 0.5 meter wide and lives in many types of soils, especially light ones, with a PH ranging from acid to alkaline and a full sun with moderate moisture. This plant withstands frost and is distributed in a weed of cultivated lands, waste places, hedges and on damp roadsides or near dwelling. From all of these sites in the middle districts of Gaza, nettle was collected during March and April, 2005 (the end of winter and the beginning of spring in Palestine). All of the plant parts including roots, leaves, and seeds were harvested by drawing the stem from the soil. All plant parts were washed under tap water and then derided in the shadow for three to five days. Dried leaves and seeds were grounded by hand and stored in dry and clean bottles until the time of experiments.

![Figure 1. Photograph of *Urtica pilulifera* in its habitat, shot by the researcher, April 2005.](image)

Kingdom: *Plantae* – Plants  
Subkingdom: *Tracheobionta* – Vascular plants  
Superdivision: *Spermatophyta* – Seed plants  
Division: *Magnoliophyta* – Flowering plants  
Class: *Magnoliopsida* – Dicotyledons  
Subclass: *Hamamelidae*  
Order: *Urticales*  
Family: *Urticaceae* – Nettle family  
Genus: *Urtica* L. – nettle P  
Species: *Urtica pilulifera* L. – Roman nettle P
2. Bottle gourd (*Lagenaria siceraria*)

Figure 2 shows the vigorous annual herb Bottle gourd, locally named “Yaktein” (يقطين), which is a member of the *Cucurbitaceae* family. The stems prostrate or climb up to 5m long, and the leaves are simple, shortly and softly hairy, and non-aromatic when crushed. *Lagenaria siceraria* is growing mainly on alluvial sandy soil and red loam, on flat areas and moderate slopes, on rocky ridges, on river banks and in dry riverbeds.

*Lagenaria siceraria* was collected during May and June (beginning of the summer). Also all parts of the plant (roots, leaves, and fruits) were harvested by drawing the plant stem. The seeds were isolated form the fruits, then seeds and leaves were washed under tap water and dried in shadow places for seventh to ten days. The dried plant parts were grounded by hand and stored in dry and clean bottles until the time of experiments.

![Figure 2. Photograph of *Lagenaria siceraria* in its habitat, shot by the researcher, may 2005.](image)

3. Edible fig (*Ficus carica*)

The edible fig belongs to the family *Moraceae* (Figure 3), centers around the Mediterranean region, and is commonly cultivated in mild–temperate climates. *Ficus carica* is a tree of small dimensions; (3-9m) high with numerous branches and a trunk rarely more than (17.5cm) in diameter, and contains copious milky latex. Lateral spread of roots is extensive and, in certain soils, the roots are quit deep. Plant leaves were harvested in summer 2005, washed under tap water and
shadow-derided for a week at least. Deride leaves were grounded and stored in a dry and clean bottles until the time of experiments.

Figure 3. Photograph of *Ficus carica* in its habitat, shot by the researcher, June 2005.

B. Preparation of the crude plant extracts
Twenty grams of grounded dry parts of the upper-indicated parts of each plant were soaked in 80 ml distilled water (20% dry wt/v). The extraction was carried out by using a reflux condenser (Figure 4a) at boiling temperature for 30 min. The condenser returns the extract vapor to the boiling flask. The extract was cooled at room temperature and filtered using a Buchenr funnel (Figure 4b) with 0.4µm cellulose filter paper. Finally the extract was sterilized-filtered using vacuum filter with 0.2µm cellulose filter paper (Sartorius, UK). About 80% of the extract volume was collected after filtration, and stored in sterilized bottles at 4°C (Stock extract). The different working dilutions were prepared in the cell culture media as indicated (104, 105, 106, 107 and 108).
Figure 4. Laboratory instruments used for extract preparations.

(a) Reflux condenser and (b) Buchenr funnel.

C. Cell culture

The human cervical carcinoma cell line (Hela), hepatocellular carcinoma cell line (Hep3b) and Prostate cancer cell line PC-3 were obtained from the Hebrew university of Jerusalem. They were chosen based on their high proliferation rates and availability. The cell lines were routinely maintained as a monolayer in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum (inactivated at 55°C for 30 min), 25mM HEPES (pH 7.4), penicillin (180 units/ml), streptomycin (100µg/ml) and amphotericin-B (0.2 µg/ml)(4,109). The cells were grown to confluency in a humidified incubator with 5% CO₂ in polystyrene culture flasks. They were subcultured by removing the medium and adding 4-6 ml of 0.05% trypsin-EDTA solution. The cells were allowed to detach at (37°C) for 5-10 min. About 1/6 of the trypsinized Hep3b or 1/4 of the other cells was passed twice a week to new flasks containing fresh-medium.
D. Experimental design
- In the first (preparatory) experiments, the extracts were prepared from the desired plant parts. The working extract concentrations were then determined by testing an array of extract dilutions on one cell type.

- The working extract concentrations were tested for each plant against each of the three cell lines in terms of cellular proliferation.

- The effects of the same extracts working concentration were further analyzed by viability assay to determine the type of cellular effect.

- Observation of the morphological changes was carried out in parallel to the viability assays.

- The data was statistically analyzed and comparison of the results from different methods was done and reported.

E. Determination of growth characteristics
Cells from Hela, Hep3b and PC-3 cell lines were seeded in 6-well plats, at a density of 100000 cells/well, as indicated earlier. Three wells from each cell line were trypsinized and harvested after 24, 48 and 72 hours and the medium was changed for the cells continuing to grow at each time point. The harvested cells were counted on hemocytometer and the average number of 3 wells was used for the growth curve.

F. Determination of plant extract working concentrations
Plant extract-DMEM preparations were prepared by incorporation of sterile stock extracts into the DMEM, media preparation (the plant extract volume was included in final volume calculation). The highest extract-DMEM plant concentration achieved this way was 16% dry wt/v (Stock extract-DMEM), and the desired extract-DMEM concentrations were prepared by dilution with the proper volume of complete DMEM medium.
To determine the concentration with a 100% cytotoxic effect, 200000 cells were seeded onto 25cm² flasks containing DMEM media for 24 hours. The medium was then replaced by 8ml of 16, 8, 4 or 0.0% plant extract-DMEM. The flasks were prepared in triplicates, and the cells were incubated in extract presence for 24 hours. The viability of cells was determined as described in the following section.

G. Cell Proliferation Assay

A commercially purchased colorimetric kit was used to determin the proliferation activity of cells (Biological Industries, Biet Haemic, Israel). The method is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to orange-colored compound of formazon. The formed dye is water soluble and the dye optical intensity can be determined at 490 nm. The intensity of the dye is proportional to the number of metabolically active cells. The test procedure includes cultivation of cells in 96-well plates, addition of the XTT reagent and incubation for 2-24 hours, during which an orange color is formed. The greater the number of metabolically active cells in the well, the greater the activity of mitochondrial enzymes and the higher the concentration of the dye formed, which can then be measured and quantified (117).

The Hela and PC-3 cells were seeded in 96-well plats at a density of 7000 cells/well, whereas the Hep3b cells were seeded at density 5000 cells/well. The cells were maintained at (37°C) for 24 hours in the presence of 100 µl DMEM. The medium was replaced with 8, 4, 2, 1, 0.5 or 0.25% plant extract-DMEM concentration in triplicates. Twenty four hours later, 50 µl of the XTT reaction solution were added to each well and the plates were incubated at 37°C for 3 hours. The absorbance was measured with ELISA reader at a wave length of 490 nm. The reference absorbance (non specific background reading) was measured at 630nm. Negative control cells were incubated with no extract in the medium.
The cells proliferative activity was estimated by calculating the ratio of remaining viable cells in each well in comparison to the control and expressed as (% of control). Each assay was repeated for two additional times and the mean and standard error was calculated for each extract concentration (113, 114, 115, and 116).

H. Trypan-blue dye exclusion Viability assay

The trypan-blue dye exclusion assay was used to determine the plant extract-mediated cell death (89, 110, 111, and 112). 200,000 cells/well were seeded in 6-well plates and grown as previously described. After 24 hours the medium was replaced with different plant extract-DMEM concentration (0.8, 0.4, 0.2, 0.1, 0.05, and 0.025%) in triplicates. After 48 hours incubation the medium was discarded and the cells were harvested by trypsinization and washed twice with PBS. A volume of 0.4% trypan-blue stain that is equal to the residual PBS was then added. After 5 min incubation, the cells were counted with a hemocytometer by compound light microscope. The unstained (viable) cells and the blue-stained (dead) cells were counted separately.

Negative control cells were incubated with DMEM media without any extract and treated the same way. Positive control cells were incubated for 10 min with 0.5mM H₂O₂ before being harvested and counted as described.

The % cell viability was calculated using the following equation:

\[
\text{% cell viability} = \frac{\text{total viable cells (unstained)}}{\text{total cells (stained + unstained)}} \times 100
\]

Each experiment was carried out in triplicate and repeated for one more time and the average of 6 wells was considered for each extract concentration.

I. Determination of morphological changes of the cells in culture

The different cell lines normally grown in 6-well plates or incubated with the desired extract concentration for the purpose of viability testing were monitored by an inverted microscope in 24 hours intervals. Any morphological changes in
the cells shape, level of adhesion and any other alterations were observed and documented by photography before end of experiment (104, 114, 117 and 118).

**J. Statistical analysis**

All values expressed as mean ± standard error of the mean by Microsoft Excel. The data were statistically analyzed by SPSS by performing the correlation, regression and one-way ANOVA tests.

For each experiment the data obtained was blotted against extract concentration and the obtained curve equation and R² value were calculated by the Microsoft Excel software. The extract concentration that gives 50% or 100% reduction in the number of metabolically active cells or in viability of cells (IC₅₀ and IC₁₀₀ respectively), was determined by substitution in the obtained equation (89.119).
RESULTS

I. Determination of the cell lines growth characteristics

Figure 5 shows the growth curve of each of the hepatocellular carcinoma Hep3B, cervical epithelial Hela and Prostate PC-3 cell lines in normal DMEM culture media. The three cell lines maintained exponential growth characteristics until the end of experiments (96 hours). The Hep3b cells grew faster than the other two cell lines. However, no cell line growth curve reached a plateau at the end of the experiments (four days). It should be emphasized that the time of all of the following experiments did not exceed this time.

![Growth curves of Hep3b, Hela and PC-3 cell lines.](image)

Figure 5. Growth curves of Hep3b, Hela and PC-3 cell lines.

Hepatocellular carcinoma Hep3b, Cervical epithelial Hela and Prostate PC-3 Cells were seeded at a density of 100000 cells/well. The wells were prepared in triplicates for each time point and were incubated at 37°C in 5% CO2. Three wells from each cell line were harvested and counted at each time point and the rest of wells allowed continuing growing. The numbers of cells were blotted against the growth duration.
II. Determination of the different plant extracts working concentrations

Plant extracts-media were prepared by introducing sterile plant extracts into the DMEM culture media (the plant extract volume was included in final volume calculation). The plant extract sterilization by a 0.2µl filter was difficult to perform as the filter was blocked by the fine pieces of extract. Thus the highest plant concentration achieved (stock concentration) was 20% (wt/v). The examined extract concentrations were 0, 4, 8 and 16% for each plant type. The effect of such concentrations from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* on the viability of Hep3b cells is illustrated in Figure 6. *Urtica pilulifera* concentrations less than 4 % (wt/v) showed a profound effect on the viability of cells, while 4% and higher concentrations gave maximal effect of 100% cell mortality. According to these result the gap between 0% and 4% is critical and a wider range of concentrations in this interval are necessary. *Lagenaria siceraria* gave weak reduction of Hep3b cells viability and the testing concentrations did not give more than 70% effect. *Ficus carica* had lower but considerable effect on the viability of Hep3b cells, and the highest concentrations did not reach the 50% inhibition.

From Figure 6 it’s noticed that concentrations lower than 4% are needed to be introduced into the experiment to assess cell death before reaching maximal values. Moreover, it seems futile to examine concentrations higher than 8% as all of the extract effects reach plateaus at those concentrations. Therefore the concentrations (0.25, 0.5, 1, 2, 4 and 8%) of each plant extract were used to be examined in the rest of the study. Any effect within this range of concentrations would be amplified theoretically if we increased the concentrations.
Figure 6. A preliminary experiment of the effect of *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* on the viability of Hepatocellular carcinoma Hep3b cells. The percent viability of cells was calculated as stated previously and plotted against the plants concentrations.
III. Effect of plant extracts on cancer cells proliferation activity in culture

A. Hepatocellular carcinoma cell line Hep3b

The Human hepatocellular carcinoma cell line Hep3b was maintained in the presence of increasing concentrations of each of the three plant extracts as indicated in each experiment. The following sections describe the effect of each plant extract on this cell line in terms of proliferation activity (Figure 7).

1. Effect of *Urtica pilulifera*

Extracts from the plant *Urtica Pilulifera* were able to inhibit the proliferation of the Hep3b cells in a dose response manner. The proliferation activity of the cells was found to be significantly, inversely related to increasing the extract concentration in the medium (P =0.013, R =-0.86). This inhibitory effect was initiated at low extract levels and reached a 50% proliferation inhibition (IC$_{50}$) when the cells were grown in media with 1.9% extract concentration (R$^2$ =0.83). The highest proliferation inhibition level was about 85% of the control group, however was obtained at the maximum extract concentration tested (4%).

2. Effect of *Lagenaria siceraria*

A dose response effect was seen when the Hep3B cells were incubated with increasing concentrations of the *Lagenaria Siceraria* extracts in the culture medium. The degree of proliferation is also inversely related to the increase in medium extract content. This relationship is significant with P-values of less than or equal to 0.01 and R values equal to -0.98. However, the extract inhibitory effect was initiated at higher concentrations than in the case of *Urtica Pilulifera*. Higher concentrations were thus needed to reach a 50% proliferation inhibition (IC$_{50}$=3.4%, R$^2$ = 0.97). The maximum extract concentration tested (8%) was able to reach proliferation inhibition levels as high as in the case of *Urtica pilulifera* (>85% of the control group).
3. Effect of Ficus carica

The results indicate that the proliferation of the Hep3B cells is inversely related to the increased levels of Ficus carica extracts in there culture media. This behavior is significant with P-values of ≤0.005 and R =-0.908. The extract concentrations needed to elicit a considerable inhibitory effect were higher than the Urtica pilulifera and Lagenaria Siceraria extracts (IC$_{50}$=5.7%, R2=0.87). The maximal inhibition observed (60% of the control group) was seen in media with 8% extract content.
Figure 7. Reduction of proliferation activity of Hep3b cells in response to plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*.

5000 Hep3b cells/well were seeded in 96-well plates. The cells were grown in DMEM culture media containing 8, 4, 2, 1, 0.5 or 0.25% of the indicated plant extract in triplicates. The cells’ proliferation activity was determined by the tetrazolium salt XTT assay as described in the material and methods. Each experiment was repeated for additional two times and the average of a total of 9 wells was calculated for each concentration. The results were calculated as percent of the control group with no plant extract and blotted against the respective extract concentration.
B. Cervical epithelial cell- line Hela

The cervical epithelial cell line Hela was grown in the presence of increasing concentrations of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. The following sections depict the effects of each plant extract on Hela cells proliferation activity (Figure 8).

1. Effect of *Urtica pilulifera*

*Urtica pilulifera* extract showed a steep inhibitory effect on the proliferation of Hela cells (figure 8). The proliferation activity of the cells was found to be inversely related to the increase in extract concentration. A dose response effect was initiated at the lowest extract level used in the experiments (0.25%), and a 50% inhibition of the cells proliferation activity was achieved at extract level of (0.132%).

2. Effect of *Lagenaria siceraria*

The proliferation activity of Hela cells was inhibited in a dose response manner by *Lagenaria siceraria*. The proliferation activity of cells was found to be significantly, inversely related to the increase in extract concentrations (P<0.01, R=-0.938). The inhibitory effect of this extract was also started at the lowest extract concentration used (0.25%) however higher concentrations were needed (3.1%) to reach a 50% inhibition, than in the case of *urtica pilulifera*. Higher concentration also were needed to reach the maximum levels of inhibition than in the case of *urtica pilulifera*.

3. Effect of *Ficus carica*

The results indicate that *Ficus carica* extract is also capable of reducing the Hela cells proliferation in a concentration dependent manner. The proliferation activity is also inversely related to the increase in extract concentration. This relationship is significant with P ≤ 0.05 and R= to -0.87. Although a considerable inhibitory effect was induced by lower concentrations, IC$_{50}$ was reached at 3.1% extract concentration. The effect of *Ficus carica* is slightly different from that of *Lagenaria siceraria*, but with minor differences.
Figure 8. Reduction of proliferation activity of Hela cells in response to plant extracts from Urtica pilulifera, Lagenaria siceraria and Ficus carica.

7000 Hela cells/well were seeded in 96-well plates. The experiment conditions were as in figure 7.
C. Prostate cell line PC-3

The prostate cells PC-3 were incubated in the presence of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* of different concentrations. The inhibition of proliferation activity of PC-3 is illustrated in the following sections (Figure 9).

1. **Effect of *Urtica pilulifera***

*Urtica pilulifera* extract showed a strong inhibition of cell proliferation in a dose response manner. The proliferation activity is inversely related to the increase in extract concentration. This relationship is significant with $P=0.004$ and $R=-0.916$. The 50% proliferation inhibition ($IC_{50}$) was achieved at 2.3% extract content in the medium. 4% extract concentration was able to reduce the proliferation activity to reach about 10% only.

2. **Effect of *Lagenaria siceraria***

The results indicate that the proliferation activity of PC-3 cells is inversely related to the increased levels of *Lagenaria siceraria* extracts concentration. Although this proliferation inhibition was weaker than *Urtica pilulifera* effect, a significant dose response manner was shown ($P=0.00$, $R=-0.995$). A relatively high extract concentration 7.9% was necessary to reach 50% proliferation inhibition.

3. **Effect of *Ficus carica***

*Ficus carica* extract showed a significant inhibition of PC-3 proliferation activity $P=0.001$, $R=-0.954$. The lowest concentrations of this extract were able to induce inhibitory effects more than the other extracts; despite of this noticeable effect, $IC_{50}$ was 5.5% extract concentration.
Figure 9. Reduction of proliferation activity of PC-3 cells in response to plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*.

7000 PC-3 cells/well were seeded in 96-well plates. The experiment conditions were as in figure 7.
IV. Effect of plant extracts on cancer cell lines viability in culture

A. Hepatocellular carcinoma cell line Hep3B

Hep3b cells viability was determined in the presence of increasing concentrations of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. Figure 10, depicts the percent viability of Hep3b cells after incubation for 48 hours with different extracts at different concentrations. Hep3b cells which were maintained in DMEM without any plant extract have a percent viability ranging from 92 to 94.5% according to the three experiments.

1. Effect of *Urtica pilulifera*

The percent viability of Hep3b cells was found to be significantly, inversely related to the increase in *Urtica pilulifera* extract concentration (P=0.03, R=-0.787). The lowest concentration 0.25% of this extract was capable to reduce the percent viability of the cells by slightly less than 30%. The 50% viability reduction was obtained by 2.1% plant extract concentration. At 4% extract concentration 100% reduction in cell viability was already obtained.

2. Effect of *Lagenaria siceraria*

The percent viability of Hep3b cells was reduced in a significant, dose response manner due to treatment with *Lagenaria siceraria* (P=0.002, R=-0.937). Although there is no noticeable inhibitory effect at the lowest concentration, the highest concentration examined (8%) was able to elicit viability reduction by 60%. In contrast with *Urtica pilulifera* behavior *Lagenaria siceraria* effect is weaker, and IC$_{50}$ was obtained at 7.1% extract concentration.

3. Effect of *Ficus carica*

The results show that increasing the levels of *Ficus carica* in the DMEM media is inversely related to the percent viability of Hep3b cells. This behavior is significant with P= 0.001 and R=-0.94. Despite of that both *Ficus carica* and *Lagenaria siceraria* behave similarly at most of the concentrations examined, higher *Ficus* extract concentration (18.1%) are estimated to be needed for...
reaching the 50% reduction in cell viability, than *Lagenaria siceraria* extract (6.7%). The difference between both extracts arrows at high concentration (8%).

![Graph showing cell viability reduction](image)

Figure 10. Reduction of Hep3b cells viability in response to extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*.

20000 Hep3b cells/well were seeded in 6-well plates. The cells were grown in DMEM culture media containing 8, 4, 2, 1, 0.5 or 0.25% of the indicated plant extract in triplicates. The percent viability was determined by the Trypan-blue test as described in the materials and methods. Each experiment was repeated for one additional time and the average ± Standard error of the mean, of a total of 6-wells was calculated for each concentration. The results were calculated as percent of the control group with no plant extract and blotted against the respective extract concentration.
B. Cervical epithelial cells Hela

The percent viability of Hela cells was determined in the presence of increasing concentrations of *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* plant extracts. Figure 11 shows the effect of each plant extract on this cell line in terms of percent viability. In normal conditions without any extract treatment the percent viability ranges from 90 to 95% according to the results of the three experiments.

1. Effect of *Urtica pilulifera*

*Urtica pilulifera* extract shows a steep and strong reduction of cell viability in a dose response manner. This effect is significant with $P=0.004$, $R=-0.911$. This effect was noticeable at 1% extract concentration, whereas to reach 50% viability reduction, 2.8% extract concentration was required. A complete elimination of viable cells was obtained at 4% extract concentration, and maintained at higher concentrations.

2. Effect of *Lagenaria siceraria*

The results illustrated in Figure 11 indicate that *Lagenaria siceraria* extract causes a significant viability reduction with $P=0.00$ and $R=-0.982$. This inhibitory effect is weaker than the effect of *Urtica pilulifera* with $IC_{50}$ equals to 14.1% *Lagenaria siceraria* extract concentration.

3. Effect of *Ficus carica*

*Ficus carica* behaved similar to *Lagenaria siceraria* extract in causing some reduction in percent viability of Hela cells. A dose response effect was observed and significant with $P=0.003$, $R=-0.921$. The fifty percent inhibition of cell viability was at 15.7%. No cell viability reduction was achieved as much as in the case of *Urtica pilulifera* even with the concentration 8% of both *Lagenaria siceraria* and *Ficus carica*.
Figure 11. Reduction of Hela cells viability in response to extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*.

The experiments’ condition as in figure 10.
C. Prostate cell line PC-3

Figure 12 shows the percent viability of PC-3 cells grown in the presence of increasing concentrations of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. The following sections describe the effect of each plant extract on this cell line in terms of percent viability. In normal conditions without any extract addition, PC-3 cells showed a lower percent viability than Hep3b and Hela cells. According to the three different experiments the percent viability of PC-3 at normal DMEM was ranging between 85.5 and 90.5%.

1. **Effect of *Urtica pilulifera***

Percent viability of PC-3 affected by *Urtica pilulifera* extract was significantly inhibited in a dose response manner (P= 0.00 and R= -0.992). A smooth and a steep relationship was initiated at the lowest extract concentration. The 50% viability was obtained by 3.6% extract concentration, while 8% extract concentration resulted in 100% elimination of any viable cells.

2. **Effect of *Lagenaria siceraria***

The percent cell viability was found to be significantly, inversely related to the increase in extract concentrations (P= 0.001, R= -0.955). Despite of that this effect is weaker than the previous extract effect, IC$_{50}$ of *Lagenaria siceraria* was achieved at 9.4% extract concentration.

3. **Effect of *Ficus carica***

A dose response effect was also seen when PC-3 was grown in increasing concentrations of *Ficus carica*. Percent viability was significantly, inversely related to the increasing concentrations of this extract (P=0.00, R =-0.974). Higher concentrations were needed to reach a 50% viability inhibition (IC$_{50}$ =13.8% extract concentration).
Figure 12. Reduction of PC-3 cells viability in response to extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*

The experiment conditions were as in figure 10.
V. Determination of morphological changes of cell lines in response to treatment with different plant extracts

The morphological characteristics of the Hep3b, Hela and PC-3 cell lines were evaluated in parallel to each time performing viability testing assay. In each viability testing experiment the 6-well plates were examined for any morphological changes in response to treatment with the three plant extracts (Urtica pilulifera, Lagenaria siceraria and Ficus carica) compared to the normally maintained cells (no treatment). Representative wells were pictured and shown in this study (Figures 13, 14 and 15). A few cell culture flasks were frequently monitored at short time intervals.

A. Hepatocellular carcinoma cell line Hep3B

The normally maintained Hep3b cells were proliferating with high rate, and formed a monolayer growth with no less than 90% confluency within 48 hours (Figure 1a).

1. Effect of Urtica pilulifera

*Urtica pilulifera* showed noticeable morphological alterations with weaker adhesion level at different concentrations as evident by the cells floating. After 48 hours, the 2% extract concentration showed a potent activity. It induced Hep3b cells to round and form a grape-shaped cluster. At 24 hours after treatment of hep3b with 4 and 8% extract most of cells were detached and aggregated in clusters (Figure 16b).

2. Effect of Lagenaria siceraria

Hep3b cells treated with *Lagenaria siceraria* 4 and 8% concentrations for 24 hours suffered from noticeable alterations such as shrinkage, sparse, and spindle shape formation (Figure 16c). At the highest concentration (8%) the level of adhesion was reduced and many cells were detached.
3. Effect of *Ficus carica*

*Ficus carica* treated cells showed a high proliferation rate at low extract concentrations (0.25 and 0.5%). While at 4% extract concentration their growth was inhibited and slight cell shrinkage was observed (figure 16d). On the other hand 8% *Ficus carica* extract concentration induced obvious alterations to the cells morphology such as, the rounding up and detachment of many cells.

![Figure 13. Morphology of Hep3b cells in culture with or without different plant extracts.](image)

Hep3b cells were grown in DMEM culture medium without any extract (a) or with 4% *Urtica pilulifera* extract for 24 hours (b), 8% *Lagenaria siceraria* extract for 48 hours (c) and 4% *Ficus carica* for 48 hours (d). Cells in a, c and d were visualized by inverted microscope at 10X and in b at 20X.
B. Cervical epithelial cells Hela

The proliferation rate of Hela cells maintained in normal media was lower than Hep3b, as about 80% confluency was obtained after 48 hours of growth (Figure 17a). Apparent alterations in cell morphology and detachment of cells from the culture surface were observed after 24 and 48 hour of treatment with different extract concentrations.

1. Effect of Urtica pilulifera

Five to ten hours after treatment of Hela cells with *Urtica pilulifera* (2, 4 and 8% concentrations) the cells began to round up and the level of adhesion was influenced. Many cells detached easily from the surface of the plastic flasks and moved into medium. After 24 hours of treatment with 2% *Urtica pilulifera*, most of cells were rounded and formed sparse clusters (Figure 17b). Higher concentrations (4 and 8%) had more pronounced effects and nearly all cells were rounded up and detached.

2. Effect of Lagenaria siceraria

When Hela cells were treated with 2% *Lagenaria siceraria* extract for 24 hours a number of cells were dead as evident by cellular rounding up, floating and fragmentations. The time and concentration dependent extract effect was observed in the degree of confluency. Figure 7c illustrates the effect of 8% extract concentration after 48 hours of incubation.

3. Effect of Ficus carica

Treatment of Hela cells with *Ficus carica* 4 and 8% for 48 hours resulted in reduced density of the monolayer, and some of the cells were round and detached (Figure 17d).
Figure 14. Morphology of Hela cells in culture with or without different plant extracts.

Hela cells were grown in DMEM culture medium without any extract (a) or with 2% *Urtica pilulifera* extract for 24 hours (b), 8% *Lagenaria siceraria* extract for 48 hours (c) and 8% *Ficus carica* for 48 hours (d). Cells in all were visualized by inverted microscope at 20X.
C. Prostate cell line PC-3:
Figure 18a shows PC-3 cells which are characterized by a considerably lower proliferation rate than both Hela and Hep3b cell lines in normal conditions.

1. Effect of *Urtica pilulifera*
After 24 hours of PC-3 treatment with 4% *Urtica pilulifera* the cells were rounded up, and detached from the monolayer (Figure 8b). There were relatively large amounts of cellular fragments and cytoplasm condensation that appeared at 4% *Urtica pilulifera* concentration.

2. Effect of *Lagenaria siceraria*
PC-3 cells treated with 4 and 8% *Lagenaria siceraria* showed a noticeable decrease in confluency after 48 hours of treatment, but no evident morphological changes (Figure 18c).

3. Effect of *Ficus carica*
The reduction of confluency degree was visible in PC-3 cells when incubated for 48 hours in the highest extract concentration used, but also with no noticeable morphological changes (Figure 18d).
Figure 15. Morphology of PC-3 cells in culture with or without different plant extracts.

PC-3 cells were grown in DMEM culture medium without any extract (a) or with 4% *Urtica pilulifera* extract for 24 hours (b), 8% *Lagenaria siceraria* extract for 48 hours (c) and 8% *Ficus carica* for 48 hours (d). Cells in all were visualized by inverted microscope at 20X.
DISCUSSION

Cancer is a term describing conditions characterized by unscheduled and uncontrolled cellular proliferation (120). It is a very common disease, and its incidence is increasing at an average annual rate of 1.2% (120). Lately, there has been improvement in the treatment strategies of cancer, which has resulted in prolonged survival of patients with chronic cancer disease. However there is a growing need for additional means of cancer therapy, in the form of both palliative and curative treatments. The strategies available today, are sophisticated, and are only able to affect 50 to 60% of cancer patients, while the others will eventually die from the disease (121, 122 and 123).

Chemotherapy has been used for cancer treatment for more than 50 years; sometimes in combination with or parallel to surgery and radiotherapy. After surgical ablation of progressive cancer, metastasized tumor cells continue to progress and this is one of the faultiest associated with surgery. One the other hand, radioactive rays and most anticancer chemotherapeutic agents damage DNA or suppress DNA duplication to kill the rapidly growing tumor cells. At the same time, they also affect normal cells causing serious adverse effects, such as bone marrow function inhibition, bone necrosis, lung fibrosis, skin devascularization, ulceration, nausea, vomiting, renal damage and alopecia (124). Thus it is evident that a wide array of selective and potent components is needed to match the growing problems associated with cancer.

Plants and natural products play an important role in medicine and provide important prototypes for the development of novel drugs (125). They offer a valuable source of compounds with a wide variety of biological activities and chemical structures. Many anti cancer agents have been derived from natural sources; directly as pure native compounds, or as semi-synthetic analogues (126, 127 and 128).
Arabic and Islamic tradition is particularly rich in medical plants that have been used by pioneer Arabic physicians to establish the basis for modern therapies. But a few of these plants have been examined scientifically. In this extent we studied the potential effect of three Arabic and Islamic traditional plants as anticancer agents. These were Fig (Ficus carica) and Bottle gourd (Lagenaria siceraria) that were mentioned in the holy Quran in more than one occasion and Roman nettle (Urtica pilulifera) which is traditionally used as a popular medication (8). To fulfill this aim, the proliferation, viability and morphological characteristics of three cell lines (Hep3b, Hela and PC-3) were studied in response to treatment with extracts from the aforementioned plants.

The cellular proliferation activity was tested by a colorimetric method which is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to orange-colored compounds of formazon. The intensity of the formed water soluble dye is proportional to the number of metabolically active cells. The proliferation activity of the treated cells was normalized to that of normally growing cells from the same type with no treatment. This normalized value was expressed as percent of the control group. Theoretically, any reduction in the number of metabolically active proliferating cells might mean that the proliferation pathway itself was halted (cell cycle arrest), or that a fraction of the cells went through a death pathway. Therefore, a viability test was necessary to determine which of these two options may play a role in this study. Therefore, the trypan-blue dye exclusion test was used to determine the plant extract-mediated cell death. The unstained cells (viable) and the blue stained (dead) were counted separately, and the percent cell viability was calculated as previously described. The viability assay was performed with the same cell-extract combinations as in the proliferation assay. The percent viability of normally grown cells from the same types was determined for comparison with extract-treated cells. If we consider the results of the proliferation and viability assays in parallel, then we would be able to say whether reduction of the number of the metabolically active cell has resulted from death of part of them or due to any other reason. Any conclusion drawn from such comparison may be confirmed morphologically
following up the treated cells in comparison with normally maintained control cells. In this study a combination of the three different assays was performed in parallel for each plant extract and each cell line.

In order to assess the degree of proliferation inhibition and viability reduction obtained by each extract in comparison to the others, the IC\textsubscript{50} values were summarized in tables 3 and 4. The findings of this study indicated that the proliferation activity of Hep3b cells was inhibited by all of the three plant extracts (\textit{Urtica pilulifera}, \textit{Lagenaria siceraria} and \textit{Ficus carica}). A dose response effect was obtained with all of these extracts, but the degree of this effect varied from one extract to the other. The results indicated that \textit{Urtica pilulifera} is the most potent extract, even at low concentrations with IC\textsubscript{50} of (1.9%) followed by \textit{Lagenaria siceraria} with IC\textsubscript{50} of (3.4%) and \textit{Ficus carica} with of IC\textsubscript{50} of (5.7%). At the highest extract concentration used in the study (8%) both \textit{Urtica pilulifera} and \textit{Lagenaria siceraria} reached a similar proliferation inhibition level of about (90%). However this degree of inhibition was reached at lower concentration of \textit{Urtica pilulifera} extract than of \textit{Lagenaria siceraria} extract (Figure 7). \textit{Ficus carica} on the other hand did not reach the same degrees of proliferation inhibition obtained by both \textit{Urtica pilulifera} and \textit{Lagenaria siceraria} even at the highest concentration. When considering the results of Heb3b cells viability assay, the three plant extracts showed various degrees of Heb3b cells viability reduction. \textit{Urtica pilulifera} showed a steep reduction of the cells viability with IC\textsubscript{50} of 2.1% extract concentration, which equals the IC\textsubscript{50} of the same extract in cell proliferation assay. These compatible results (Figure 16) suggest that reduction in the metabolically active Hep3b cells in consequence to \textit{Urtica pilulifera} extract treatment is due to cell death. However such compatibility was not evident in the case of \textit{Lagenaria siceraria} (Figure 17) and \textit{Ficus carica} (Figure 18). \textit{Lagenaria siceraria} has a less potent cytotoxic effect against Hep3b cells (IC\textsubscript{50} = 7.1%). This might indicate that this extract counteracts the proliferation activity of Hep3b cells mostly by mechanisms other than induction of their death. This deviation between the cytotoxic effect and proliferation effect was more profound in the
case of *Ficus carica* with IC$_{50}$ of 18.1% in case of cytotoxicity compared to 5.7% in the case of proliferation.

Table 3. Summary of calculated concentrations of the three plant extracts *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* that give 50% (IC$_{50}$) reduction in proliferation activity of Hep3b, Hela and PC-3 cancer cell lines. The R$^2$ value for each curve equation is illustrated.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Hep3b cells</th>
<th>Hela cells</th>
<th>PC-3 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Urtica pilulifera</em></td>
<td>ic$_{50}$ 1.98</td>
<td>0.132</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>R$^2$ 0.837</td>
<td>0.6022</td>
<td>0.8563</td>
</tr>
<tr>
<td><em>Lagenaria siceraria</em></td>
<td>ic$_{50}$ 3.4</td>
<td>3.1</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>R$^2$ 0.97</td>
<td>0.9761</td>
<td>99.82</td>
</tr>
<tr>
<td><em>Ficus carica</em></td>
<td>ic$_{50}$ 5.7</td>
<td>3.1</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>R$^2$ 0.8675</td>
<td>0.9059</td>
<td>0.9583</td>
</tr>
</tbody>
</table>

Table 4. Summary of calculated concentrations of the three plant extracts *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* that give 50% (IC$_{50}$) reduction in the % viability of Hep3b, Hela and PC-3 cancer cell lines. The R$^2$ value for each curve equation is illustrated.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Hep3b cells</th>
<th>Hela cells</th>
<th>PC-3 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Urtica pilulifera</em></td>
<td>ic$_{50}$ 2.1</td>
<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>R$^2$ 0.7056</td>
<td>0.8305</td>
<td>0.9843</td>
</tr>
<tr>
<td><em>Lagenaria siceraria</em></td>
<td>ic$_{50}$ 7.16</td>
<td>14.18</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>R$^2$ 0.8496</td>
<td>0.9585</td>
<td>0.9367</td>
</tr>
<tr>
<td><em>Ficus carica</em></td>
<td>ic$_{50}$ 18.12</td>
<td>15.7</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>R$^2$ 0.8886</td>
<td>0.852</td>
<td>0.9442</td>
</tr>
</tbody>
</table>
Figure 16: Reduction of viability and proliferation activity of Hep3b (a), Hela (b) and PC3 (c) cells in response to Treatment with *Urtica pilulifera* extract. The best-fit trend line are shown. The equation for each trend line was used to estimate the IC50, IC100 and R² values by substitution. R² values are indicated next to the respective lines.
Figure 17: Reduction of viability and proliferation activity of Hep3b (a), Hela (b) and PC3 (c) cells in response to Treatment with *Lagenaria siceraria* extract.

The best-fit trend line are shown. The equation for each trend line was used to estimate the IC$_{50}$, IC$_{100}$ and R$^2$ values by substitution. R$^2$ values are indicated next to the respective lines.
Figure 18: Reduction of viability and proliferation activity of Hep3b (a), Hela (b) and PC3 (c) cells in response to Treatment with *Ficus carica* extract.

The best-fit trend line are shown. The equation for each trend line was used to estimate the IC50, IC100 and R2 values by substitution. R2 values are indicated next to the respective lines.
These data were confirmed by the inspection of morphological alterations of Hep3b cells due to treatment with the three plant extracts (Figure 13). As shown in figure 13b *Urtica pilulifera* had the most potent effect compared to the control (Figure 13a). Most of cells were rounded up and formed a grape shaped cluster, which is one of the prominent morphological features of cellular death in culture. Such features were also noticeable to lesser extent when the cells were incubated with *Lagenaria siceraria*, and could hardly be detected in the case of *Ficus carica*. Moreover, the confluency of Hep3b cells was reduced in response to treatment with the three plant extracts and in accordance to the previously discussed results of proliferation inhibition (104, 114, 118).

Similarly Hela cells proliferative activity was inhibited with various levels due to their treatment with the three plant extracts. As shown in Figure 8, *Urtica pilulifera* has a strong inhibitory effect on the proliferation activity in extract concentration dependent manner with IC$_{50}$ of 0.13% extract concentration. Both *Ficus carica* and *Lagenaria siceraria* had a similar but weaker effect on proliferation of Hela cells, and the IC$_{50}$ for both extracts was 3.1%. The percent viability of Hela cells was also reduced in a dose response manner by all of these plant extracts. *Urtica pilulifera* extract was the most effective with IC$_{50}$ of 2.8%, while the *Lagenaria siceraria* and *Ficus carica* effect on cells viability were similarly less potent with IC$_{50}$ of 15.7% for *Ficus carica* and 14.1% for *Lagenaria siceraria*. Being the most potent plant, *Urtica pilulifera* mostly exerts its effects by inducing cell death although other effect can be present (IC50 for proliferation=0.13% and for cytotoxicity =2.8%). The difference between the IC$_{50}$ of both *Ficus carica* and *Lagenaria siceraria* in proliferation inhibition and the IC$_{50}$ of cell viability reduction indicates that these extracts exert their effect by cell cycle arrest with low cytotoxicity. The morphological observations of Hela cells maintained in increasing concentrations of the three plant extracts show that *Urtica pilulifera* had the most rapid and potent effect of these extracts. Figure 14b shows that most of the cells were rounded up and formed sparse clusters due to treatment with 2% *Urtica pilulifera*. These results suggest that most of Hela cells were dead in the case of *Urtica pilulifera*. A reduction of Hela cells confluency
was the most prominent observation when these cells were incubated with *Ficus carica* or *Lagenaria siceraria*, but cell death indications were almost not seen. These observations indicate an antiproliferative effect of both *Ficus carica* and *Lagenaria siceraria* but less likely cytotoxicity.

The proliferation activity of the prostate cell line PC-3 was inversely related to increasing the levels of the three plant extracts. The degree of proliferation reduction varied from one plant extract to the other. As shown in figure 9, *Urtica pilulifera* was again the most potent extract with IC<sub>50</sub> of 2.3%, whereas *Ficus carica* comes second with IC<sub>50</sub> of 5.5%, and *Lagenaria siceraria* comes last with IC<sub>50</sub> of 7.9%. When comparing these results with results from PC-3 cells viability assay, we find that *Urtica pilulifera* also has the strongest effect with IC<sub>50</sub> of 3.6%. These results are supported by the morphological changes shown in figure 15b including cells rounding up and detachment from the monolayer indicating that cell death might has occurred.

*Lagenaria siceraria* induced a reduction of PC-3 cell viability with IC<sub>50</sub> of 9.4% extract concentration similar to IC<sub>50</sub> from the same extract in the proliferation assay. This fact suggests that this extract also caused PC-3 cell death. The reduction of the cells confluency due to *Lagenaria siceraria* treatment observed in figure 14c gives evidence of the effect of this extract. A Higher concentration of *Ficus carica* was needed to reach a 50% viability inhibition (IC<sub>50</sub>=13.8%) than that needed to reach 50% inhibition in cell proliferation (IC<sub>50</sub>=5.5%). This again suggests that the effect of this extract did not occur only through cell death.

Based on the previous results, *Urtica pilulifera* have a potent antiproliferative effect on the three tested cancer cell lines. While there is no previous studies about the role of *Urtica pilulifera* extract as anticancer agent, many studies investigated the role of its genus member *Urtica dioica*. *Urtica pilulifera* and *Urtica dioica* are similar in many chemical and morphological aspects (75, 8). Our results on *Urtica pilulifera* are in agreement with previous studies on *Urtica dioica*. For example an aqueous extract of *Urtica dioica* roots was shown to
directly inhibit the proliferation of Hela cells and block its binding by epidermal growth factor (EGF) (129). In the same study a polysaccharide mixture from an aqueous root extract was shown to exert an anti-inflammatory activity in a rat Paw oedema test. Investigation of the effect of a 20% methanol extract of *Urtica dioica* roots on prostate cell line (LNCap) resulted in a selective and significant concentration-dependent proliferation reducing effect on prostate cells (89). In the same study the cell proliferation activity was determined by a colorimetric assay and the cytotoxicity was examined by Trypan-blue test. Our results are also comparable with data obtained from an in vitro study that aimed to investigate the effect of *Urtica dioica* leaves aqueous extract on the enzyme activity of prostate cancer tissue (130). The results of the study indicated that the extract caused a significant inhibition of adenosine deaminase activity (ADA) of these tissues in a dose dependent manner. These data might be of importance because ADA is a key enzyme in the nucleotide metabolism and DNA turn over. Extracts of *Urtica dioica* were also used in the treatment of adult mouse with bingeing prostate hyperplasia (131). Five differently prepared root extracts were tested on these rats. The 20% methanol extract was the most effective with 51.4% inhibition of induced growth.

Despite all of these data the mechanism(s) of action of *Urtica dioica* extract as an antiproliferative agent has yet to be established. Different modes of actions are proposed in this regard. For example it has been observed that some sterols and hydroxyl-fatty acids, given their low concentrations in *Urtica dioica*, can inhibit aromatase, which is a key enzyme in steroid hormone metabolism mediating the conversion of androgens into estrogens (132). Another mechanism involves a dose dependent inhibition of the binding of sex hormone binding glubin (SHBG) to its receptor in response to *Urtica dioica* extract (133). Some lignans present in *Urtica dioica* were shown to interfere with the binding of androgens to SHBG, thereby reducing the transport capacity of androgens (132). Based on the similarity between *Urtica pilulifera* and *Urtica dioica* we would suggest that *Urtica pilulifera* might exerts its antiproliferative effect via a similar fashion.
The results of this study strongly indicate a possible cytotoxic effect of *Urtica pilulifera* against cancer cell lines. This was evident from the results of Trypan-blue viability assay as well as from the inspection of cells morphology in culture. The cytotoxicity of fixed and volatile oils extracted from *Urtica pilulifera* leaves and seeds were tested on Swiss albino mice (134). The results indicated that both oils of *Urtica pilulifera* are completely nontoxic even at doses reaching 12.8 ml/kg. However this study involved only the oil fractions of *Urtica pilulifera* extract. Other components of the extract may play a role in the observed toxicity in our study. Moreover, the aforementioned study was performed in vivo on mice and the only cytotoxicity parameter considered was the mice death. No other mice toxicity indicators were analyzed such as, histopathological alterations of the different mice tissues and organs.

According to results discussed earlier the inhibitory role of *Ficus carica* on the proliferation of the three cell lines was more profound than its cytotoxic effect on the same cell lines. This conclusion is in accordance with the results of previous studies. For instance, the proliferation of different cell lines was inhibited by components of *Ficus carica* (135). Such proliferation inhibition was also obtained when cow teat papillomatosis skin surface benign tumors, were treated by *Ficus carica* latex (136). Furthermore, *Ficus carica* was found to have an in vivo antioxidant effect after being consumed by human (137). Accordingly, the dried fruits of this plant should be eaten more frequently as they are rich in phenol antioxidants and fibers. Compared with vitamins C and E, the well known antioxidants, the dried fruits of *Ficus carica* were found to be a superior one.

*Lagenaria siceraria* was found to posses both antiproliferative effects as well as a cytotoxic effect to a considerable degree. Unfortunately no literature was found regarding the anticancer activities of this plant. However, members of its family (*Cucurbitaceae*), were shown to have a class of biologically active compounds (Cucurbitacins) with well established anticancer cytotoxic activity (138,139,140). For example, Cucurbitacins were shown to have strong cytotoxic effect on KB cell line, derived from human nasopharyngeal carcinoma and on Hela cells by
different proposed mechanisms (140, 141). Moreover, (Cucurbitacins) were shown to have a proliferative inhibitory activity in vivo against several carcinoma, sarcoma, and leukemia models (142, 143 and 144).

The level of these compounds and/or any of their derivatives in Lagenaria siceraria is unknown. However the results of this thesis indicate both antiproliferative and cytotoxic activity of *Lagenaria siceraria*. Therefore we expect *Lagenaria siceraria* extracts to behave in a similar manner like other members of its family via (Cucurbitacins) involvement.
CONCLUSION

In conclusion, all of the three plants examined in this study possess varying levels of anticancer activity in vitro. This is evident by the concentration dependent manner reduction in the final number of cancer cells as a consequence to treatment. Two kinds of anticancer effects were examined and found to take part in this study. The first is anti-cell proliferation effect (decreased number of metabolically active cells) and the second is cytotoxicity (decreased number of live cells). The three plants examined possess both of the effects with various degrees. *Urtica pilulifera* possess the strongest and most profound effects on the three cell lines, probably by cytotoxicity mainly. On the other hand *Lagenaria siceraria* probably affects the three cell lines by combination of cytotoxicity and antiproliferation almost to a similar degree. *Ficus carica* most probably reduces the final number of metabolically active cells mainly by its antiproliferative effect, although cytotoxicity likely contributes to viability reduction at high concentrations.

Both *Ficus carica* and *Lagenaria siceraria* are edible plants that were chosen on the bases of being mentioned in the holy Quran. Therefore, although their effect is lower than that of *Urtica pilulifera* their amount in the diet or as a treatment can be safely scaled up when ingested in their native form. On the other hand, despite its possible toxicity *Urtica pilulifera* is frequently orally used as a medication in many conditions by traditional medicine.
RECOMMENDATIONS

1. Further studies are needed to assess the active ingredients of *Ficus carica*, *Lagenaria siceraria* and *Urtica pilulifera*, involved in the antiproliferative or cytotoxic effects of these plants. These studies must involve the establishment of in vivo cancer models and their treatment by these plants in their native crude extracts or by their purified active components. More efficient extraction techniques and instruments have to be used in order to obtain the crude extracts or their derivatives in convenient quantities and concentrations.

2. The type(s) of effects exerted by the three plants component(s) involved in their activity have to be further studied by molecular or other relevant techniques.

3. Both *Ficus carica* and *Lagenaria siceraria* may be ingested more frequently in different forms, if not to treat cancers, then as a prophylactic against them. *Urtica pilulifera* can be administered for treating relevant conditions including cancer with reasonable pre-studied amounts.

4. Many other plants are waiting to be examined for any possible therapeutic effects and if present they may represent a convenient and cheap means to cope with many types of cancer.
REFERENCES

5. Rebecca C G., 2004 -Isolation of Natural Products from Casearia Nigescens. Virginia Polytechnic Institute and State University, Master thesis in chemistry science 4-20.


27. Seeram NP., Adams L.S., Henning S.M., Niu Y., Zhang Y., Nair MG., et al,. 2005- In vitro antiproliferative, apoptotic and anti oxidant activities of punicalagin, ellagic acid and total pomegranate tannin extract are enhanced in combination with other Polyphynoles as found in pomegranate juice. Journal of nutritional biochemistry,16(6): 360-7


56. Ukiya M, Akihsa T., Tokuda H., Toriumi M., Mukainaka T., et al., 2002- Inhibitory effects of Cucurbitance Glycosides and other Tritrpenoids from the fruit of Momerdica grosvenori on Epstein- Barr Virus early antigen induced by tumor promoter 12-o- Tetradeanoylphorbol-13- acetate. agricultural food chemistry, 50(23):6710


87. Matsingou T C., Kapsokefalou M., and Safiloglou A., 2001- Aqueous infusions of Mediterranean herbs exhibits antioxidant activity towards iron-


102. Ho C-K., Huang Y L., and Chen C-C., 2002- Garcinon E, a xanthone derivative, has potent Cytotoxic effect against Hepatocellular Carcinoma cell lines. Planta medica, 68:975-979.


