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In Vitro Effect of Some Medicinal Plant Extracts on Stimulating the Immune System in Cancer Patients*

تأثير مستخلصات بعض النباتات الطبية على تحفيز الجهاز المناعي في مرضى السرطان

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Abstract: The difficulty to treat cancer without side effects by surgery, chemotherapy, radiotherapy and immunotherapy, has led investigators to look for phytotherapy as a new strategy in cancer medicine. The immune system plays an important role in anti-tumor defenses, thus, we evaluated the proliferation potential of aqueous extracts from five medicinal plants on peripheral blood lymphocytes (PBLs) from 118 properly consented volunteers. We examined the aqueous extract of Thyme, Sage, Clove, Calament and Black Seed *in vitro* on PBLs from 100 cancer patients seeking treatment at Al-Basheer Hospital in Amman and 18 apparently healthy volunteers. PBLs were isolated from blood samples collected in heparin tubes. Then, Ficoll-Hypaque density gradient centrifugation was employed to enrich for lymphocytes. Cells were collected in RPMI containing 10% human serum at 10^6/mL before culturing them at an appropriate density. Three concentrations of the aqueous extract from each plant were assayed in duplicates on cultured PBLs for 72 hours. Cell proliferation was quantified using 3-(4, 5-Dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) standard method. Phytohemagglutinin (PHA) was used as a positive proliferation control, and sterile RPMI medium was used as a negative control. Among the five different aqueous extracts used in this study, only sage aqueous extract demonstrated promising results. Sage extract was effective in proliferating PBLs of all normal controls and cancer patients tested. Proliferation of the majority of PBLs from cancer patients was highly effective. However, some samples showed a weaker index of proliferation. PBLs proliferation exhibited a dose-dependent effect. The effectiveness among cancer patients was age, sex, cancer-type, and cancer-stage independent. Our data suggest that the aqueous extract of sage contains a polyclonal mitogen(s) that enhances the immune system in a non-specific fashion.
Keywords: *Proliferation Assay, Peripheral Blood Lymphocytes, Medicinal Plants, Sage, Immunostimulation, Polyclonal Mitogen.*

المستخلص: إن صعوبة معالجة السرطانات بدون تأثيرات جانبية بالجراحة أو العلاج الكيميائي أو العلاج النووي أو العلاج المناعي قاد الباحثين إلى تجربة العلاجات المستخلصة من النباتات كاستراتيجة حديثة في طب السرطان. وبما أن الجهاز المناعي يلعب دوراً مهماً في الدفاع ضد السرطان فقد تم في هذا البحث دراسة تأثير مستخلصات 5 نباتات طبية على القدرة التكاثرية للخلايا اللمفاوية الطرفية المأخوذة من عينات دم أشخاص مصابين بالسرطان، ومن أشخاص عاديين.

وقد تم دراسة تأثير المستخلص المائي لكل من: الزعتر، المريمية، كبش قرنفل، الزعتمان، والحبة السوداء على عينات خلايا لمفاوية طرفية مأخوذة من 100 شخص مصابون بالسرطان (ذكور وإناث)، وهؤلاء الأشخاص يعالجون في قسم الطب النووي في مستشفى البشير في عمان إضافة الى 18 شخصا من الأصحاء.لقد تم عزل الخلايا اللمفاوية الطرفية من عينات الدم المأخوذة في أنابيب تحوي مانع التجلط (هيبارين)، وتم استخدام التنبيذ المتدرج في مادة الفيكول لفصل الخلايا اللمفاوية ومن ثم زراعة الخلايا وتكثيرها في وسط غذائي مناسب (RPMI)، واستخدمت ثلاثة تراكيز مختلفة من مستخلصات النباتات الطبية المذكورة آنفا، وتركت الخلايا لتتكاثر لمدة 72 ساعة. وقد وجد بأن المستخلص المائي لنبات المريمية له تأثير واضح على تكاثر الخلايا اللمفاوية الطرفية عند الأشخاص المصابين بالسرطان وكذلك عند غير المصابين (الأصحاء) عند تحديد معدل تكاثر الخلايا باستخدام طريقة MTT. وقد استخدمت مادة PHA كضابط إيجابي، ومادة RPMI كضابط سلبي في هذه التجارب. أما بخصوص مستخلصات النباتات الأربعة الأخرى فلم يلاحظ لها تأثير يذكر على تكاثر الخلايا اللمفاوية. وقد وجد بأن تكاثر الخلايا اللمفاوية يزداد بشكل واضح بزيادة تركيز مستخلص نبات المريمية، أي انه يعتمد عل مقدار الجرعة المستعملة، ولكنه لا يعتمد على جنس المريض او نوع السرطان المصاب به الشخص أو مرحلة السرطان. ومن الجدير بالذكر أن نتائج هذا البحث تشير إلى وجود مواد (مركبات) محفزة لتكاثر الخلايا اللمفاوية في مستخلص نبات المريمية، أي أنها تزيد فعالية الجهاز المناعي بشكل عام.

كلمات مدخلية : *فحص تكاثر الخلايا اللمفاوية، المستخلص المائي، النباتات الطبية، المريمية، التحفيز المناعي، محدث للانقسام متعدد النسائل.*

INTRODUCTION

For centuries natural products with specific medicinal properties were used to treat diseases. These medicinal properties were identified by trial and error. In recent years alternative medicine gained more popularity and scientists started investigating these natural products. Many natural products are believed to stimulate the body disease-fighting ability in close proximity with infectious agents and neoplastic cells by activating the body non-specific defenses (Block and Mead, 2003). Stimulation of the humoral immunity is measured by increased lymphocyte proliferation and production of specific types of antibodies. In contrast, changes in the cellular immunity is assessed in terms of increase in the number and the activity of many cells, i.e., natural killer cells, macrophages, lymphokine-activated killer cells and proliferation of specific T lymphocytes subsets (Whiteside and Herberman 1994). Also, immune enhancement is measured by the stimulation of certain cytokines such as tumor necrosis factor (Cho and Leung 2000), cellular release of interferon g and interleukin 2.

The therapeutic use of plants or any of its components for the treatment of diseases is collectively called herbal therapy or phytomedicine. Importantly, over 25% of our common medicines contain at least some compounds obtained from plants (University of Maryland Health Library 2007). East of the Mediterranean countries (Fertile Crescent) has over 2000 of the world biota. This represents about 1% of the world recorded plants. Many of these plants are considered folkloric herbs. These herbs have a long history of usage in healing many diseases especially in underdeveloped countries. Indeed, the World Health Organization estimates that 80% of the people in underdeveloped countries rely on plant-based medicines for primary health care (Block and Mead, 2003).

Several reports evaluated the potential of phyto-products for their immune-modulating potential against cancer and infections (Cho and Leung 2007; Lamm and Riggs 2001; Kimura, *et al.* 2004), anti microbial effect (El Astal, *et al.* 2003), and their pharmacological use such as diuretics, laxatives, analeptic and lactagogues (Block and Mead, 2003). Kimura, *et al.* (2004), reported an anti-tumor active ingredient isolated from Angelica keiskei roots. Al-Kofahi and others demonstrated that several Jordanian plant extracts displayed *in vitro* anti-tumor effect against human cell lines (Alkofahi, *et al.* 1988; Alkofahi, *et al.* 1990; Duda, *et al.* 1999). They reported that these extracts were cytotoxic against A-549, a human lung carcinoma, MCF-7, a female breast carcinoma and HT-29, a colon adenocarcinoma.

Sage is an evergreen perennial herb from the mint family. Sage takes its name from the

Latin "Salvare" which roughly translates "to rescue" or to "heal". Sage originally grows along the Mediterranean coast up to and including the east side of the Atlantic, and Southern Europe. Phenolic extract of sage showed antibacterial activity against limited bacterial species such as *Staphylococcus aureus* and *Enterococcus* (El Astal, *et al.* 2003). In contrast, the aqueous extract of sage was highly effective against a wider range of microorganisms (El Astal, *et al.* 2003). Sage has been reported to enhance memory in healthy young volunteers (Tildesley, *et al.* 2003). Akhondzadeh, *et al.* (2003) showed (in double blind, randomized and placebo-controlled trials) that sage extract was effective in the treatment of patients with mild to moderate Alzheimer's disease. Also, sage aqueous extract was found to exhibit a remarkable capacity in retarding lipid oxidation. (Masuda, *et al.* 2002). Antioxidant property of extracts from different sage strains showed different active compounds, such as estrogen, saponins, and volatile oil (Masuda, *et al.* 2002).

MATERIALS AND METHODS

Subjects

The project was approved by the Ethics Committee of the Islamic Hospital in Amman and informed consent was obtained from each individual. Blood samples were collected from 100 properly consented cancer patients seeking treatment at the Radiotherapy Center, Al-Basheer Hospital in Amman. Al-Basheer Hospital is the main referral center for all areas in Jordan. Similarly, blood samples were collected from 18 apparently healthy control individuals who were properly informed and consented to the procedure.

Lymphocytes Preparation

Lymphocytes were isolated using Ficoll-Hypaque density gradients as described in the followings: Blood was collected in a vacutainer tube containing 20U/ml sodium heparin. Blood was diluted 2:1 with sterile RPMI, and layered onto Ficoll-Hypaque with ratio of blood/RPMI: Ficoll maintained 3:1. The blood was centrifuged at 1800 rpm for 35 minutes at room temperature. The lymphocyte layer (Buffy coat) was removed and washed twice in RPMI 1640 medium (Gibco, Carlsbad, CA) at 1200 rpm for 10 min each. Cells were then washed twice with RPMI 1640. Cell density was determined using a hemocytometer (Rose, *et al.* 2002).

Proliferation Assay and Tissue Culture

Freshly obtained lymphocytes were diluted in RPMI containing 10% human serum. Our empirical experiments showed that 200,000 lymphocytes per well were sufficient to get adequate absorbance in MTT assay (next section). Therefore, 200,000 cells were cultured in 0.2ml round bottom microtiter plates. Phytohemagglutinin (PHA) prepared in sterile RPMI (Epstein, *et al.* 1971), pH 7.4 at a concentration of 500mg/ml (Sigma, Saint Louis, MO). It was added at a final concentration of 1% in positive control wells. Cultured lymphocytes in negative control wells received a similar volume of sterile RPMI. Filter-sterile water extract from tested plants were added to duplicate wells. Cells were cultured for 72 hours before 10ml of MTT solution (final MTT concentration was 0.5mg/ml) to each well were added (Rodgers, *et al.* 1992).

MTT Colorimetric Assay

MTT assay depends on its uptake and reduction by viable cells. The yellow tetrazolium salt (MTT) is reduced in metabolically active cells to form insoluble purple formazan crystals by the mitochondria of viable cells (Epstein, *et al.* 1971; Rodgers, *et al.* 1992). Then, MTT crystals were solubilized by the addition of a dimethyl sulphoxide (DMSO) detergent. The color can then be quantified by spectrophotometry. After culturing cells for 72 hour, 20ml of MTT reagent was added at a final concentration of 0.5mg/ml for approximately 2 to 4 hours. Then 100ml DMSO detergent solution was added to lyse the cells and solubilize the colored crystals. The samples were read using an ELISA plate reader at a wavelength of 570nm. The amount of color produced is directly proportional to the number of viable cells. Specific proliferation was determined by subtracting the negative control readings from the reading of the experimental wells (Bakri and Douglas, 2005). The effect of chromagens in all

medicinal water extracts was excluded by adding the water extract before ELISA reading at OD_{570}. The MTT reagent was kept at 4°C in the dark. The DMSO detergent reagent was prepared fresh at room temperature and gently mixed by inverting before use.

Extract Preparation

Commercially available dry plants were obtained from the household. They were: Sage, Salvia triloba; Calament, *Calamintha incana*; Clove, *Syzygium aromaticum*; Black seed, *Nigella sativa;* and Thyme, *Origanum syriacum*. Two grams of each plant were soaked in 20 ml of double distilled water for 1 hour before boiling for 10 minutes. The fluid extract was collected, cooled then filtered using 0.45 micron filters (Millipore, Billerica, MA). The aqueous extract was spectrophotometrically read at 600nm. Different batches of the extract were adjusted to the same A_{600} reading to normalize the concentration of the different batches used. The extract was stored at 4°C till it was used.

RESULTS

We focused our studies on solid cancers and we excluded lymphoma and leukemia. Our samples were obtained from Al-Basheer Hospital, Amman because it is the main center for cancer radiotherapy in Jordan. The samples studied are a reflection of the types of cancer in Jordan (Freedman, *et al.* 2003). Colon cancer (32 cases) and breast cancer (31 cases) represented the most prevalent cancers (63%) of the tested samples. Other cancers such as lung (9 cases), prostate (7 cases), and ovary (4 cases) are the second highest group among the studied volunteers from both sexes as shown in Table 1. The Patients came to Al-Basheer Hospital from all districts of the country seeking treatment with radiotherapy. About half the patients resided in greater Amman area.

Only sage aqueous extract demonstrated immunostimulation of peripheral blood lymphocytes

Thyme, sage, calament, clove and black seed grow and are folkloric to the populace of the Fertile Crescent area (Block and Mead, 2003). Traditional herbalists in the area prescribe these plants or their products to treat many maladies and in preventive medicine (University of Maryland Health Library 2007). To evaluate the efficacy of immuno-stimulation for these plants, we tested their water extract for the potential to proliferate peripheral blood lymphocytes (PBLs) from cancer patient *in vitro*. Among the five aqueous extracts, only sage water extract demonstrated promising results in proliferating PBLs (Figure 1).

Table 1. Colon and Breast Cancers are the Most Prevalent Types among Jordanian Population. The distribution of cancer types among one hundred male and female volunteers tested. The volunteers were seeking treatment at Al-Basheer Hospital, Amman.

Cancer Type	Number of Cases	Males	Females
Breast	31	1	30
Colon	32	14	18
Lung	9	7	2
Prostate	7	7	0
Ovary	4	0	4
Stomach	3	2	1
Multi-organ	3	2	1
Larynx	3	3	-
Sinuses	2	1	1
Uterus	2	-	2
Brain	2	1	1
Testis	1	1	-
Muscle	1	1	-
Total	100	40	60

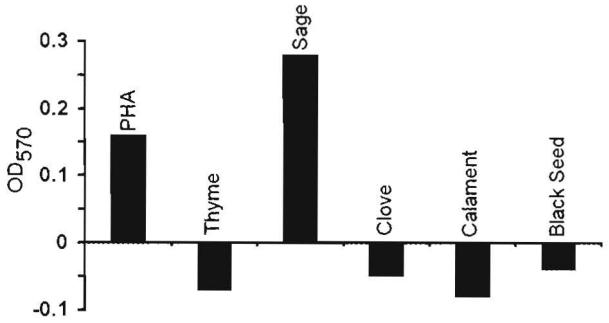

Fig. 1. Only Sage Aqueous Extract Induced Proliferation in Peripheral Blood Lymphocytes from Volunteers. The PBL of a representative individual is shown in the figure. Induction was measured using MTT assay. Average OD570 reading of the negative control using RPMI was subtracted from each sample. Each sample was cultured in duplicate wells. PHA as a positive control is shown on the graph.

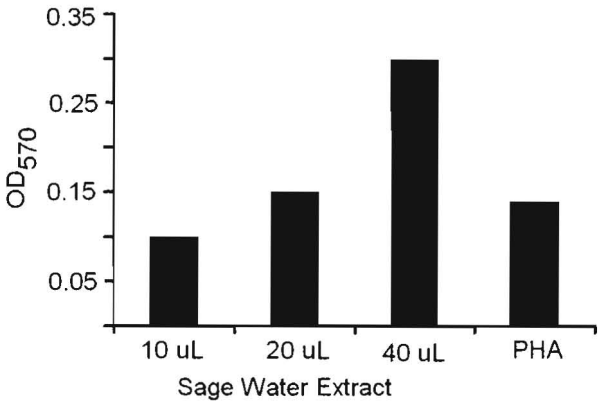

Fig. 2. PBLs Proliferation is Specific. Proliferation was Concentration-Dependant. Three concentrations of sage water extract were tested and the positive control (PHA). Average OD570 reading of the negative control using RPMI was subtracted from each sample. Each sample was cultured in duplicate wells. Induction of cell proliferation was measured using MTT assay.

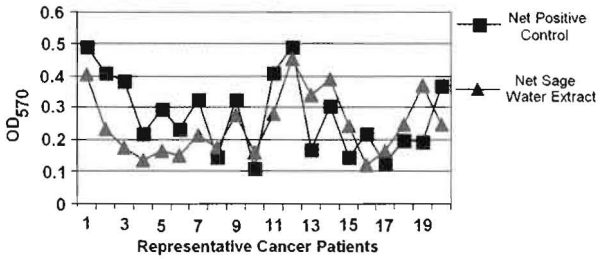

Fig. 3. Proliferation is Specific: Age, Sex, Cancer-type and Cancer-stage Independent. A representative sample shows immuno-stimulation of PBLs of randomly selected twenty patients. Average OD570 reading of the negative control using RPMI was subtracted from each sample. Triangles represent sage treatment and squares are the PHA (positive control). Each sample was cultured in duplicate wells. Induction of cell proliferation was measured using MTT assay.

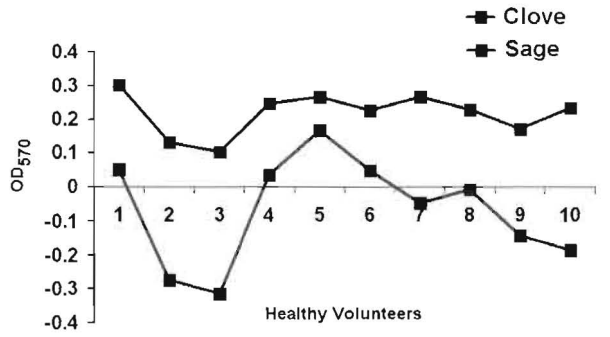

Fig. 4. Sage Water Extract Proliferate PBLs from Healthy Volunteers. PBLs from ten normal individuals were tested for their potential to proliferate after incubation with 40ml of sage water extract or clove water extract in a standard MTT assay. Average OD570 reading of the negative control using RPMI was subtracted from each sample. Each sample was cultured in duplicate wells. Induction of cell proliferation was measured using MTT assay.

Sage Water Extract Proliferative Effect is Specific

In order to find out whether PBL proliferation was specific and systemic, we tested a large pool of volunteers using three different concentrations of all five extracts. First, we found that PBL proliferation using sage water extract was concentration dependent as shown in (Figure 2). Second, sage extract stimulated the proliferation of PBLs in all cancer volunteers irrespective of their age, sex, type of cancer and stage of cancer as shown in (Figure 3). In a parallel control experiment we tested the proliferation of PBLs obtained from apparently healthy volunteers. Similarly, only sage water extract stimulated the proliferation of peripheral blood cells from normal individuals as shown in (Figure 4).

DISCUSSION

We investigated only solid cancers and excluded all types of blood cancers. The sample studied was a reflection of the highly prevalent types of cancers in Jordan and the area (Freedman, *et al.* 2003). Our results demonstrate that sage water extract contains a fraction that has the potential to stimulate the immune defenses of healthy individuals and cancer patients *in vitro*. Our data suggest that this fraction is likely a non-specific polyclonal mitogen(s). Evidence supporting this assumption comes from the fact that this fraction induces mitosis in PBLs from different clonal origins, and a wide range of HLA haplotypes (taken from a large pool of volunteers). Additionally, we observed this effect on healthy individuals and cancer patients. Moreover, the proliferation was cancer-type and cancer-stage independent. Importantly, it remains to identify this fraction and find out the mechanism of action for the immuno-modulating activity. It is well established that natural killer cells, lymphokine activated cells (LAK), macrophages and lymphocytes play a major role in the responses against tumors (Cho and Leung 2007).

Phytomedicine mechanisms of action are not well known. *In vitro* studies on ginseng suggest that it may enhance the effects of medications used to treat breast cancer (Alkofahi, *et al.* 1988). Garlic has antioxidants that scavenge free radicals thereby it protects damage to cell membranes and genetic material. Also, garlic has properties that fight aging, the development of heart disease and cancer (Alkofahi, *et al.* 1990; Duda, *et al.* 1999). Other investigators reported that garlic has a direct toxic effect to several cancer cells in tissue culture, but these effects cannot explain the inhibition of growth of transplanted cancer in animal models (Lamm and Riggs, 2001). However; recent reports demonstrate the presence of an anti-protease activity in garlic extract, suggesting the inhibition of metastasis of cancer could be through proteolytic inhibition (Bakri and Douglas, 2005). Similarly, a diet rich in lutein (from celery, spinach, broccoli, lettuce, tomatoes, oranges, carrots, and greens) is reported to prevent the formation of cancerous tumors in mice (Zheng, *et al.* 1993). Importantly, a large epidemiological study found that consumption of a diet rich in lutein was significantly effective against the development of colorectal cancer (Slattery, *et al.* 2000). Another anticancer mechanism may be related to inhibition of cancer. Curcumin was reported to inhibit proliferation of cancer cells and metastasis by induction of apoptosis, and inhibition of angiogenesis (Dorai, *et al.* 2001).

Many natural products stimulate the body disease-fighting ability in close proximity with infectious agents and neoplastic cells by activating the body non-specific defenses (Block and Mead 2003; Whiteside, *et al.* 1994; Cho and Leung, 2007). Most T cells are stimulated by PHA, a lectin extracted from red kidney beans (Epstein, *et al.* 1971) or by concanavilin-A, another lectin extracted from castor beans (Roitt, *et al.* 2001). The mechanism of action of these molecules depends on binding to T cell surface molecules including the T cell receptor complex causing them to cluster on the cell surface mimicking the clustering caused by antigen presentation. Such mitogens however, will activate T cells regardless of their antigen specificity (Roitt, *et al.* 2001). Our data suggest that the immuno-stimulant fraction in sage water extract is likely polysaccharides. This suggestion is supported by its ability to survive relatively high temperature during extraction. In a recent study that is in line with our results, Ebringerová, et. al., found that water extracted polysaccharides from the aerial parts of *Salvia officinalis* and extracts from other plants demonstrated immunomodulatory activities using the *in vitro* mitogenic and comitogenic rat thymocyte tests. They have shown that these chemically active compounds act as adjuvants (Ebringerová, *et al.* 2003).

Our results are in agreement with those reported in the literature earlier about sage and other plants (Block and Mead 2003; Cho and Leung 2007; Ebringerová et al 2003; Zozulia and Iurchenko, 2000). Several investigators reviewed the effects of three commonly used plants in the west on the immune system (Block and Mead 2003; Cho and Leung, 2007). They reported that Ginseng, Astragalus and Echinacea have several immuno-modulating activities of several types, including enhancement of levels of activity

of specific cell types associated with disease resistance and cancer. It is noted that limited studies reported that these herbs have tumoricidal potential (Cho and Leung, 2007). Other herbs are reported to boost the immune system (Quan, *et al.* 2007). Spirulina has been used in Russia to treat the victims, especially children, of the nuclear disaster at Chernobyl. In these children, whose bone marrow had been damaged from radiation exposure, spirulina seemed to boost the immune system (Zozulia and Iurchenko, 2000).

The explanation for the presence of an immuno-stimulating fraction in sage and not in the four other herbs tested in our study could be related to the method of extraction rather than the absence of these immune-modulators in the other herbs. In fact, El-Asatl and his team prepared aqueous, ethanolic, methanolic and phenolic extracts from different folkloric plants and tested them against several microorganisms. The aqueous extracts of sage and thyme were effective against most of the tested microorganisms. In contrast, sage and thyme phenolic extracts showed antibacterial activity against limited number of microorganisms.

In conclusion, the results of this investigation indicate the presence of a thermophilic fraction from sage water extract that has the potential to stimulate cell division in peripheral blood lymphocytes from cancer patients as well as healthy individuals. The activity is specific and dose dependent. The exact mechanism by which this fraction exerts its action is not yet known. However; in order to translate our findings into possible in vivo therapy, it is paramount to identify this fraction and to test its ability to induce cytokines such as tumor necrosis factor-α, interleukins and interferons before this proliferative activity could be studied on animal models.

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REFERENCES

Akhondzadeh, S, Noroozian, M, Mohammadi, M, Ohadinia, S, Jamshidi, AH, and **Khani, M** (2003) Salvia officinalis extract in the treatment of patients with mild to moderate Alzheimer>s disease: A double blind, randomized and placebo-controlled trial. *J Clin Pharm Ther* **28** (1):5359-.

Alkofahi, AS, RupprechtJ K, Smith DL, Chang CJ, and **McLaughlin JL** (1988) Goniothallamicin and Amonacin Bioreactive acetogenin from Goniothalamus giganteus (Amonaceae). *Experimentia* **44** (1):8385-.

Alkofahi, AS, Rupprecht JK, Liu XM, Chang CJ, Smith DL, McLaughlin JL, and **Gigantcin, A** (1990) A novel antimitotic and cytotoxic, with nonadjacent tetrahydrofuran rings from Goniothalamus giganteus (Amonaceae). Experimentia **46**:539541-.

Bakri, IM, and **Douglas, CWI** (2005) Inhibitory effect of garlic extract on oral bacteria. *Archives of Oral Biology* **50** (7):645-651.

Block, KI, Mead MN (2003) Immune System Effects of echinacea, ginseng, and astragalus: A review. *Integr Cancer Ther* **2** (3):247- 267.

Cho, WCS, and **Leung, KN** (2007) *In vitro* and *in vivo* anti-tumor effects of *Astragalus membranaceous*. *Cancer Letters* **252** (1):43-53.

Dorai, T, Cao, YC, Dorai, B, Buttyan, R, and **Katz, AE** (2001) Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. *Prostate* **47** (4):293-303.

Duda, RB, Zhong, Y, Navas, V, Li, MZ, Toy, BR, and **Alavarez, JG** (1999) American ginseng and breast cancer therapeutic agents synergistically inhibit MCF-7 breast cancer

cell growth. *J Surg Oncol* **72** (4):230-239.

Ebringerova, A, Kardosova, A, Hromadkova, Z, Hribalova, V. (2003). Mitogenic and comitogenic activities of polysaccharides from some European herbaceous plants. *Fitoterapia* **74** (1-2):52-61.

El Astal, ZY, Ashour, AA, Kerrit, A (2003). Antimicrobial Activity of some Medicinal Plant Extracts. *West Afr. J. Pharmacol. Drug Res* **19** (121-16:(2/.

Epstein, LB, Cline, MJ, Merigan, TC (1971). The Interaction of Human Macrophages and Lymphocytes in the Phytohemagglutinin-Stimulated Production of Interferon. *J Clin Invest* **50** (4):744–753.

Freedman, LS, Barchana, M, Al-Kayed, S, Qasem, MB, Young, JL, Edwards, B, Ries, L, Roffers, S, Harford, J, and **Silbermann, M** (2003) A comparison of population-basedcancer incidence rates in Israel and Jordan. *European Journal of Cancer Prevention* **12** (5):359-365.

Kimura, Y, Taniguchi, M, Baba, K (2004) Antitumor and antimetastatic activities of 4-hydroxyderricin isolated from Angelica keiskei roots. *Planta Med* **70** (3):211- 219.

Lamm, DL, and **Riggs, DR** (2001) Enhanced immunocompetence by garlic: Role in bladder cancer and other malignancies. *J Nutr* **131** (Supl.3):1067- 1070.

Masuda, T, Inaba, Y, Maekawa, T, Takeda, Y, Tamura, H, Yamaguchi, H (2002) Recovery mechanism of the antioxidant activity from carnosic acid quinone, an oxidized sage and rosemary antioxidant. *J Agric Food Chem.* **50** (21):5863- 5869.

Quan, FS, Compans, RW, Cho, YK, Kang, SM (2007) Ginseng and Salviae herbs play a role as immune activators and modulate immune responses during influenza virus infection. *Vaccine* **25** (2):272-282.

Rodgers,JR,Shawar,SM,Guenther,MM,Rich, RR. (1992). Kinetics of Killing by Monoclonal Cytotoxic T Lymphocytes. I. Colorimetric Detection of Cryptic CTL Determinants on Adherent Target Cells and Survivorship Analysis. *Jr. Immunol Methods.* **152**:159-169.

Roitt, E, Brostoff, J, Male, D (2001) *Immunology, 6th edition.* Mosby, New York, USA.

Rose, RR, Hamilton, RG, Detrick, B (2002) *Editors Manual of Clinical Laboratory Immunology, 6th Edition.* American Society for Microbiology, Washington, USA.

Slattery, M.L, Benson, J, Curtin, K, Ma, KN, Schaeffer, D, and **Potter, JD** (2000) Carotenoids and colon cancer. *American Journal of Clinical Nutrition* **71** (2): 575-582.

Tildesley, NT, Kennedy, DO, Perry, EK, Ballard, CG, Savelev, S, Wesnes, KA, and **Scholey, AB (2003).** Salvia lavandulaefolia (Spanish sage) enhances memory in healthy young volunteers. *Pharmacol Biochem Behav.* **75** (3):669- 674.

University of Maryland Health Library (2007) *Introduction to Complementary and Alternative Medicine.* University of Maryland, Baltimore MD, USA. Available at: http://www.umm.edu/altmed/ConsLookups/Uses.html

Whiteside, TL, Herberman, RB (1994) Role of human natural killer cells in health and disease. *Clin Diag Lab Immunol.* **1** (2):125133-.

Zheng, GQ, Kenney, PM, Zhang, J, and **Lam LK** (1993) Chemoprevention of benzo[a] pyrene-induced forestomach cancer in mice by natural phthalides from celery seed oil. *Nutr Cancer.* **19** (1):77–86.

Zozulia, IS, Iurchenko, AV (2000) The adaptive potentials of those who worked in the cleanup of the aftermath of the accident at the Chernobyl Atomic Electric Power Station under the influence of different treatment methods. *Likarska Sprava* (3-4) :18-21.

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