Anti-tumor and Antioxidant Effects of *Daucus carota* Water extract

by

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Who have always been their for me as sources of inspiration, moral support, and hope

I love you all very much.

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ABSTRACT

Anti-Tumor and Antioxidant Effects of *Daucus carota* water extract

By Farah S. El Ghaziri Tabbarah

Daucus carota (Linnaeus) ssp. *carota*, also known as wild carrot, is listed by the council of Europe as a natural source of food flavoring. It is confined to temperate regions of Europe, Asia and South Africa. The plant is known in the Lebanese folklore for its protective effects against gastric ulcer and diabetes without affecting other body functions. The present study is considered to be the first research on the effectiveness of *Daucus carota* water extract in inhibiting the promotion phase of carcinogenesis in mouse skin. The anti-tumor promoting effects of *Daucus carota* water extract on skin carcinogenesis was established through the DMBA (7, 12-dimethyl benze-{a}-anthracene)–initiation TPA (12- O- tetradecanoalyl phorbol-13- acetate) promotion mouse skin carcinogenesis model. The extract was either applied topically to mouse skin at concentrations of 50, 250, 500 mg/kg, or injected intraperitoneally or given by gavage (force feeding) at 100% twice per week for 17 weeks, 20 minutes prior to each promotion treatment with TPA. At week 15, the anti-tumor effect was only observed with the intraperitoneal mode of treatment, where the % inhibitions of papilloma incidence, yield and volume were 28 %, 23% and 86.4% respectively. Gavage treatment failed to inhibit tumor incidence, yield and volume. Topical application using a cream appeared to be an inappropriate mode of delivery to study the effect of the extract since the control failed to show significant papilloma formation. The *Daucus carota* water extract was also studied for its antioxidant activity and phenolic compound content. The extract exhibited a relatively high free radical scavenging activity (65.4%) and antioxidant power (508 μmol $FeSO_4$), but a low phenolic compound content (10.8 mg gallic acid/g extract). In conclusion, our results indicate that *Daucus carota* water extract has a suppressive activity against tumor promotion in mouse skin which we suspect to be related to the antioxidant properties of the extract.

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GLOSSARY

AP: apurinic sites

BCC: Basal cell carcinoma

DC: *Daucus carota*

DMBA: 7,12, dimethyl benza-anthracene

DPPH: 2,2-diphenyl -1- picryhydrazyl

FRAP: ferric reducing antioxidant power

GAE: Gallic acid content

I.P: Intraperitoneal

LC-MS: liquid chromatography- mass spectrometry

LD_{50} : lethal dose 50

LDL: Low density lipoprotein

PAPS: papilloma

SCC: squamous cell carcinoma

SEM: standard error of the mean

TPA: 12-O-tetrad-ecanoylphorbol-13-acetate

UV: ultra-violet

Chapter1

INTRODUCTION AND LITERATURE REVIEW

Phytotherapy (from Greek "Phyton" a part of a flowering plant and "therapy" treatment) is treatment by means of herbs. Until the 19th century phytotherapy was considered the primary treating method where herbs were nearly the only available source of medication. Nowadays, plant materials play a vital role in fighting illnesses such as infectious diseases. Many of these plants have been used as templates for the development of new therapeutic agents. For the sake of improving the efficacy of this medicine, scientists are working to find pharmacological and biological basis for many materials and practices used in folk medicine. In order to benefit from nature it is essential to distinguish the different types of plants, and develop ways to maximize their benefit through modern understanding of drug design regulations and through pharmacokinetics testing. This science should be preserved, since its benefits have proven useful and relevant and applicable to many diseases.

1.1 The *Daucus carota* Plant:

1.1.1 Plant Information:

Daucus carota (Linnaeus) ssp. carota also called wild carrot, belongs to the family UMBELLIFERAE (Apiacae). Wild carrot is a tall robust biennial spiny-fruited herb that grows in dried out in fields (Mitich, 1996). Other common names for *Daucus carota* are Quees Anne's Lace, Bird's nest Weed, Devil's plague, Garden Carrot, Bee's nest plant, Bird's nest root and the common name in Arabic is "Khilli" (Mitich, 1996).

The white flowers have 5 regular parts with a tiny maroon flower in the middle that may vary in color between yellow and brown. A *Daucus carota* umbel may contain 100 of flowers (Hoffmann, 1990). The clusters are up to 11 cm wide (Figure 1.1). Leaves of the plant are alternate and finely divided. The flower blooms in the summer and continue till mid fall.

Figure 1.1 *Daucus carota* plant umbel (www.emmitsburg.net).

1.1.2 Distribution:

Wild carrot was first found in Afghanistan then it spread to China in the 13-14th century and reached England in the 15th century. It is grown widely in temperate and tropical regions of the world. It is native to western Asia and it is also present in the Mediterranean region, southwest Asia, tropical Africa, Australia and North and South America (Reed, 1976). *Daucus carota* is reported to be an important weed in Algeria, Arabic countries, Belgium, Brazil, Poland, Iran, and Jordan. It is a widespread weed in Austria, Canada, Egypt, Germany, Iraq, and USA. Similarly, it is a common weed almost present in all fields throughout Lebanon (Ross, 2005).

1.1.3 Ecology:

Ranging from rainy to wet forest life zones, wild carrot tolerates annual temperatures of 3.6 to 28.5 ^{o}C and pH of 4.2 to 8.7 (Duke, 1978; Duke 1979) . For seed production warm dry areas and fall showers are desirable (Reed, 1976).

1.1.4 Chemical composition:

Daucus carota contains 4 polyacetylenes, the major one being falcarinol (Lund and White, 1990). A Study conducted by Saad et al. (1995) showed that fruit, stem, and leaf oils of *Daucus carota* from Lebanon differ in composition. Phenyl propanoids and sesquiterpine hydrocarbons, (E)- methylisoeugenol (37%), β- bisobolene (35%), are major constituents of the fruit oil.. The leaf and stem oils were characterized by the presence of oxygenated sesquiterpenes and particularly 2 monocyclic ketones, shybunone and preisocalamendiol (18% and 33%) (Saad et al., 1995; Gonny et al., 2004).

Seeds are composed of ascrone, carotol and bisabolene and quaternary base (Gambhir et al., 1966). The leaves include 2 bases, pyrolidine and daucine in addition to the essential oil. The roots contain numerous vitamins including vitamin A, B, C, and D (Dandiya & Chopra, 1970). The oil extracted from edible carrot umbel (*Daucus carota* L. ssp. *Sativus*) revealed different compositions when compared to wild carrot (Kula et al., 2006). The main constituents of the oil were monoterpene hydrocarbons (66%-85%) represented mainly by □ pienene (40-46%) and myrcene (12-24%). β caryophyllene and carotol are the most abundant sesquiterpene present in the oil extract of *Daucus carota.*

1.1.5 Medicinal Properties:

Roots and seeds have diuretic, stimulant, and deobstruent effects. It is known to be a remedy for heart failure, chronic kidney diseases and infections of the bladder (Hoffmann, 1990). In addition, they are used for the relief of flatulence and colic, dysentery, chronic coughs (Hoffmann, 1990). The roots, rich in β- carotene a precursor of vitamin A, has been important in preventing blindness. The plant is bactericidal, a hypotensive agent and effective in expelling intestinal worms (Hoffmann, 1990).

The root has been used as a protection against various forms of cancer including skin cancer, mammary carcinoma and tumor of the testicles. The juice of the root is used in treating carcinomatous ulcers of the neck and the uterus, cancer of bowels and stomach cancers (Dandiya and chopra, 1970). In addition to being an active urinary antiseptic,

wild carrot volatile oil is used for the treatment of cystitis, prostatitis and kidney stones (Crelin, 1995).

1.1.6 Pharmacological Studies:

Alcoholic and aqueous extracts of seeds showed anti-fertility and abortifacient activity in rats (Kapoor, 2001). Aqueous extract had antiestrogenic, antipregestation, antihistaminic and antiactylcholine activity. The water soluble fraction of alcoholic extracts of seed appeared to have a hypotensive activity. Similarly, the essential oil causes transient drop of arterial blood pressure without affecting respiration in lower doses (Kapoor, 2001).

Unlike the abortifacient effect observed in pregnant rat with the subcutaneous treatment with petroleum ether extract of dried seeds, the ethanol extract was ineffective when given by gastric intubation (Ross, 2005). Intraperitoneal injection of the petroleum ether extract of the seed of carrot (3mg/kg dry weight for 7 days) exhibited anti-tumor activity by inhibiting the growth of Ehrlich ascites tumor in mice (Majumder & Gupta, 1998). Recently, the *Daucus carota* aqueous extract prepared in our labs showed potent anti-ulcerogenic and anti-inflammatory effect in rats (Wehbe et al., 2009).

Importantly, the *Daucus carota* water extract exhibited anti-leukemic activity when using human promyelocytic leukemia HL-60 cell line. The effects of non-cytotoxic concentrations of *Daucus carota* were evaluated against HL-60 cells. The anti-proliferative effects were tested by determining the changes in the expression of transforming growth factors (TGF-alpha, TGF-beta1 and TGF-beta2). The degree of differentiation was assayed by the ability of treated cells to reduce NBT to insoluble blue black formazan. The results of this study showed that *Daucus carota* extract possesses anti proliferative effects and stimulates differentiation of cells (Diab-Assaf et al., 2007).

Other pharmacological activities of the plant include agglutinin activity; water extract of the *Daucus carota* root at variable concentrations was active on streptococcus

mutants (Ross, 2005). The methanol extract of *Daucus carota* exhibited an effective antibacterial activity against *Bacillus aureus* (a food borne pathogen) where the MIC (minimal inhibitory concentration) was estimated to be 0.01mg/ml (Kumarasamy et al., 2002., Valero and Satmeron, 2003). However, i*n vitro* studies showed that *Daucus carota* extracts had no effect against *Vibrio cholera* (Guevara et al., 1994).).

In a murine model of helicobacter infection, bacteria were only cleared from 20% of carrot seed-oil-treated animals (Bergonzelli et al., 2003). Gambhir et al. (1966) indicated the presence of a cardiotonic principle and cholinergic and papaverine-like activity. Later on in 1979 they showed that the nitrogenous base present in the seeds of *Daucus carota* is responsible for the papavarine like smooth muscle relaxant and spasmolytic activity.

In Europe, *Daucus carota* is a popular remedy for jaundice and hepatic disorders. Bishayee et al. (1995) revealed that *Daucus carota* has a significant protective action in the alleviation of CCL4-induced hepatocellular injury. Similar studies on the pesticide lindane (20 mg/kg/day) induced hepatotoxicity in rats showed that the oral administration of *Daucus carota* extract (25ml/kg/day; for 30 days) produced significant hepato-protection (Balasubramaniam et al., 1998).

1.2 Skin Cancer:

Skin cancer is a growing concern worldwide. The risk of skin cancer is higher with lighter skin and increased sun exposure (Buckmaster, 2007). More than 50% of all cancers that occur are skin-related (Nouri, 2007). Skin cancers are usually locally destructive if left untreated, so early detection is considered vital (Nouri, 2007).

The three most common types of skin cancer are: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (Rajpad & Marsden, 2008). The most common and least lethal type of skin cancer is basal cell carcinoma. It is a malignant epithelial cell tumor that begins as a small papule and then enlarges peripherally to develop into a crater that erodes, crusts and bleeds (Rigel et al., 2004).

Squamous skin cancer is the second type of skin cancer. It is a form of cancer that occurs in different regions including: lips, mouth, esophagus, lungs, prostate, urinary bladder, vagina and cervix. SCC is a malignant tumor of squamous epithelium (Lane & Comac, 1998). It usually appears as scaly patches on the skin with a red inflamed base, a budding tumor or a non healing ulcer (Miller et al., 1996). The 2 main risk factors for SCC are sunlight exposure and immuno-suppression, with chronic sun exposure being the strongest environmental risk factor. It is possible for SCC to spread to other areas of the body which makes early treatment vital. SCC can destroy much of the tissue surrounding the tumor and the possibility of spreading to the lymph nodes and other organs is quite high and may result in death (Lane & Comac, 1998).

Melanoma, third type of skin cancer, is considered to be the most lethal type of skin cancers. It is a malignant tumor of melanocytes which are found in skin, bowel and the eyes. One of the major risk factors for melanoma is the ultraviolet radiation (UVA, UVB) exposure. UV radiation causes damage to the DNA of cells; resulting in thymine dimerization which when unrepaired, prevents normal DNA proliferation and creates mutations in the cell's genes (Nathanson, 1987). If mutations occur in oncogenes or tumor suppressor genes, the rate of mitosis in the mutation-bearing cells becomes uncontrolled leading to abnormal proliferation, hence formation of tumor (McClay et al., 2003). The first sign of a melanoma is usually a change in skin color or a new freckle or a mole. At an early stage melanomas are few millimeters in diameter, but they may grow to several centimeters in later stages. Its color may range from dark brown to black, red, blue and occasionally light grey. Once melanomas reach the dermis, they may easily spread to other tissues via the lymphatic system to the local lymph nodes or via the blood stream to other organs such as the lungs or brain. This is known as secondary spread or metastatic disease (McClay et al., 2003).

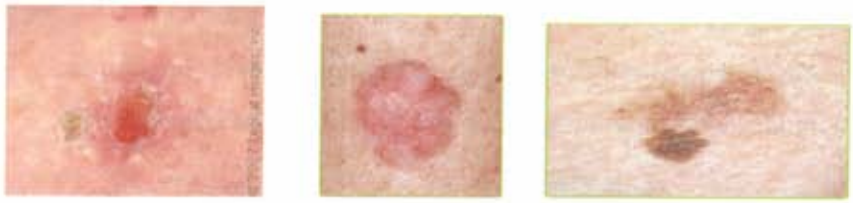

Figure1.2. (a) Squamous call carcinoma, (b) Basal cell carcinoma, (c) Melanoma (www.skin-cancers.info/)

1.3 Multistage carcinogenesis in mouse skin:

1.3.1 A brief history:

Three stages characterize skin carcinogenesis during which genetic changes build up leading to a more invasive and less controllable form of cancer (Boutwell, 1964; Digiovanni, 1992). The term carcinogenesis was first used in 1924 when Deelmman revealed that treated mice with coal tar develop skin tumor if wounded (Digiovanni, 1992). In 1775, Percival Pott was the pioneer in describing chemically induced skin cancer caused by the constant exposure to coal tar emitted from the chimney sweeps in London. In 1915, scientist started skin cancer induction by consistent application of coal tar to mouse skin (Boutwell, 1964). Later, the active ingredient from coal tar was purified and it was named dibezanthracene (Rudden, 1987; Eccles, 1987). In 1940, Rous demonstrated that the latent tumor cells initiated in the rabbit skin could be promoted to reveal themselves by agents such as wounding, turpentine and chloroform. Initiation, first described by Rous, describes the appearance of latent tumor cell due to the exposure of sufficient amounts of carcinogen. Treatment of the same area of the skin with a non-specific tumor enhancing factor causes latent tumor cells to appear in a stage named as promotion (Boutwell, 1964; Digiovanni, 1992). Croton oil extracted from Croton Triglium was discovered to be a potent promoting agent (Berenblum, 1941). Studies have shown that only few mice develop papillomas when their skin was painted once with croton oil, however, almost all mice develop skin carcinoma when the same area was painted repeatedly (Ruddon, 1987). Mottram, in 1944, demonstrated the two stage carcinogenesis in mouse skin when he showed that a single application of a non

GTPase activity of Ras, leaving Ras active for prolonged periods of time. This has dramatic consequences in the ras signal transduction pathways within the cell (Digiovanni, 1992; Lawley, 1987). Initiation, an irreversible mutational change, often remains latent or unexpressed until a promoter causes the cells to grow and form a tumor. Despite the fact that the epidermis renews itself once every 6-8 days, initiation is indeed a heritable event (Digiovanni, 1992).

1.3.3 Molecular basis of "Promotion":

Promotion is a slow and gradual process that entails the prolonged exposure of the promoting agent to the initiated cells, permitting them to expand and divide. A proven promoting agent is the croton oil; the active ingredient being 12-O-tetradecanoayl phorobol-13-acetate (TPA). TPA doesn't require any metabolic activation; on the contrary, it binds to the membrane associated cellular receptors resulting in cellular changes including chronic hyperplasia, localized edema, and inflammatory responses (Boutwell, 1964; Rudden, 1987; katiyar, 1997).

The stimulation of the proinflammatory cytokine, IL-1□, mediates TPA's effect on keratinocytes. The basal activity level of the enzyme ornithine decarboxylase (ODC) in papillomas thus experiences a 28 fold increase. Ornithine decarboxylase is the rate limiting step in the polyamine biosynthetic pathway leading to the synthesis of putrescine, spermine and spermidine which are implicated as regulators of cell cycle and keratinocytes proliferation (O'Brien, 1997). The second action of Il-1□ is the activation of phospholipase A_2 which results in the phospholipid metabolism and the production of arachidonic acid. Arachidonic acid is converted by the inducible enzyme cyclooxygenase-2 (Katiyar, 1997) to prostaglandins, PGE_2, PGD_2, $PGF_{2□}$, which are the major mediators of inflammation (Boutwell, 1982).

Moreover, one of the major effects of TPA is activating the calcium dependent protein kinase C which results in a cascade of events; alterations in the membrane channels and receptors, changes in gene expression and alterations in cellular proliferation and differentiation are some of the results of this activation (Digiovanni,

1992; Weistein, 1988). Furthermore; the mouse epidermal microsomes goes through lipid peroxidation caused by the TPA which generates peroxy radicals (ROO) that form hydroperoxides, in turn altering the DNA structure and chromosomal proteins (Katiyer, 1997). This leads to a decrease in the basal activity of epidermal dismutase and catalase (Digiovanni, 1992).

In conclusion, TPA is the proliferative agent that completes the procedure that was initiated by DMBA to form papillomas (Boutwell, 1964). Papillomas are described as heterogenous because some persist and progress to squamous cell carcinoma, while others regress and disappear (Digiovanni, 1992). Thus, skin carcinogenesis at the promotion stage may be partially reversed (Boutwell, 1964; Perchellet, 1992).

1.3.4 Molecular basis of progression:

Progression involves the conversion of the papillomas to the squamous cell carcinoma (Digiovanni, 1992). Progression involves trisomy of the chromosomes 6 and 7, amplification of the mutation Ha-ras allele and recessive mutation in the tumor suppressor gene p53 (Digiovanni, 1992). After that, there is vascularization of the growing tumor and the escape from the host's immune system occur leading to metastasis of neoplastic cells (Rudden, 1987).

1.4 Free radicals and antioxidant and skin cancer:

Studies have shown that reactive oxygen species are important in skin cancer development. It is well established that antioxidants could be useful chemopreventive agents (Baumann, 2005). More research have shown that naturally occurring compounds in the human diet such as green tea polyphenols and resveratol provide protection against development of skin cancer, both in vitro and in vivo (Baumann, 2005).

Free radicals are a major cause of human cancer and other diseases. Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2^{\cdot}$) and hydroxyl radicals, as well as non free-radical species such as hydrogen peroxide (H_2O_2) (Halliwel,

1995; Squadriato et al., 1998). Free radicals form in two main sources: endogenous sources and exogenous sources (Kruawan et al; 2006). Endogenous sources include normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes in living organisms. Exogenous sources of free radicals include tobacco smoke, ionising radiation, certain pollutants, organic solvents, and pesticides (Halliwel et al., 1989; Robinson et al., 1997).

Furthermore, free radicals lead to lipid peroxidation in foods, leading to their deterioration (Sasaki et al., 1996; Miller at al., 1995). In addition, reactive oxygen species have been contributing to more than a hundred diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (Tanizawa et al., 1992). There is a reciprocal relationship between ROS and tissue injury, each leading to the other (Auroma, 1998). While this may be the case, antioxidant defenses that protect against such damage are present in all aerobic organisms, including humans by using repair enzymes to remove or repair damaged molecules (Granelli et al., 1995). However, this natural antioxidant mechanism may not always produce the desired results; hence, ingesting antioxidant compounds is vital (Duh, 1998; Halliwel, 1994; Teroe et al., 1994). Although synthetic antioxidant compounds are found in processed food, these may have some side effects (Branien, 1975; Ito et al., 1983). Still, there exists an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease (Rice-Evans, 1997). Therefore, determining natural antioxidants sources is important. Common sources of antioxidants are herbal drinks prepared from a wide variety of plants. These herbal drinks, known as tea, have been commonly used since thousands of years until the present time. The majority of herbal drinks contain polyphenols which possess both antioxidant and chemo preventive potential (Kruawan et al; 2006).

1.5 Purpose of the study:

Mice are the sample of choice for skin carcinogenesis studies because they are easy to maintain and breed, and they are considered convenient representatives of mammals that offer us a close approximation to human subjects. The DMBA-initiation-TPA promotion mouse skin carcinogenesis model is well established and has been used to test the efficiency of several drugs. The purpose of the present study is to test the efficacy of *Daucus carota* water extract as an anti-tumor promoting agent through the analysis of tumor appearance, incidence, yield and volume using the DMBA-initiation-TPA promotion mouse skin carcinogenesis model. The study included three experimental groups to identify the best administration route of the water extract. These groups were: topical application, gavage (force feeding), and intraperitoneal injections. In order to reveal other benefits of the plant water extract in vitro studies were conducted to evaluate the phenolic content and antioxidant activity.

Chapter 2

MATERIALS AND METHODS

2.1 Animals:

Adult Balb/c mice bred in the animal room at the Lebanese American University weree used to test anti-tumor promoting activity of *Daucus carota* extract. Mice were kept under optimum conditions (temperature 20-22 ^{0}C and light 12 hour light-dark cycle), housed in plastic cages (10 mice per cage) and had unrestricted access to a commercial mouse diet and tap water.

2.2 Collection of the Samples:

Daucus carota umbels were collected during the months of September to October. Collection was done from Bshamoon area, Mont Lebanon. The taxonomic identification of the plant material was confirmed through botany and plant taxonomy books (Juneidi, 1999; Mouterde, 1970).

2.3 *Daucus carota* water extraction:

The plant umbels were left in the shade in a non humid environment until they became dry. The yield of the chopped dry umbel was determined and it appeared that every 1g of umbel gives 0.112 grams of pure extract (11.2%). All experimental doses were calculated and computed accordingly. Water extract was prepared by soaking the dried umbels in pre-boiled water for half an hour and then after filtration the filtrate was administered to the animals. Extract used for intraperitoneal injections was subjected to syringe filtration using Millipore filters (0.45 µm pores size) after simmering the appropriate amount of the plant in 0.9% NaCl solution. Water extract was stored in aliquots in the deep freeze at –80 ^{o}C.

2.4 Cream Preparation:

For the topical application, a cream was prepared where the main constituents are 3g cholesterol, 3g of stearyl alcohol, 8g white wax and 86 g of white petroleum. The

stearyl alcohol, white wax and petroleum are melt together on a hot plate. The cholesterol is added to the mixture and stirred on a hot plate until congealed. Using the mortar and pestle 150 ml of the water extract are mixed with the cream and then the resulting cream is stored at 4 °C. Different concentrations were applied: 50 mg/kg, 250mg/kg, 500mg/kg body weight.

2.5 Anti-tumor effect:

2.5.1 Induction of Papillomas in BALB/c mouse skin: The dorsal surface of mice was shaved several times until no hair growth is seen. Skin cancer initiation was performed by a single topical application of 190 nmol DMBA (7,12- dimethyl benz{a} anthracene) dissolved in 0.2 ml acetone. Three weeks following initiation, mice were promoted twice a week with a topical application of 8nmol TPA (12-O-tetradecanoyl phorbol-13-acetate) dissolved in 0.2 ml acetone. TPA application continued all over the duration of the experiment (17 weeks).

2.5.2 Treatment of mice: Daucus carota water extract was administered through three different ways: a) Intraperitoneal injections (250 mg/kg body weight) b) Gavage or force-feeding (250 mg/kg body weight) and c) topical application (50, 250 and 500 mg/kg body weight).

A- Intraperitoneal injections: *Daucus carota* extract was diluted with isotonic saline (0.9% NaCl) to obtain a concentration of 250mg/kg body weight. The water extract (0.27 ml) was injected into the intrapritoneal cavity using 1ml plastic syringe. Care was taken not to perforate any of the abdominal organs. TPA was applied on the dorsal skin 20 minutes after the treatment.

B- Gavage or force-feeding experiment: *Daucus carota* water extract was forced into the pharyngoesophageal region by introducing a micropipette tip into the pharynx and injecting *Daucus carota* water extract into the esophagus. TPA was applied on the dorsal skin 20 minutes after the treatment.

C- Topical application: Different concentrations of *Daucus carota* water extract cream were prepared (table 2.1). 290 mg of *Daucus carota* water extract cream was applied to the dorsal skin of the mouse 20 min prior to TPA solution application.

Experimental group	Treatment	Number of mice
Control	290 mg of cream	5
Dose 1 (50 mg/kg body weight)	290 mg of cream containing 1.5 mg *Daucus carota* water extract	10
Dose 2 (250mg/kg body weight)	290 mg of cream containing 7.5 mg *Daucus carota* water extract	10
Dose 3 (500mg/kg body weight)	290 mg of cream containing 15 mg *Daucus carota* water extract	10

Table 2.1: Doses of *Daucus carota* water extract applied topically to mice skin

2.5.3 Data collection: The incidence and yield of skin tumors were recorded on a weekly basis for the duration of the experiment (17 weeks). Tumor yield was expressed as the number of tumors divided by the number of mice in each group. Tumor incidence was assessed as the % of mice bearing tumors in each group. Tumor volume was recorded at week 15. Tumor volume was estimated using specific reference volume models provided by our labs.

2.6 Antioxidant Activity:

2.6.1. Preparation of water extract of *Daucus carota*

1 g of chopped *Daucus carota* was extracted with 150ml of boiling water on a hot plate for 10 minutes. This extract was then filtered through a filter paper Whatman no.1. The filtrate was used directly for antioxidant assay without storage.

2.6.2. Antioxidant activity assay

A- Free radical scavenging activity (DPPH assay):

The antioxidant activity of the water extract from *Daucus carota* was tested using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The extract was allowed to react with DPPH in order to evaluate the free radical scavenging activity. An aliquot of 22 µl of the extract was mixed with 200µl of DPPH (0.00591g of DPPH was dissolved 100ml 80% methanol) and incubated at 37°C for 30 minutes and the absorbance was read at two intervals 0 minutes and 30 minutes at 520 nm. The activity was monitored by a decrease in an absorbance at 520 nm. The radical scavenging activity was calculated as a percentage of DPPH scavenging activity using the equation:

% scavenging activity = 100x [1-(A_E/A_D)]

where A_E is the absorbance of the DPPH solution with an extract added, and A_D is the absorbance of the DPPH solution with nothing added (Kahkonen et al; 1999).

B- Ferric reducing antioxidant power (FRAP) assay:

The reducing power was determined by using FRAP assay. Briefly the FRAP reagent contained 2.5 ml of 10mM tripyridyltriazine (TPTZ) solution in 40 mM HCL plus 2.5 ml of 20 mM FeCl3 and 25ml of 0.3 M acetate buffer, pH 3.6. Dilutions ranging from 20-500 µMol of FeSO4 solution were prepared to obtain a calibration curve. Aliquots (0.2 ml) of extract were mixed with 2.8 ml of FRAP reagent. The absorbance was measured at 595nm. The antioxidant activity was measured by its ability to reduce the Fe 3+/ ferric cyanide complex by forming ferrous products. Fe2+ can be monitored by measuring the formation of Perl's Prussian blue. Increased absorbance indicates a strong reducing power. In order to make comparison, Ascorbic acid (0.1 gr/100ml and dilutions ranging from 10x to 1000 x) was also tested under the same conditions as a

standard antioxidant compound. All readings were done at 0 minutes and 8 minutes with minimal exposure to light (Kahkonen et al; 1999).

C- Determination of the phenolic content:

Total phenolic content of the *Daucus carota* extract was determined using the Folin-Ciocalteu's reagent (FCR). 100 µl extract, 16 µl water, 100 µl FCR and 200 µl saturated Na_2CO_3 were mixed together. After incubation for 30 minutes at room temperature the absorbance is read at 750 nm. The total phenolic content were calculated as a gallic acid equivalent from a calibration curve of gallic acid standard solutions (ranging from 25 to 800 mg/ml) and expressed as mg of gallic acid per gram of dry sample (Kahkonen et al; 1999).

2.7 Statistical analysis

Values of the different tested parameters within each group are presented as mean ± SEM. Comparison of each group with the control was done using the student t-test. A *p*-value lower than 0.05 was considered significant.

Chapter 3

RESULTS

3.1 Tumor Experiment:

3.1.1 Gavage Treatment:

Daucus carota water extract administered by gavage had no effect on tumor incidence and tumor yield. As shown in tables 3.1 and 3.2 and figures 3.1 and 3.2, no inhibition was recorded for the tumor yield, tumor incidence and the tumor volume at week 15.

3.1.2: Intraperitoneal Treatment:

The ability of intraperitoneal injections to inhibit tumor promotion by TPA was determined using the *Daucus carota* water extract of concentration 250 mg/kg. Referring to figures 3.1 and 3.2, it is clear that the intraperitoneal injections caused a decrease in the tumor incidence and tumor yield. Referring to table 3.1, *Daucus carota* water extract injections caused inhibition for the tumor yield and tumor incidence by 28.8% and 23 % respectively at week 15. In terms of tumor volume, week 15 showed an 86.4% inhibition as compared to control.

3.1.3 Topical treatment:

No results were recorded in the topical treatment. Almost no papillomas were seen in both experimental and control groups during the duration of the experiment.

Table 3.1: The effects of different concentrations of *Daucus carota* water extract administered by various means on the promotion of mouse skin papillomas (PAS) at week 15.

	Mice #			Papilloma	Yield	Papilloma	Incidence
Treatments	-	% Survival	Weeks of 1st PAS	PAS/ Mouse	% Inhibition	% Mice with PAS	% Inhibition
Control (TPA)	10	100	6	13.7±1.89	0	100	0
Gavage	10	100	6	15.5 ±2.32	0	100	0
Intraperitoneal	10	80	6	9.77 ±1.46	28.8	77	23

Table 3.2: The inhibitory effects of *Daucus carota* water extract treatment on papilloma volume at week 15.

Treatment	Tumor Volume (mm^3)	% Inhibition
Control (TPA)	45.08 ± 9.53	0
Gavage	109.7 ± 39.9	-143
Intraperitoneal	8.47 ± 2.7 *	86.4

* $P<0.05$ significant compared to control.

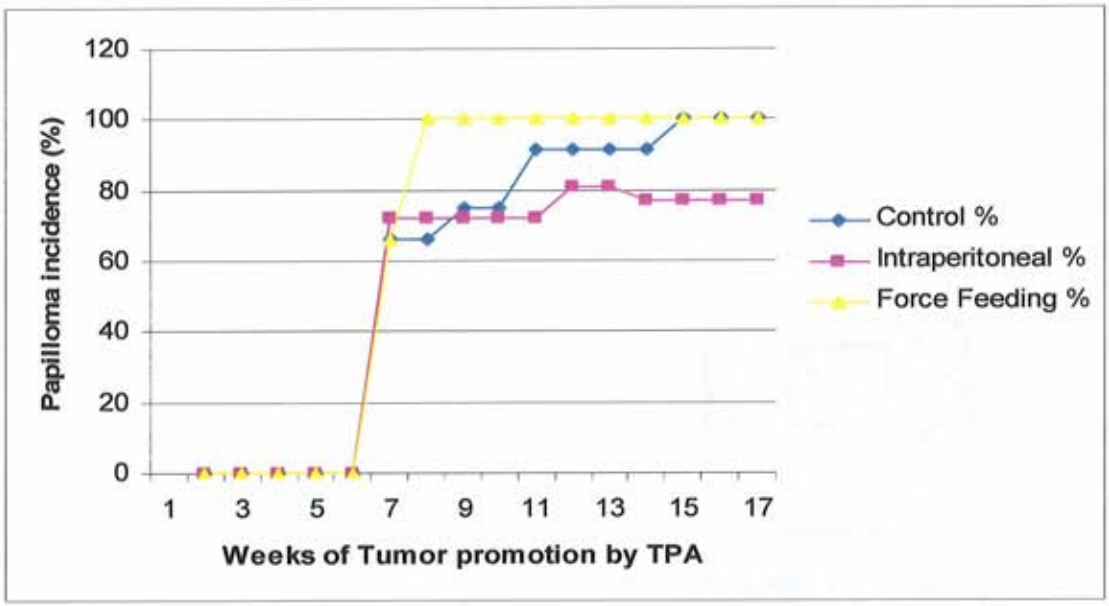

Figure 3.1: The effect of gavage and intraperitoneal injections of *Daucus carota* water extract on papilloma incidence % over the study period.

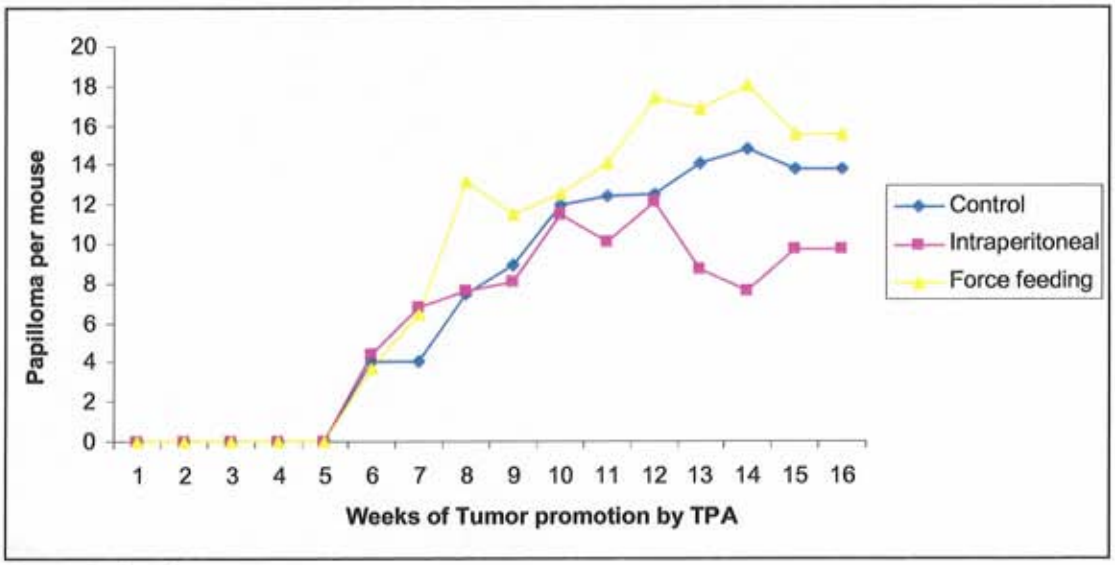

Figure 3.2: The effect of gavage and intraperitoneal injections of *Daucus carota* water extract on the number of papillomas per mouse over the study period.

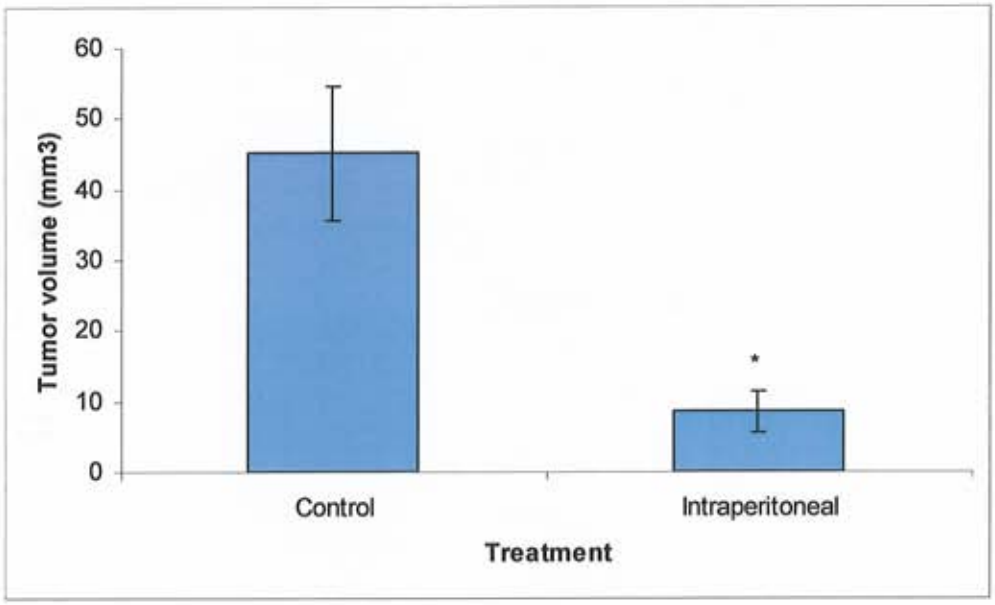

Figure 3.3: The effect of intraperitoneal injections of *Daucus carota* water extract on tumor volume at week 15. * P<0.05 significant compared to control.

3.2. Papilloma Morphology

Figure 3.4 show the papilloma morphology at week 15 for a representative mouse from each group, in comparison to the controls. Differences between the groups concerning tumor yield, incidence, and volume discussed before are obvious in this figure.

3.4 Mice Body Weight

Mice were subjected to this experiment for the duration of 17 weeks. Figure 3.5 shows the average body weight in grams measured at two main intervals during the experiment week 5 and week 17.

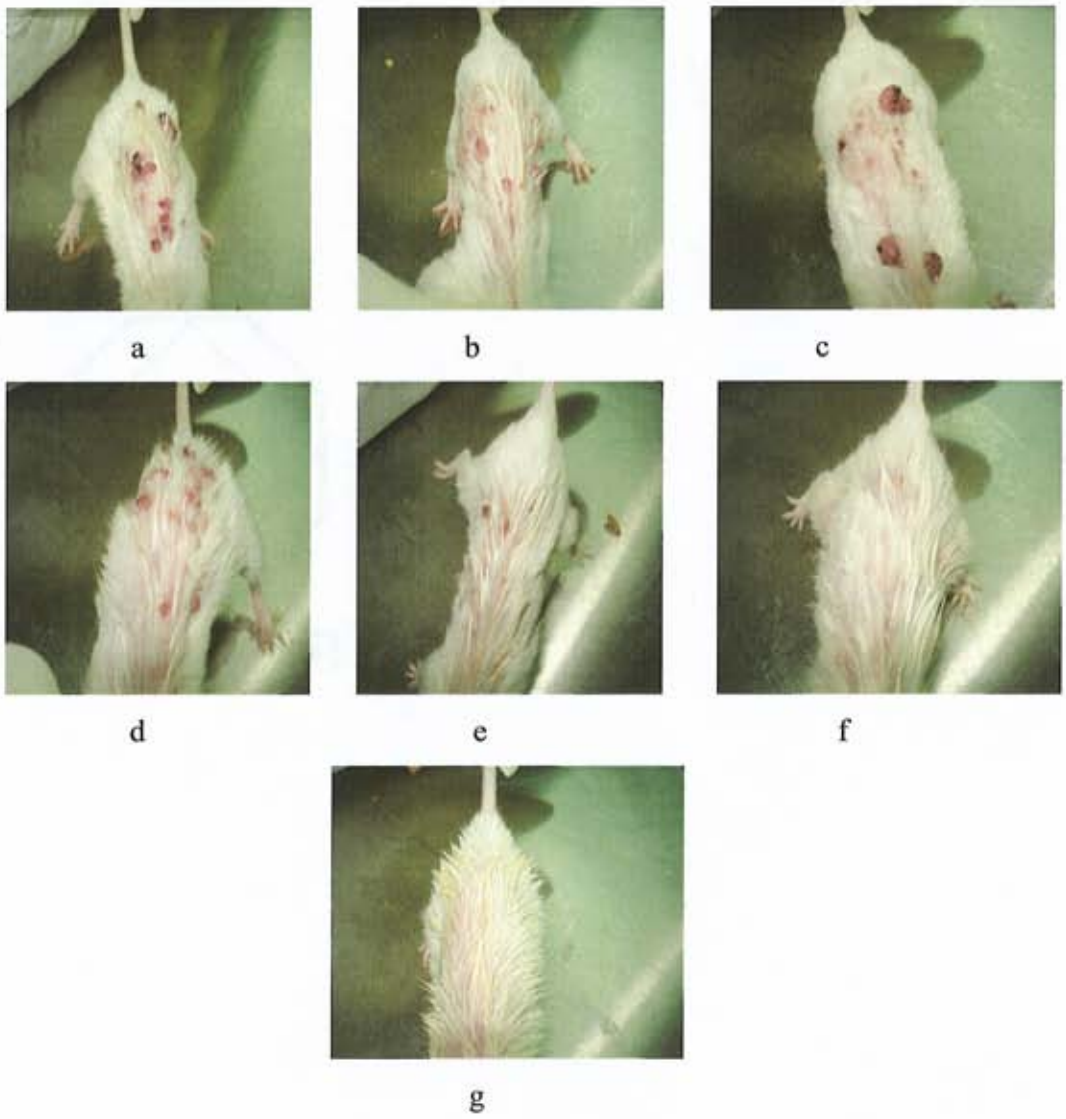

Figure 3.4: Papilloma morphology at week 15 (a, b) control; (c, d) gavage; (e,f) intraperitoneal injection; (g) Topical group.

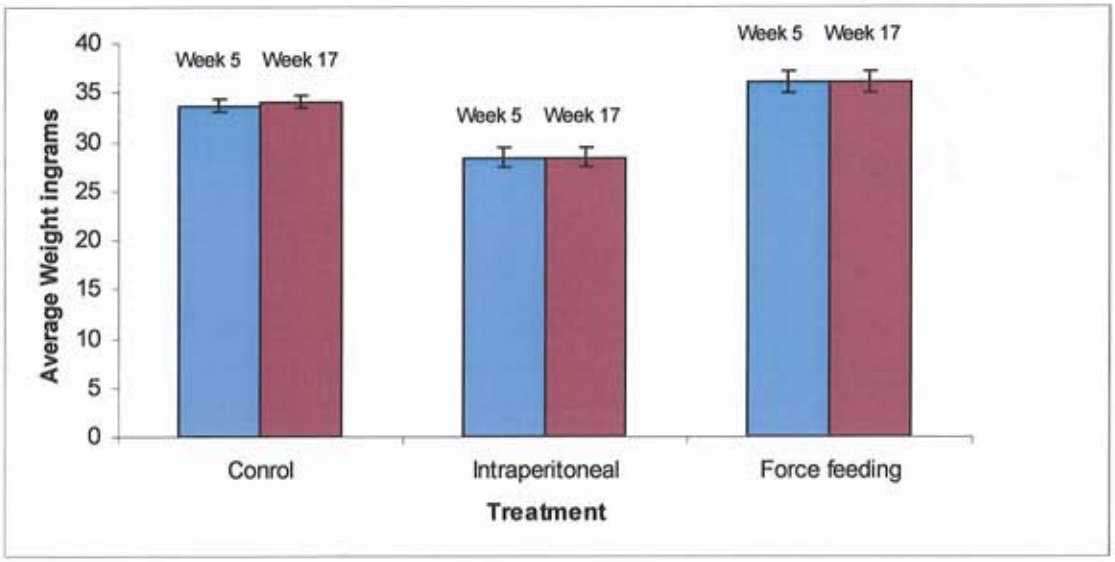

Figure 3.5 Average body weight of the mice of the three groups measured at week 5 and week 17.

3.3Antioxidant activity of *Daucus carota* water extract

3.3.1 Total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent and is expressed as Gallic acid equivalents (GAE) per gram. The total phenolic contents calculated using the standard curve of Gallic acid was 10.6µg/ml (fig. 3.6).

3.3.2 FRAP assay

FRAP method was used to evaluate the reducing power of *D. carota* water extract. In this method the reduction of ferric-tripyridyltriazine complex to its ferrous form is evaluated in the presence of antioxidants (Zhishen et al; 1999). Based on the results, the crude extract have the ability of reducing Fe^{3+} to Fe^{2+} by FRAP value of 508 µM/mg dried extract (Fig.3.7).

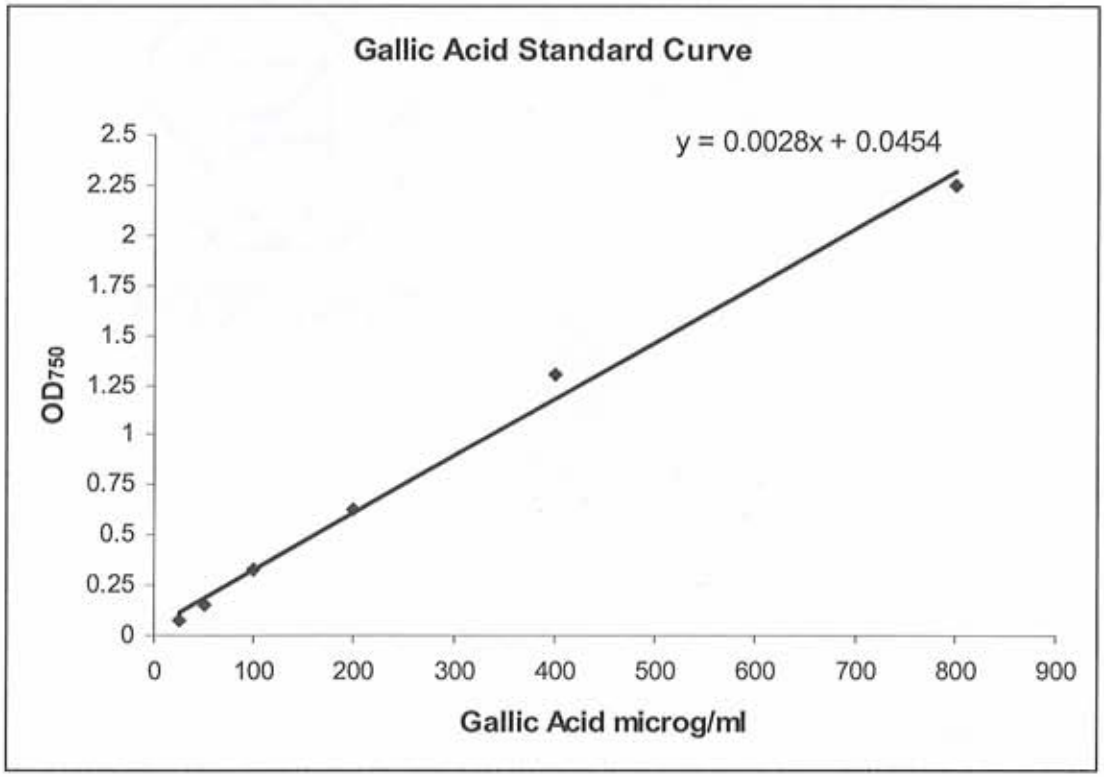

Figure 3.6: Gallic acid standard curve

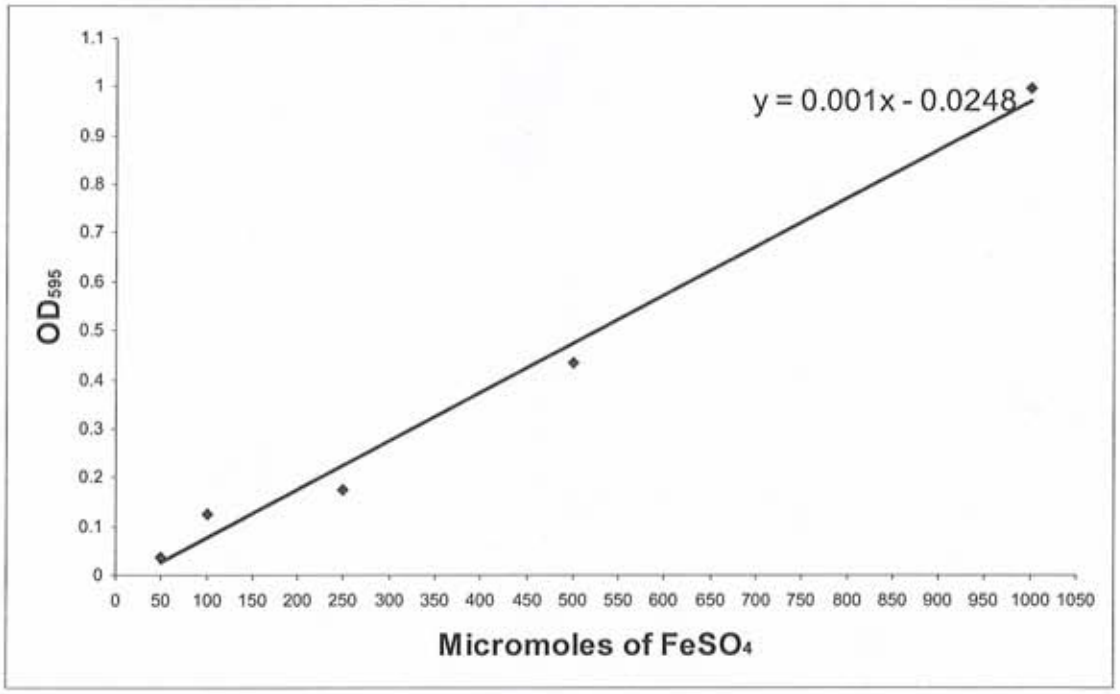

Figure 3.7 Ferrous Sulfate Standard curve

3.3.3 DPPH assay

DPPH assay was used to evaluate the antioxidant activity of the water extract. A stable radical 2,2-diphenyl-1-picrylhyrazyl (DPPH). The extract was allowed to react with DPPH in order to evaluate the free radical scavenging activity. The activity was monitored by a decrease in absorbance at 520nm. The *Daucus carota* water extract demonstrated a % scavenging activity = 65.4%.

Table 3.3: Antioxidant properties of *Daucus carota* water

Plant	DPPH assay	FRAP µM/mg	Phenolic content µg/ml
Daucus carota	65.4%	508	10.6
Ascorbic Acid		1324	

Chapter 4

DISCUSSIONS AND CONCLUSIONS

Wild carrot is listed by the council of Europe as a natural source of food flavoring (Barnes et al., 2002). The plant is commonly used in the Lebanese folklore medicine as a remedy against gastric ulcer, diabetes, muscle and back pain and to enhance liver function and immune system. It is worth noting the distinction between the edible carrot, *Daucus carota L. ssp. sativus*, and the wild carrot, *Daucus carota L. ssp. carota*, which has inedible whitish tough roots. Numerous studies have been conducted on *Daucus carota L. ssp. sativus*, while little was done on *Daucus carota L. ssp. carota*. A recent study by Assaf et al. (2007) on human promyelocytic leukemia HL-60 cells concluded that DC (*Daucus carota*) reduces cell proliferation and induces cell differentiation. Other studies highlighted the beneficial effects of the plant on a wide range of conditions, for example the petroleum ether extract of the seeds administered intraperitonealy showed antitumor activity, inhibiting the growth of Ehrlich ascites tumor in mice (Majumder et al., 1998). The present study is considered to be the first research on the effectiveness of *Daucus carota* water extract on inhibiting the promotion phase of carcinogenesis in mouse skin. Consequently, it could be considered a first step for further investigations about the inhibitory mechanisms and relate them to other types of cancer.

Evaluation of the anti-tumor promoting effects of *Daucus carota* water extract on skin carcinogenesis was established through the DMBA-initiation-TPA promotion mouse skin carcinogenesis model (Indra et al., 2007). The anti-tumor activity was studied using three different routes of administration: topical application, gavage, and intraperitoneal injections. The anti-tumor effect was only observed with the intraperitoneal mode of treatment. The % inhibitions of papilloma incidence, yield and volume were 28 %, 23% and 86.4% respectively. Phytochemical and biological investigation of the roots of the wild carrot, *Daucus carota* L. ssp. *carota*, indicated the presence of four sesquiterpene daucane esters (Ahmed et al., 2005). β-caryophyllene, one of the sesquiterpene present, has several biological function such as anti inflammatory, antibiotic, antioxidant and

anticarcinogenic activities (Tambe et al., 1996). The potentiating effect of β-caryophyllene on the anticancer activity of α-humulene, isocaryophyllene, and paclitaxel in 3 human tumoral cell lines (MCF-7, DLD-1, L-929) was evaluated by Legault & Pichette (2007). A non-cytotoxic concentration of β-caryophyllene significantly increased the anticancer activity of α-humulene and isocaryophyllene on MCF-7 cells. Moreover, β-caryophyllene potentiated the anticancer activity of paclitaxel on MCF-7, DLD-1 and L-929 cell lines. However, β-caryophyllene and other sesqueterpines are barely soluble in water (Hiltpold et al., 2005); therefore, the anti-tumor effect of the water extract due to β-caryophyllene is minimal in the present study. On the other hand, anthocyanins, water soluble antioxidants (Xavier et al., 2008), are found in high amounts in DC and proved to inhibited colon cancer cell proliferation at varying degrees (Jing et al., NA). Consequently, the anti-tumor effects of the water extract may be due to the high amount of the anthocyanins present. This study raises the possibility of considering DC as a target therapeutic agent for cancer. Nevertheless; it is important to detect the composition of *Daucus carota* water extract by LC-MS. This may give us the opportunity to isolate the different ingredients which may be tested instead of the whole crude extract to detect any specific anti-tumor activity.

For this study, large quantities of the *Daucus carota* plants were collected from the same location and at the same time because the composition of all plants vary according to environmental and seasonal changes including temperature, altitude, humidity and soil conditions (Pirzard et al., 2006)

The efficacy of the various means of *Daucus carota* water extract administration was evaluated. It was quite obvious that gavage treatment neither had an effect on the papilloma yield nor on the papilloma incidence as compared to the control. The papilloma incidence coincides with that of the control while the papilloma yield and volume were higher. Therefore, gavage seemed not to be an appropriate way for the treatment of skin cancer. This may be explained by several theories: first, palatability is considered a major factor that could have participated in such contribution. We are forcing a volume of 27 µl in the mouth of each mouse, a high risk of vomiting back is

present (Gali & Affara, 2000). Second, the active ingredients that are present in this extract might be lost during their passage through the gastrointestinal (GI) tract. They might be either degraded by digestive enzymes or initially not absorbed through the digestive system or inactivated by the digestive organs after absorption, or affected by the drastic PH changes present between the stomach and the GI tract.

In case of intraperitoneal injections, there was no delay in the appearance of papillomas. As for the papilloma yield the % inhibition was 28.8 % as compared to the control. On average, each mouse in the intraperitoneal group had 10 papillomas as compared to the control where each had 13 papillomas. As for the papilloma incidence, the number of mice having papillomas in the intraperitoneal group was less than that of the control group (by 23 %). As for the tumor volume, significant results were recorded. The % inhibition for the tumor volume was 86.4% at week 15. The skin tumors were barely protruding out of the skin forming exceptionally tiny papillomas. As a result *Daucus carota* water extract had an inhibitory effect on papilloma yield, incidence and volume.

These outcomes were additionally investigated by testing the presence of antioxidants in the water extract. Antioxidants have been used as chemo preventives against skin cancer (Ahmad et al., 2001). The water extract of *Daucus carota* used in the present study was assessed for its antioxidant activity and total phenolic contents. Relatively high antioxidant activity was determined by DPPH (65.4%) and FRAP (508µmol/g) assay. A good correspondence is found between antioxidant activity and phenolic content which was proved in Bulgarian medicinal plants (Ivanonva et al., 2005), Chinese medicinal plants (Zheng et al., 2001) , some fruits, vegetables and grain products (Velioglu et al., 1998). The phenolic hydroxyl groups present in plant antioxidants have redox properties (Shahidi et al., 2000) allowing them to act as a reducing agent and a hydrogen donor in the two assays (Shahidi et al., 2000); thus phenolic compounds could be the major antioxidant in herbal drinks. Nevertheless, a high phenolic content doesn't always suggest a high antioxidant activity such as the case in *Schefflera leucantha* which had a high phenolic content but it showed moderate antioxidant activity. This finding was in agreement with the study of Kahklnen et al. (1999) who found no correspondence between antioxidant activity and phenolic content.

In our study, *Daucus carota* water extract exhibited exhibited a relatively high FRAP value, a relatively high DPPH value but moderately low phenolic compound content value. High antioxidant activity may be due to non-phenolic antioxidant molecules including anionic substituted cinnamic acid conjugates, avenanthramides, which also are potential antioxidants (Dimberg et al., 1993). This study indicates the presence of non phenolic antioxidants in *Daucus carota* water extract which may help protect against free radicals.

In conclusion, intraperitoneal injections seemed to be an effective mean against skin cancer. Nevertheless, it is worth mentioning that D.C water extract had no effect on the average body weigh of the mice during the duration of the experiment. Although two of the mice died in the intraperitoneal group, this can be attributed to internal bleeding resulting from the injections and not to the water extract since the remaining mice were healthy. As a result, *Daucus carota* water extract doesn't affect the health of the mice negatively. Having this in mind, DC water extract when administered intraperitonealy may have promising role in the treatment of cancer.

The third route of administration was the topical application. Three different concentrations were tested in addition to a control group. The three concentrations were 50mg/kg, 250 mg/kg, and 500mg/kg. The control group received pure cream with no DC water extract. Apparently, at week 6 of the experiment papillomas were not observed either in the control group or in the other three experimental groups. Moreover, no papillomas appeared during the duration of the experiment. Since the control group had no papillomas at week 6, we suspected that the cream was acting as a barrier for the TPA treatment. TPA was not being absorbed by the skin of the mice hence no papilloma induction was observed. A period twenty minutes was not enough for the cream to be absorbed by the skin of the mouse, to allow appropriate TPA absorption by the skin. Quite often we noticed a huge amount of the volume applied was sliding on the skin surface. Modifications that could make the topical treatment successful include: More time must be given for the cream to be absorbed and then apply the TPA. In conclusion,

further research should be done in this field in order to enhance the results of the topical group.

In conclusion, the gavage treated groups showed no inhibitory effects on the chemically induced tumors. As for the topical route, the method used was not valid since no papillomas appeared during the duration of the experiment. Only the intraperitoneal group showed promising results, especially when it comes to tumor volume. Moreover, DC had no negative effects on the health of the mice. Further research is needed to determine the compound in the extract that is responsible for the decrease in the tumor volume, yield and incidence. Nevertheless, the antioxidant assays performed on the water extract lead to the fact that the plant possesses antioxidant properties which may help protect against free radicals and against cancer. Further research is recommended to know the compounds responsible for the antioxidant properties of the extract. Future work including in vitro and in vivo studies is needed to come up with solid conclusions regarding the effectiveness of the D.C water extract as an anti-tumor remedy. It is also important to do histological studies on the tumors to support the observed results.

Chapter 5
BIBLIOGRAPHY

Ahmed, A. A, Bishr, M. M, El Shanawany, M. A, Attia, E. Z, Ross, S. A, & Pare,W. (2005). *Phytochemistry ,66,* 16-80.

Auroma, O.I. (1998) Free radicals, oxidativestress, and antioxidants in human health and disease . *Journal of American Chemical Society, 75*, 199-212.

Balasubramaniam, P, Pari, L, & Venugopal, P. M. (1998). Protective effect of carrot (*Daucus carota* L.) against Lindane- induced hepatotoxicity in rats. *Phytotherapy Research, 12*, 434-436

Baumann, L. (2005). How to prevent photoaging. *Journal of Investigative Dermatology, 25*, xii-xiii.

Bergonzelli, G. E, Donnicola, D, Porta, N, & Corthesy-Theulaz, I. E. (2003). Essential oils as components of a diet-based approach to management of *Helicobacter* infection. *Antimicrobial Agents and Chemotherapy, 47*(10), 3240-3246.

Bishayee, A, Sarkar, A, & Chatterjee, M. (1995). Hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. *Journal of Ethnopharmacology, 47*, 69-74.

Boutwell, R. K. (1964). Some biological aspects of skin carcinogenss. *Progress in Experimental Tumor Research, 4,* 207-250.

Braithwaite, E, Wu, X, & Wang, Z. (1998). Repair of DNA lesions induced by polycyclic aromatic hydrocarbons in human cell-free extracts: Involvement of two excision repair mechanism in vitro. *Carcinogensis, 19*(7), 1293-1246.

Branien AL. (1975). Toxicoly and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *Journal of American Chemical Society*, *52*, 59-63.

Buckmaster, M. (2007). *Skin cancer*. New York: Benchmark Books.

Dandiya, P. C, & Chopra, Y. M. (1970). CNS-active drugs from plants indigenous to India. *Indian Journal of Pharmacology*, *2*(3), 67-90.

Diab-Assaf, M., Mroueh, M., & El-Sharif, S. (2007). Evaluation of anti-cancer effect of *Daucus carota* on the human promyelocytic leukemia HL-60 cells. ACCR International Conference on Molecular Diagnostics in Cancer Therapeutic Development, Atalanta, USA.

Digiovanni, J. (1992). Multistage carcinogensis in mouse skin. *Journal of Clinical Pharmacy and Therapeutics, 54*, 63-128.

Duh P-D. (1998) Antioxidant activity of Burdock: Its scavenging effect on free-radical and active oxygen. *Journal of American Chemical Society ,75*, 455- 463.

Duke, J. A. (1978). The quest for tolerant germplasm. In *ASA Sspecial Symposium 32, Crop Tolerance to Suboptimal Land conditions*. Madison, WI: American Society of Agronomy.

Fukushima, S, Hassegawa, A, Shibata, M. & Ogiso, T. Carcinogenicity of butylated hydroxyanisole in F344 rats. *Journal of National Cancer Institute*, 70, 343-347.

Gambhir, S. S., Sen, S. P., Sanyal, A. K., & Das, P. K. (1979). Antispasmodic activity of the tertiary base of *Daucus carota*, Linn seeds. *Indian Journal of Physiology and Pharmacology, 23*(3), 225-228.

Gambhir, S. S., Sanyal, A. K., Sen, S. P. & Das, P. K. (1966). Study on *Daucus carota* Linn, Part (I). Pharmacological studies with the water soluble fraction of the alcoholic extract of the seeds, A preliminary report. *Indian Journal of Medical Research*, *54*, 178-187.

Gonny, M, Bradesi, P, Casanova, J. (2004). Identification of the components of the essential oil from wild Corsican *Daucus carota* L.using 13C-NMR spectroscopy. *Journal of Flavenoid Fragrance,* 19, 424- 433.

Guevara, J. M., Chumpitaz, J., & Valencia, E. (1994). The in vitro action of plants on *Vibrio cholerae. Revue of Gastroenterology of Peru*, *14*(1), 27-31.

Granelli, K. Bjrck, L. Appelqvist, L-A. (1998). The variation of SOD and XO activities in milk using an improved method to quantitate SOD activity. *Journal of Science, Food, and Agriculture*, *67*, 85-91.

Halliwel, B. (1994). Free radicals, antioxidants and human disease: Curiosity, cause or consequence. *The Lancet*, *344*, 721- 724.

Halliwell, B. (1995). How to characterize an antioxidant: An update. *Biochemical Social Symptoms*, *61*, 73- 101.

Halliwell, B. & Gutteridge, JM. (1989). *Free radicals in biology and medicine* (pp.23-30) Oxford: Clarendon Press.

Hiltpold, I. Turlings, T. (2005). Is (*E*)-Caryophyllene An Ideal Belowground Signal? University of Neuchâtel, Switzerland.

Hoffman, D. (1990). *The new holistic herbal* (3rd ed.). Great Britain: Element Books.

Indra, A. K, Casaneda, E., Antal, M. C., Jiang M., Messaddeq, N., & Meng, X. (2007). Malignant transformation of DMBA/TPA-induced papillomas and nevi in

the skin of mice selectively lacking retinoid-X receptor in epidermal keratinocytes. *The Society for Investigative Dermatology, 127,* 1250-1260.

Ivanova, D. Gerova, D. Chervenkov, T. Yankova, T. (2005).Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology, 96*,145-150.

Juneidi, M.J. (1999). *Wild plants of Jordan;* Amman ,Jordan: [n.p.].

Kahkonen, M.P. Hopia, A. Vulorela, J. Rauha, P. Pihlaja, T. Kujula, S. Heinoen. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agriculture, 47*, 3954-3962.

Kapoor, L. D. (2001). *Handbook of Ayurvedic medicinal plants: Herbal reference library.* [n.p.]: CRC Press.

Katiyar, S., & Moukhtar, H. (1997). Inhibiton of phorbol ester tumor tetradecanoylphorbol-13-acetate caused inflammatory responses in SENCAR mouse skin by black tea polyphenols. *Journal of Carcinogenisis, 18* (10), 1911-1916.

Kumarasamy, Y., Cox, P. J., Jaspars, M., Nahar, L., & Sarker, S. D. (2002). Screening seeds of Scottich plants for antibacterial activity. *Journal of Ethnopharmacology, 83* (1-2), 73-77.

Kruawam, K.,& Kangsadalampai, K. (2006). Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extracts of herbs. *Journal of Pharmacology, 30*, 28-35.

Lane, W. I., & Comac, L. (1998). *The skin cancer answer: The Natural treatment for basal and sqamous cell carcinomas and keratoses.* New York: Avery Publication.

Legault, J., & Peichette, A. (2007). Potentiating effects of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. *Journal of Pharmacy and Pharmacology, 59*(12), 1643-1647

Lund, E., White, M. (1990). Polyacetylenes in normal and water stressed "Orlando Gold" carrots (*Daucus carota*). *Journal of Science of Food and Agriculture, 51*(4), 507-516.

McClay, E. F., McClay, M. E. T. & Smith, J. (2003). *100 Questions & answers about melanoma & other skin cancers*. Texas: Jones and Bartlett Publishers.

Majumder, P. K., & Gupta, M. (1998). Effect of the seed extract of carrot (*Daucus carota* Linn.) on the growth of Ehrlich ascites tumour in mice. *Phytotherapy Research, 12*, 584-585.

McGuffin, M., Hobbs, C., Upton, R., & Goldberg, A. (1997). *Botanical safety handbook.* [n.p.]: [n.p.].

Miller, N.J. Diplock, A.T. & Rice-Evans, C.A.(1995). Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *Journal of Agriculture and Food Chemistry, 43*, 1794-1801.

Miller, M., Webr, R. S., & Goepfert, H. (1996). *Basal and squamous cell skin cancers of the head and neck.* Baltimore, MD: Williams & Wilkins.

Mills, S. (1994). *The complete guide to modern herbalism.* Great Britain: Thorsons.

Mitich, L. W. (1996). Intringuing world of weeds: Wild carrot (*Daucus carota* L.). *Weed Technology, 10*, 455-457.

Mouterde, P. (1970). *New flora of Lebanon and Syria*. Beirut: Dar El-Mashreq.

Nathanson, L. (1987). *Basic and clinical aspects of malignant melanoma. Cancer Treatment and Research*. USA: Springer.

O'Brien, T., Megosh, L. C., Gilliard, G., & Solar, A. (1997). *Herbal medicine, a guide for health-care professionals*. London: The Pharmaceutical Press.

Perchellet, J. P., Gali, H., Perchellet, E., Klish, D., & Armbrust, A. (1992). *Antitumor promoting activities of Tannic Acid, Ellagic Acid, and several Gallic Acid derivatives in mouse skin*. New York: Plenuim Press.

Pirzars, A, Alyari, H, Shakiba, M R, Zehtab-Salmasi, S, &Mohammadi, A. (2006). Essential oil content and composition of German Chamomile (Matricaria chamomilla L.) at different irrigation regimes. *Journal of Agronomy*, 5(3), 451-455

Rajpad, S., & Marsden, J. (2008). *ABC of skin cancer*. Massachusetts: Blackwell Publishing.

Reed, C.F. (1976). Information summaries on 1000 economic plants. Typescripts submitted to the USDA.

Rice-Evans, C.A. Sampson, J, Bramley, P.M. Hollowa, D.E. (1997).Why do we expect carotenoids to be antioxidants in vivo. *Free Radical Research, 26*, 381-398.

Rigel, D., Friedman, R., Dzubow, L. M. Reintgen, D., Marks, R., & Bystryn, J. C. (2004). *Cancer of the skin*. Philadelphia: Saunders Publications.

Robinson, E.E., Maxwell, S.R.J., & Thorpe, G.H.G. An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. (1997). *Free Radical Research, 26*, 291-302.

Ross, I. (2005). *Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses* (3rd ed.). Farmington Hills, MI: Gale.

Ruddon, R.W. (1987). Causes of cancer: Chemical and physical carcinogenisis. In *Cancer Biology*. (pp 297-336). New York: Oxford University Press.

Saad, H. E. A., El-Sharkawy, S. H., and Halim, A. F. 17. (1995). Essential oils of *Daucus carota* ssp. Maximus. *Pharmaceutica Acta Helvetiae, 70* (1), 79-84.

Sandarc, M,. Ashok, M, Kalkura, N. (2002). Obsevation of cholesterol nucleation in a magnetic foeld. *Journal of Biological Crytallography,* 51(10), 1711-1714.

Sasaki,S., Ohta, T., and Decker, EA. (1996). Antioxidant activity of water soluble fractions of salmon spermary tissue. *Journal of Agriculture and Food Chemistry, 44*, 1682-1686.

Shahidi, F. Wanasundara, P.K. (1992). Phenolic antioxidants. *Critical Review of Food Science and Nutrition, 32*, 67-103.

Squadriato, G.l., and Pelor, W.A. (1998) Free Rad. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. *Biology and Medicine 25*, 392-403.

Tambe, Y., Tsujiuchi, H., Honda, G., Ilkeshiro, Y.,& Tanaka, S. (1996). Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β-caryophellene. *Journal of Planta Medica, 62*, 469-470.

Tanizawa, H., Ohkawa, Y., Takino, Y., Miyase, T., Ueno, A., Kageyama, T., & Hara,

S., (1992). Studies on natural antioxidants in citrus species I: Determination of antioxidative activities of fruits. *Chemical Pharmacology Bulletin, 40,* 1940-1942.

Valero, M., & Salmeron, M. C. (2003). Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *International Journal of Food Microbiology*, *85*(1-2), 73-81.

Velioglu, Y., Mazza, G., Gao, L.,& Oamah, B. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agriculture*, *46*, 4113- 4117.

Wehbe, K , Mroueh, M., Baroody, G, & Daher, C.(2009). The role of aqueous and methanolic extract of *Daucus carota* in lipemia, glycemia, inflammation, gastric ulcer and bacterial growth. *Journal of Complementary and Integrative Medicine*, *6(1).*

Xavier, M. F., Lopes, T., Quadri, M.,& Quadri, M.B. (2008). Extraction of red cabbage anthocyanins: Optimization of the operation conditions of the column process. *Brazilian Archives of Biology and Technology,* 51(1), 143-152.

Zheng, W., & Wang, S. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agriculture*, *49*, 5165-5170.