

# Antiproliferative activity of chloroformic extract of Persian Shallot, *Allium hirtifolium*, on tumor cell lines

Hamideh Ghodrati Azadi · Seyed Mahmood Ghaffari ·  
Gholam Hossein Riazi · Shahin Ahmadian ·  
Fatemeh Vahedi

Received: 13 January 2008 / Accepted: 28 March 2008  
© Springer Science+Business Media B.V. 2008

**Abstract** *Allium hirtifolium* (Persian Shallot) belongs to *Allium* genus (*Alliaceae* family). We investigated the in vitro effects of chloroformic extract of *A. hirtifolium* and its Allicin on the proliferation of HeLa (cervical cancer), MCF7 (human, caucasian, breast, adenocarcinoma) and L929 (mouse, C3H/An, connective) cell lines. Our results showed that components of *A. hirtifolium* might inhibit proliferation of tumor cell lines. This inhibition in HeLa and MCF-7 cells was dose-dependent. The presence of Allicin was evaluated by TLC method in bulbs and the extract of *A. hirtifolium* was analyzed by HPLC. MTT test was performed 24, 48 and 72 h after cell culture. A significant decrease in cell lines was observed in HeLa and MCF-7 as compared to L929 cell lines. DNA fragmentation analysis revealed a large number of apoptotic cells in treated HeLa and MCF-7 cell groups, but no effects in L929 cells. Therefore *A. hirtifolium* might be a candidate for tumor suppression.

**Keywords** Allicin · *Allium hirtifolium* · Antiproliferative · Cancer · Tumor cell lines

## Abbreviations

DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
PBS	Phosphate-buffered saline

## Introduction

Antibacterial, antifungal, antiviral, antiprotozoal, and antihelmintic properties of *Allium* genus have been reported recently (Cavallito and Bailey 1944; Ariga and Seki 2006; Ankri and Mirelman 1999; Yamada and Azuma 1977; Moore and Atkins 1977; Taran et al. 2006). It is believed Alliums can treat diabetes, arthritis, colds and flu, stress, fever, coughs, headache, hemorrhoids, asthma, arteriosclerosis, cancer, rheumatic and inflammatory disorders (Eidi et al. 2006; Abdou et al. 1972; Lawson 1996; Bordia et al. 1997; Kojuri and Vosoughi 2007; Hirsch et al. 2000). In the invading organisms and microorganisms, *Allium* genus extracts were shown to decrease the oxygen uptake, reduce the growth of the organism, inhibit the synthesis of lipids, proteins, and nucleic acids and damage the membranes (Borek 2001; Dirsch and Kiemer 1998).

Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety

H. Ghodrati Azadi · S. M. Ghaffari (✉) ·  
G. H. Riazi · S. Ahmadian  
Institute of Biochemistry and Biophysics (IBB),  
University of Tehran, P.O. Box 13145-1384, Tehran, Iran  
e-mail: ghaffari@ibb.ut.ac.ir

H. Ghodrati Azadi  
e-mail: ghodrati@ibb.ut.ac.ir

F. Vahedi  
Vaccine and Serum Razi Research Institute, Mashhad,  
Iran

of allyl sulfides (Lee et al. 2003). Allicin (diallyl thiosulfinate) was isolated and identified. It is responsible for the remarkable antibacterial activity of crushed garlic cloves (Cavallito and Bailey 1944). Alliin was found to be the stable precursor that was converted to Allicin by the action of allinase which was more than 10% of the total protein content in garlic (Baghalian et al. 2005). Allicin was reported to possess diverse biological actions such as antimicrobial, antiparasite, and antifungal activities. It has been found that lipid peroxidation is inhibited and OH is scavenged (Borek 2001; Lawson and Hughes 1992).

Garlic consumption reduces the risk of cancer, as its extract blocks effectively induced tumors in skin, breast, uterine cervix, and colon (Oommen and Anto 2004; Vainio and Weiderpass 2006). Aqueous garlic extract might exert its chemopreventive effect by inducing apoptosis (Balasenthil and Rao 2002). Many organosulfur compounds, the major active principles in garlic, inhibit the proliferation of cancer cells, and some of them cause apoptosis in tumor cells of different tissue origin (Kwon and Moon 2005; Thatte and Bagadey 2000; Arditti and Rabinkov 2005; Jakubikova and Sedlak 2006). Hence, apoptosis could be a potential general mechanism providing a mechanistic basis for the anticarcinogenic activities of individual garlic components, although the actual mechanism is not known (Thatte and Bagadey 2000).

*Allium hirtifolium* with the common Persian name of 'Moosir', a native edible plant in Iran, has been widely used as medicine and condiment predated. It belongs to the same biological genus as *Allium sativum* (garlic) and other onions (Mozaffarian 1996). Therefore, the aim of this study was to investigate the anticancer effects of *Allium hirtifolium*.

## Materials and methods

### Reagents

Allicin (standard) was obtained from Nopex Company as Allisure™ (Nopex, England). Fetal calf serum (FCS) was obtained from Gibco BRL (Grand Island, NY, USA). RPMI 1640, Ampicillin, MTT (3-(4,5-dimethylthiazoyl)-2,5-diphenyltetrazolium bromide), Trypsin, and other reagents were obtained from Sigma (St. Louis, MO, USA).

### Samples of *A. hirtifolium*

The bulbs of *A. hirtifolium* were collected from Isfahan province, Iran in June 2006. The plant specimen was identified by The Iranian Research Institute of Forests and Rangelands, Tehran, Iran.

### Preparation of chloroformic extract of *A. hirtifolium* for cell culture

The bulbs of *A. hirtifolium* were washed with tap water and cut into small slices. The slices were powdered after air-drying. Then 100 g of powder was added to 1 l chloroform and the mixture kept for 24 h at 10°C. It was filtered through cotton cloth and evaporated in a rotating evaporator under reduced pressure until completely dried. The dried extract was preserved at 4°C and added to RPMI medium up to a final concentration of 200 µg ml<sup>-1</sup>. For preparation of Allicin, RPMI was used up to a final concentration of 18 µg ml<sup>-1</sup>.

### Analysis of Allicin by thin layer chromatography method (TLC)

The presence of Allicin was evaluated in bulbs by TLC. Chloroformic extract of *A. hirtifolium* was tentatively identified on silica gel GF254 pre-coated plate. Pure Allicin was used as a control for comparison. Briefly, the toluene–ethyl acetate (100:30) was used as mobile phase and UV detection was performed at 254 nm and R<sub>f</sub> value was calculated by Vanillin–glacial acetic reagent (Sreevidya and Mehrotra 2003). Total thiosulfates and Allicin contents of *A. hirtifolium* bulbs were determined using a spectrophotometric method (You et al. 1989; Miron et al. 1998; Miron et al. 2002; Ogra and Suzaki 2005).

Reversed-phase HPLC was a method of choice for detection and quantitation of Allicin in *A. hirtifolium* extract. The HPLC Reversed-phase HPLC/UV (methanol/water/formic acid (40:60:0.1), λ: 240 nm, flow rate: 0.9 mlmin<sup>-1</sup>) was used (Lawson and Wood 1991).

### Cell culture

The human cervical carcinoma cell line (HeLa, NCBI-115), estrogen receptor—positive human breast cancer cell line (MCF-7 NCBI C135) and

mouse fibroblast cells (L929 NCBI C161) were purchased from the National Cell Bank, Pasteur Institute of Iran. All the cell lines were maintained in RPMI 1640 medium supplemented with 10% FCS serum, streptomycin ( $100 \mu\text{g ml}^{-1}$ ) and penicillin ( $100 \text{ IU ml}^{-1}$ ) in culture flasks at  $37^\circ\text{C}$  in 5% humidified  $\text{CO}_2$  incubator. The cells were fed until confluence ( $2 \times 10^6$ ) and expanded by trypsinization and subculture at lower numbers in new culture flasks.

The cultured cells were treated with Allicin and *A. hirtifolium* extract and examined their effects in different concentrations. The cells were evaluated after 24, 48 and 72 h of the treatment.

#### DNA fragmentation analysis

The DNA from cultivated cells was isolated (Herrmann and Lorenz 1994). In brief, cells ( $2 \times 10^6$ ) were treated with the extract and Allicin then collected by centrifugation (2,000 g, 10 min). The pellet was resuspended in 0.5 ml DNA lysis buffer (2% SDS, 10 mM EDTA, 10 mM Tris-HCl, pH = 8.5). The lysate was incubated with  $0.1 \text{ mg ml}^{-1}$  proteinase k and then incubated for 4 h at  $37^\circ\text{C}$ . The DNA was precipitated with 70% ethanol after addition of isopropanol. The suspension was centrifuged and DNA treated with RNase A in 10 mM Tris-HCl (pH = 7.5) at  $37^\circ\text{C}$  for 18 h. The samples were analyzed by electrophoresis on a 1.5% agarose gel and assessed under UV illumination.

#### Morphological changes of cells after treatment

After treating of cells with chloroformic extract and Allicin, shrinkage, detachment, and colony forming of cells were evaluated under inverted microscope.

#### Evaluation of cultured cells by MTT colorimetric method

A colorimetric assay using MTT was performed (Mosmann 1983). Cells seeded in 96-well microplates ( $5,000 \text{ cells well}^{-1}$   $200 \mu\text{l}^{-1}$ ) and routinely cultured in a humidified incubator for 24 h. The medium was aspirated off after a 24 h pre-culture and exchanged for medium containing *A. hirtifolium* extract and Allicin at various concentrations ranging from 0 to  $44 \mu\text{g ml}^{-1}$  and 0 to  $0.44 \mu\text{g ml}^{-1}$ ,

respectively. Cells were then re-incubated for 24, 48 and 72 h. A control group (RPMI without extract) and a blank group (without cells or medium) were also included. This assay was performed in triplicate. The medium replaced with  $100 \mu\text{l}$  of medium containing MTT solution ( $0.5 \text{ mg ml}^{-1}$  in RPMI). Cells were re-incubated for an additional 2 h. Then after addition of 0.15 ml DMSO, the plates were shook for 10 min to dissolve the formazan crystals. Then, optical density of 96-well culture plates was measured using an enzyme-linked immunosorbent assay (ELISA) reader at 540 nm. The optical density of formazan formed in untreated control cells was taken as 100% viability. The obtained optical densities from the treated wells were converted to a percentage of living cells (cell survival rate) against the control using the following formula:

$$\text{Absorbance of treated cells in the each well} \times 100 / \text{Mean absorbance of control cells}$$

#### Statistical analysis

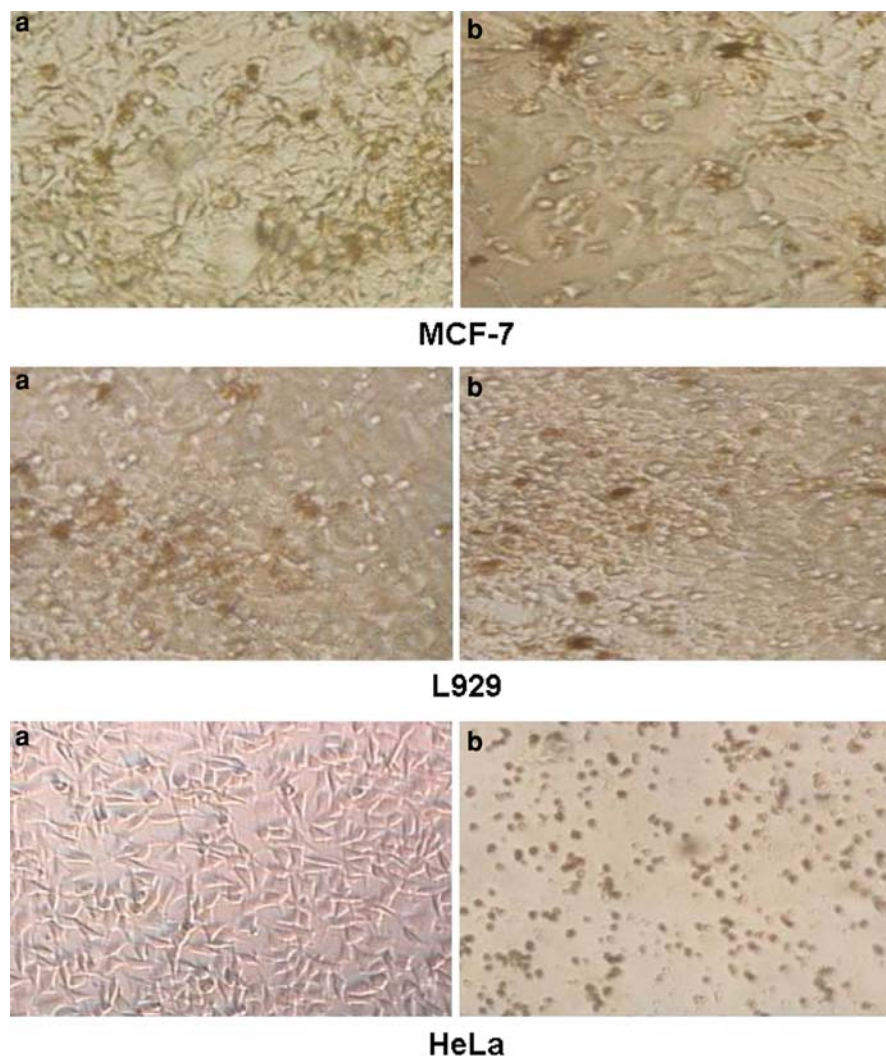
Computer program (Graph Pad Prism) was used to calculate the IC<sub>50</sub> (50% inhibition of cell proliferation) values. Student's *t*-test was used for statistical analyses while *P*-values < 0.05 were considered to be statistically significant.

## Results and discussion

Allicin was identified by observing the characteristic violet-brown zone. The R<sub>f</sub> of Allicin was determined 0.6 based on TLC experiment. In *A. hirtifolium* chloroformic extract, Allicin was quantified in the amount of  $3.4 \pm 0.1 \text{ mg g}^{-1}$ .

Shrinkage, granulation of cytoplasm and detachment were observed in treated cells with chloroformic extract of *A. hirtifolium* and Allicin. Particularly in higher concentrations of extract and Allicin and longer period of incubation the number of colony forming cells was decreased. Most sensitive cells were HeLa and MCF-7 cell lines, respectively (Fig. 1). The inhibitory effect of chloroformic extract of *A. hirtifolium* was stronger than Allicin. The growth of L929 cells was suppressed only in high doses of *A. hirtifolium* and Allicin ( $200 \mu\text{g ml}^{-1}$  and  $18 \mu\text{g ml}^{-1}$ , respectively).

**Fig. 1** Morphological changes of L929, MCF-7, and HeLa cells after treating with *A. hirtifolium* extract for 72 h. Shrinkage and detachment effects were visible in treated cells (**a**, untreated; **b**, treated cells)



Fifty percent inhibition of cells occurred with  $20 \mu\text{g ml}^{-1}$  *A. hirtifolium* and  $0.15 \mu\text{g ml}^{-1}$  Allicin in HeLa cells,  $24 \mu\text{g ml}^{-1}$  *A. hirtifolium* and  $0.20 \mu\text{g ml}^{-1}$  Allicin in MCF-7 cells and  $250 \mu\text{g ml}^{-1}$  *A. hirtifolium* and  $1.25 \mu\text{g ml}^{-1}$  Allicin in L929 cells. The IC<sub>50</sub> values of *A. hirtifolium* extract and Allicin for HeLa and MCF-7 and L929 cell lines are shown in Table 1. At the highest concentration, the inhibitory rate of *A. hirtifolium* on HeLa cell was 100% while that of Allicin was 94%.

The first MTT assay performed after 24 h. The cell viability had an inverse correlation with extract concentration. In contrast, viability of L929 cells increased in higher extract concentrations. There was significant difference in percent of surviving cells between consecutive days (day 1, 2, and 3)

for both HeLa and MCF-7 cells ( $P < 0.05$ ) as shown in Fig. 2.

HeLa and MCF-7 cell lines exhibited decreased growth in a dose-dependent manner under experimental condition used in the 72 h treatment. According to 50% inhibition of cell proliferation (IC<sub>50</sub>), the order of sensitivity of the cell lines to this extract was HeLa > MCF-7 > L929 cells.

Incubation of MCF-7 and HeLa cells with extract and Allicin showed typical DNA ladder by electrophoresis after 72 h. We did not find the DNA ladder shape in L929 cells (Fig. 3).

Several pieces of evidences suggested that *Allium* genus possess anticancer properties as shown by their ability to suppress tumor proliferation in vivo and in vitro (Ahmed et al. 2001; Miron et al. 2002;

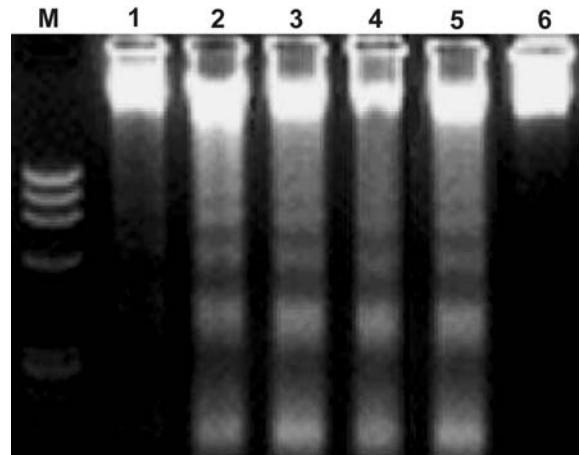
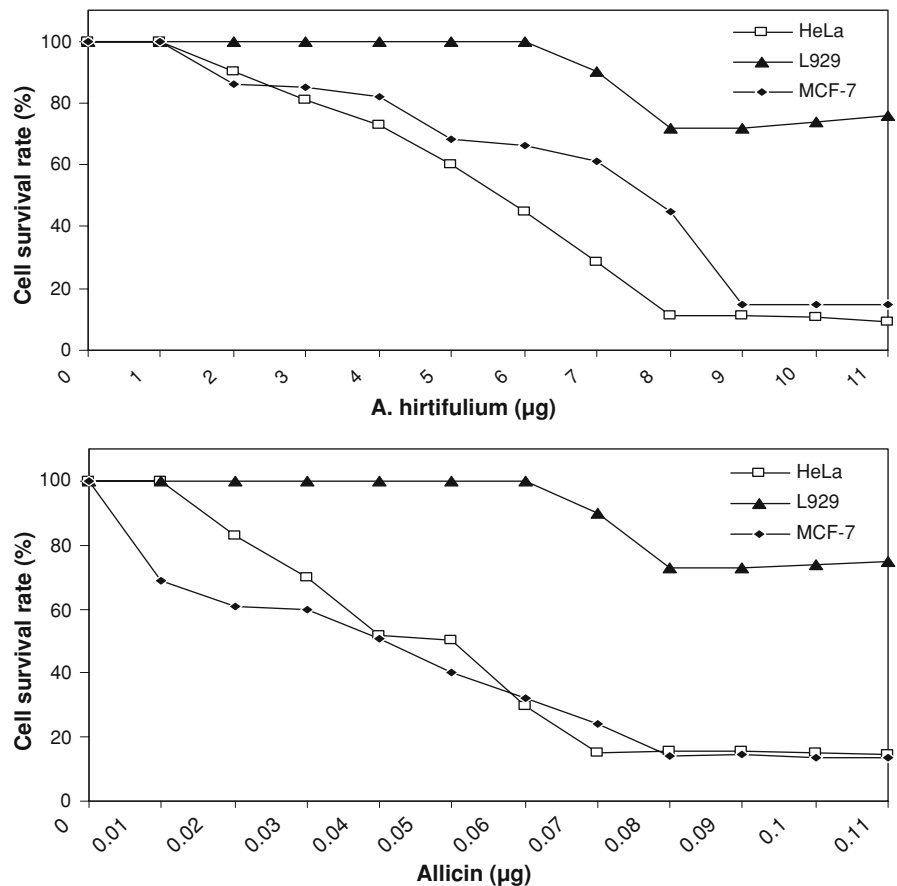
**Table 1** Growth-inhibitory effects of Allicin and *A. hirtifolium* extract on HeLa, MCF-7, and L929 cells

Cell line	IC50 Allicin ( $\mu\text{g ml}^{-1}$ )	IC50 <i>A. hirtifolium</i> ( $\mu\text{g ml}^{-1}$ )
HeLa	0.15	20
MCF-7	0.20	24
L929	1.25	250

The cells were incubated with various concentrations of Allicin and *A. hirtifolium* extract in RPMI medium for 72 h. Cell proliferation was measured by the MTT reduction assay. The IC50 value was the estimated concentration that results in 50% inhibition of cell proliferation under the specified experimental conditions

Zhang et al. 2007). It was shown that concentration and duration of the exposure to allyl sulfides increased the antiproliferative effects. In our study the amount of Allicin formed from *A. hirtifolium* was  $3.4 \pm 0.1 \text{ mg g}^{-1}$  and the theoretical amount of Allicin formed from garlic is reported as  $3.5 \text{ mg g}^{-1}$  (Lawson and Wood 1991).

**Fig. 2** The effect of the extent of confluence on cell survival after treatment with Allicin and *A. hirtifolium* extract. The cells were seeded on a 96-well plate. The cells were then treated with Allicin and *A. hirtifolium* for 72 h and cell survival was measured by MTT assay. Percentage of surviving HeLa and MCF-7 cells in all concentrations of Allicin and *A. hirtifolium* extract was significantly decreased when compared with L929 cells ( $P < 0.001$ ). *A. hirtifolium* and Allicin were shown as  $\mu\text{g}$  per each well microplate. Each point represented the mean  $\pm$  SD ( $n = 3$ )



**Fig. 3** Agarose gel electrophoresis of DNA extracted from cells treated with *A. hirtifolium* ( $44 \mu\text{g ml}^{-1}$ ) and Allicin ( $0.44 \mu\text{g ml}^{-1}$ ). Lane M: DNA marker; 100 bp, Fermentas. DNA laddering, typical for apoptotic cells, was visible for treated HeLa with *A. hirtifolium* (lane 2), HeLa with Allicin (lane 3), MCF-7 cells with *A. hirtifolium* (lane 4), and MCF-7 with Allicin (lane 5), but it was not found for L929 cells with *A. hirtifolium* and Allicin, respectively (lane 1, 6)

This antineoplastic effect was greater for lipid-soluble than for water-soluble allyl sulfides (Rabinkov et al. 1998; Tattelman 2005). Diallyl disulfide suppresses the growth of human colon tumor cell xenografts in athymic nude mice (Miron et al. 2003; Zhang et al. 2007).

*Allium hirtifolium* (Moosir) belongs to the same biological genus as *Allium sativum* (garlic) and other onions. This species is a native plant in Iran that its antiproliferative effects have received little attention (Taran et al. 2006). No previous literature was found to show the effectiveness of chloroformic extract of *A. hirtifolium* on HeLa, MCF-7, and L929 cells.

In the present study we assessed the antiproliferative effect of *A. hirtifolium* extract on HeLa, MCF-7, and L929 cell lines by MTT method and the DNA fragmentation analysis. This investigation clearly showed a cell growth inhibition on cancer HeLa and MCF-7 cell lines at concentrations about  $44 \mu\text{g ml}^{-1}$  of *A. hirtifolium* extract. With respect to the cell lines studied,  $\text{IC}_{50}$  values varied from  $20 \mu\text{g ml}^{-1}$  in HeLa to  $24 \mu\text{g ml}^{-1}$  in MCF-7. In contrast, *A. hirtifolium* extract had less effect on a normal cell (L929) (Table 1).

The effect of *A. hirtifolium* on these cells started from 24 h and became more prominent in 48 and 72 h. Therefore, maximum morphologic changes and antiproliferative effect appeared after 72 h. In MTT assay, the statistical analysis indicated that *A. hirtifolium* extract significantly inhibited the proliferation of HeLa and MCF-7 cells. But this inhibition could not occur in non-tumor L929 cells. Interestingly the *A. hirtifolium* extract affected the tumor cells much stronger than Allicin.

The results showed that *A. hirtifolium* could inhibit proliferation of tumor cell lines in a dose-dependent manner. The inhibitory effect on tumor cell lines was 25 times stronger than that in normal cells. Therefore, our results provided important insights into the use of *A. hirtifolium* as an additive to food or as a drug without any side effects.

The present study was the first report to provide evidence of *A. hirtifolium* activity on tumor cell lines. Further investigations were needed to elucidate subcellular mechanisms involved in the suppression of growth in tumor cell lines.

**Acknowledgments** This work was supported by the Institute of Biochemistry and Biophysics (IBB), Tehran, Iran. We thank

Dr. H. Dehghani for the critical reading of the manuscript and Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

## References

- Abdou A, Abou-Zeid AA, el Sherbeeney MR, Abou-El ZH (1972) Antimicrobial activities of *Allium sativum*, *Allium cepa*, *Raphanus sativus*, *Capsicum frutescens*, *Eruca sativa*, *Allium kurrat* on bacteria. *Plant Foods Hum Nutr* 22:129–135
- Ahmed N, Laverick L, Sammons J, Zhang H, Maslin DJ (2001) Ajoene, a garlic-derived natural compound, enhances chemotherapy-induced apoptosis in human myeloid leukaemia CD34-positive resistant cells. *Anticancer Res* 21(5):3519–3523
- Ankri S, Mirelman D (1999) Antimicrobial properties of allicin from garlic. *Microbes Infect* 1(2):125–129
- Arditti FD, Rabinkov A (2005) Apoptotic killing of B-chronic lymphocytic leukemia tumor cells by allicin generated in situ using a rituximab-allylase conjugate. *Mol Cancer Ther* 4(2):325–331
- Ariga T, Seki T (2006) Antithrombotic and anticancer effects of garlic-derived sulfur compounds: a review. *Biofactors* 26(2):93–103
- Baghalian K, Ziai SA, Naghavi MR, Naghdi BH, Khalighi A (2005) Evaluation of allicin content and botanical traits in Iranian garlic (*Allium sativum* L.) ecotypes. *Sci Hortic* 103(2):155–166
- Balasesenthil S, Rao KS (2002) Altered cytokeratin expression during chemoprevention of experimental hamster buccal pouch carcinogenesis by garlic. *J Oral Pathol Med* 31(3):142–146
- Bordia A, Verma SK, Khabia BL, Vyas A, Rathore AS, Bhu N, Bedi HK (1997) The effect of active principle of garlic and onion on blood lipids and experimental atherosclerosis in rabbits and their comparison with clofibrate. *J Assoc Phys Ind* 25:509–516
- Borek C (2001) Antioxidant health effects of aged garlic extract. *J Nutr* 131(3s):1010S–1015S
- Cavallito CJ, Bailey JH (1944) Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. *J Am Chem Soc* 66:1944–1952
- Dirsch VM, Kierner AK (1998) Effect of allicin and ajoene, two compounds of garlic, on inducible nitric oxide synthase. *Atherosclerosis* 139(2):333–339
- Eidi A, Eidi M, Esmaeili E (2006) Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine* 13(9–10):624–629
- Herrmann M, Lorenz HM (1994) A rapid and simple method for the isolation of apoptotic DNA fragments. *Nucleic Acids Res* 22(24):5506–5507
- Hirsch K, Iron DM, Giat J, Miron T, Rabinkov A, Wilchek M, Mirelman D, Levy J, Sharoni Y (2000) Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutr Cancer* 38(2):245–254
- Jakubikova J, Sedlak J (2006) Garlic-derived organosulfides induce cytotoxicity, apoptosis, cell cycle arrest and

- oxidative stress in human colon carcinoma cell lines. *Neoplasma* 53(3):191–199
- Kojuri J, Vosoughi AR (2007) Effects of *Anethum graveolens* and garlic on lipid profile in hyperlipidemic patients. *Lipids Health Dis* 6:5
- Kwon SK, Moon A (2005) Synthesis of 3-alkylthio-6-allylthiopyridazine derivatives and their antihepatocarcinoma activity. *Arch Pharm Res* 28(4):391–394
- Lawson DL (1996) The composition and chemistry of garlic cloves and processed garlic. In Koch HP, Lawson DL (eds) *Garlic: the science and therapeutic applications of Allium sativum L. and related species*, 2nd edn. William & Wilkins, Baltimore, pp 37–107
- Lawson LD, Hughes BG (1992) Characterization of the formation of allicin and other thiosulfates from garlic. *Planta Med* 58:345–350
- Lawson LD, Wood SG (1991) HPLC analysis of allicin and other thiosulfates in garlic clove homogenates. *Planta Med* 57(3):263–270
- Lee S-N, Kim N-S, Lee D-S (2003) Comparative study of extraction techniques for determination of garlic flavor components by gas chromatography–mass spectrometry. *Anal Bioanal Chem* 377(4):749–756
- Miron T, Mironchik M, Mirlman D, Wilchek M, Rabinkov A (2003) Inhibition of tumor growth by a novel approach: in situ allicin generation using targeted alliinase delivery. *Mol Cancer Ther* 2(12):1295–1301
- Miron T, Rabinkov A, Mirlman D, Weiner L, Wilchek M (1998) A spectrophotometric assay for allicin and alliinase (Alliin lyase) activity: reaction of 2-nitro-5-thiobenzoate with thiosulfates. *Anal Biochem* 265(2):317–325
- Miron T, Shin I, Feigenblat G, Weiner L, Mirlman D (2002) A spectrophotometric assay for allicin, alliin, and alliinase (alliin lyase) with a chromogenic thiol: reaction of 4-mercaptopyridine with thiosulfates. *Anal Biochem* 307(1):76–83
- Moore GS, Atkins RD (1977) The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. *Mycologia* 69(2):341–348
- Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65:55–63
- Mozaffarian V (1996) *A dictionary of Iranian plant names*. Tehran. Farhange Moaser Publications, Tehran
- Ogra Y, Suzuki KT (2005) Speciation of selenocompounds by capillary HPLC coupled with ICP-MS using multi-mode gel filtration columns. *J Anal At Spectrom* 20:35–39
- Oommen S, Anto RJ (2004) Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. *Eur J Pharmacol* 485(1–3):97–103
- Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirlman D, Weiner L (1998) The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochim Biophys Acta* 1379(2):233–244
- Sreevidya N, Mehrotra SH (2003) A new spray reagent for detection and differentiation of sulfur compounds in plant extracts. *J Planar Chromatogr* 16(6):468–469
- Taran M, Rezaeian M, Izaddoost M (2006) In vitro antitrichomonas activity of *Allium hirtifolium* (persian shallot) in comparison with Metronidazole Iranian. *J Public Health* 35(1):92–94
- Tattelman E (2005) Health effects of garlic. *Am Fam Physician* 72(1):103–106
- Thatte U, Bagadey S (2000) Modulation of programmed cell death by medicinal plants. *Cell Mol Biol* 46(1):199–214
- Vainio H, Weiderpass E (2006) Fruit and vegetables in cancer prevention. *Nutr Cancer* 54(1):111–142
- Yamada Y, Azuma K (1977) Evaluation of the in vitro antifungal activity of allicin. *Antimicrob Agents Chemother* 11(4):743–749
- You WC, Sheng CY, Ershow A, Tian YZ, An Q, Henderson BE, Fraumeni JF, Wang TG (1989) Allium vegetables and reduced risk of stomach cancer. *J Natl Canc Inst* 81(2):162–164
- Zhang ZM, Yang XY, Shu-ha D, Wei X, Hai-qing G (2007) Anti-tumor effects of polybutylcyanoacrylate nanoparticles of diallyl trisulfide on orthotopic transplantation tumor model of hepatocellular carcinoma in BALB/c nude mice. *Chin Med J* 120(15):1336–1342