

STUDY OF ANTITUMOR EFFECT OF METHANOLIC AND AQUEOUS EXTRACTS OF *ALLIUM SATIVUM* L. (GARLIC) CLOVES USING POTATO DISC BIOASSAY

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Summary

In this study the methanolic and aqueous extracts of *Allium sativum* (garlic) cloves were tested for possible antitumor activity using potato disc assays. The Minimum inhibitory concentration (MIC) of extracts were determined using microplate method. In potato disc assay, discs were cut of potato with specific diameter and transferred on 1.5% agar under a laminar air cabinet. 50 µl of a mixture containing suspension of *Agrobacterium tumefaciens* and the solution of extracts were inoculated on potato discs in plates. The plates were incubated in 25 °C for 21 days until the tumors were counted. The extracts did not show any antibacterial activity at the concentration 0.25-4 mg/ml. The IC₅₀ values of aqueous and methanolic in water were 0.92 mg/ml (0.58-1.45) and 0.72 mg/ml (0.47-1.09), respectively. The IC₅₀ values of aqueous and methanolic in DMSO were 0.75 mg/ml (0.47-1.19) and 0.59 mg/ml (0.36-0.94), respectively. This study indicates that the aqueous and methanolic extracts of *A. sativum* have antitumor activity.

Key words: *Allium sativum*, Garlic, *Agrobacterium tumefaciens*, Potato disc assay, Antitumor, Anticancer

Introduction

Allium sativum L. or garlic (family: Liliaceae) has been used as a flavoring agent, food and for its medicinal properties in many diseases all over the world (1-4). The *Allium* genus include more than 600 various species and garlic is a member of this genus and other members are leek, chive, shallot and scallion. They are different in appearance, color and flavor but similar in biochemical and phytochemical constituent (4, 5). Garlic has been applied for the treatment of several illnesses since ancient times (6). In folk remedy garlic is applied for treatment of many diseases such as respiratory illnesses, asthma, diabetes, cardiovascular diseases, rheumatism, infections, cancers and gastrointestinal disorder (spleen and liver diseases), inflammations, leucoderma, and also it is utilize as a tonic, aphrodisiac, emmenagogue and anthelmintic (1, 7-9). Recent pharmacological studies have demonstrated insecticidal (10), antihypoxic (11), antibacterial (12), antifungal (13), hypoglycemic (14), hypolipidemic (15), and antiatherosclerotic (3) and antihepatotoxic (16) activities of garlic.

Garlic contains mostly of water (60–70 g/100 g) and the main important constituents are the organosulfur compounds (11–35 mg/100 g) (5). Garlic has an extensive range of organosulfuric substances, which obtained from alliin when the tissues of garlic are cut, the enzyme of allinase liberated and catalyzes the conversion of alliin into the thiosulfinates like diallyl trisulfide and diallyl disulfide (allicin and ajoene) (5, 17, 18). The odour and flavor of garlic depends on these compounds (4, 17). Productions of these substances from alliin depend on the drying and extraction method of the garlic bulbs and also the temperature, pH, and solvent are important (18). Some study has been shown the anticarcinogenic effect of these substances (18). It was shown that garlic constituents block the initiation and promotion phases of carcinogenic process and regulate immunity of specific and non-specific antitumor (19).

Potato disc bioassay is a simple, inexpensive and sensitive technique to investigate the antitumor effect of a lot of compounds by inhibition of the formation of tumor on potato disc that is induced by *Agrobacterium tumefaciens* and regarding the high expense of the *in vivo* experiments for evaluating the antitumor effect of substances, it is a appropriated method to study the antitumor activity of many materials (20-22).

Due to anticarcinogenic constituent of garlic and its application in the cancer in folk medicine the antitumor effect methanolic and aqueous extract of garlic was investigated using potato disc bioassay.

Materials and methods

Preparation of the methanolic and aqueous extracts of garlic

Garlic was purchased from a local market in Mashhad, Iran and voucher samples were preserved for reference in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad (Voucher no. 159-0119-01). Fresh garlic cloves were peeled, and then chopped into small piece. For the aqueous extract, 500 ml distilled water was added to 500 mg of garlic and filtered through cloth. The extract was then concentrated *in vacuo* to the desired volume. For the methanolic extract, garlic was subsequently macerated in 500 ml methanol for 2 days and the mixture was subsequently filtered and concentrated *in vacuo* at 68 °C. The residue was suspended in water or DMSO, respectively.

Antitumor assay

Antitumor effect of the methanolic and aqueous extracts of garlic was evaluated with the potato disc method (22, 23). *A. tumefaciens* (strain B6) which contain the Ti (tumor inducing) plasmid was cultured on Soybean Casein Digest Agar for 24 h days at 25 °C. Vincristine sulfate and water considered as a positive and negative controls, respectively. The concentrations of bacteria were adjusted to absorbance (560 nm) values of 1.0×10^8 bacteria. All extracts, controls and solutions were sterilized using filter (0.22 μ m filter, sterile syringe filter holder CHROMAFIL, CA-20/255). Potatoes (*Solanum tuberosum* L.) were cleaned and their surface sterilized in 1% hypochlorite sodium for 15 min. Tubers were cut then immersed in 1% hypochlorite sodium for 30 min. The disc of potato were cut with specific diameter (15 mm) and height (5 mm) and the discs transferred to culture plates containing agar (1.5 %). 2 ml suspension of *A. tumefaciens* (10^8 CFU.ml) was mixed with 2 ml of the methanolic and aqueous extracts of garlic in water or DMSO (0.25, 0.5, 0.75, 1, 2, 2.5, 3, and 4 mg/ml) in separated tubes and then 50 μ l of each mixture was added on potato discs. The plates were incubated for 21 days at 25 °C under a laminar air cabinet. After 21 days, disks were stained with Lugol's reagent then the number of tumors was counted. Each test had 16 replicates. The percent of tumor inhibition was determined.

Statistical method

Data were presented as mean \pm SD. All data were analyzed using analysis of variance (ANOVA) followed by Tukey- kramer. Statistical significance was defined as $p < 0.05$. The IC₅₀ values of both extracts and the corresponding confidence limits (CL, 95%) were determined using Litchfield and Wilcoxin II program from PHARM/PCS Version 4.2 software.

Results

The inhibitory effect of the methanolic and aqueous extracts of garlic on *A. tumefaciens* induced tumor was tested using potato disc bioassay. Vincristine sulfate as a positive control blocked the tumor formation on potato disc (100% inhibition).

All doses of the methanolic extract of garlic in water or DMSO as a solvent showed antitumor activity in comparison with the negative control in a dose dependent manner (Figures 1 and 2). The IC₅₀ values of methanolic extract in water and DMSO were 0.749 mg/ml (CL, 95%; 0.1406- 0.955) and 0.58 mg/ml (CL, 95%; 0.359- 0.936), respectively.

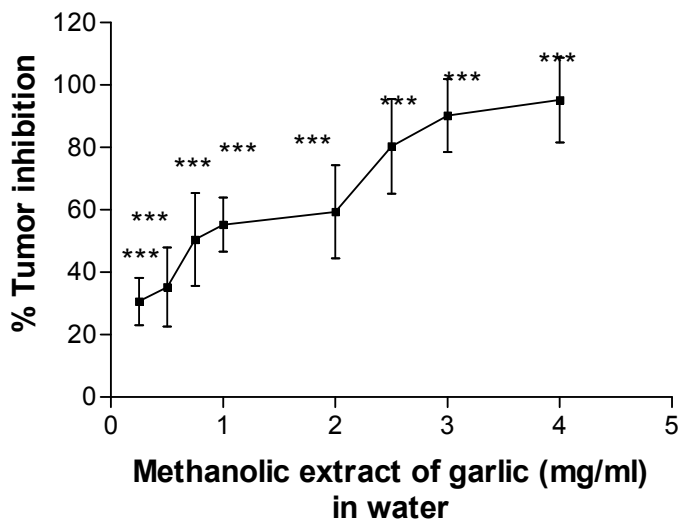


Fig 1. The effect of different concentrations of methanolic extract of garlic in water on *A. tumefaciens*-induced tumor
 Values are the mean \pm S.D. n= 16. ***P < 0.001, compared to control, Tukey–Kramer

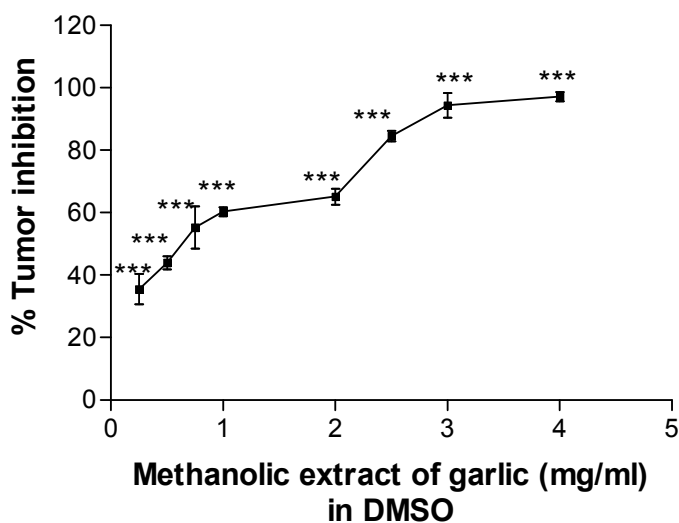


Fig 2. The effect of different concentrations of methanolic extract of garlic in DMSO on *A. tumefaciens*-induced tumor
 Values are the mean \pm S.D. n= 16. ***P < 0.001, compared to control, Tukey–Kramer

The aqueous extract of garlic in water indicated the inhibitory effect on tumor compared with negative control in doses 0.25 and 0.75 mg/ml, P<0.01 and 2, 2.5, 3 and 4mg/ml, P<0.001 and the percentage of blocking was more than 20%, (Figure 3). The IC50 values of the aqueous extract in water was 0.915 mg/ml (CL, 95%, 0.576- 1.45).

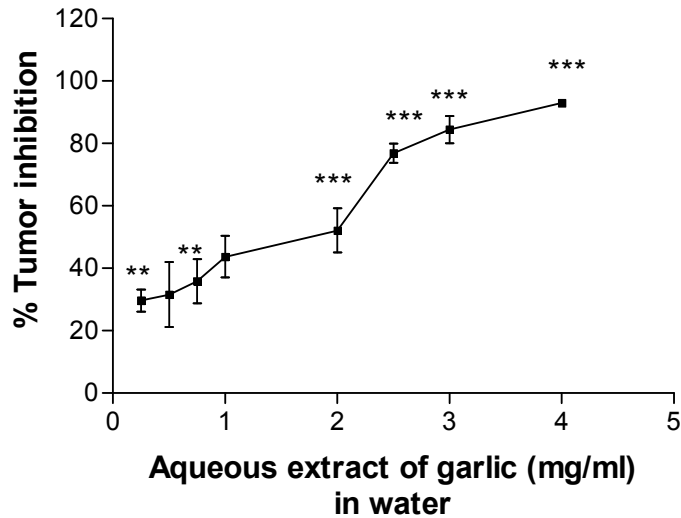


Fig 3. The effect of different concentrations of aqueous extract of garlic in water on *A. tumefaciens*-induced tumor

Values are the mean ± S.D. n= 16. **P<0.01 and ***P < 0.001, compared to control, Tukey–Kramer

Garlic aqueous extract in DMSO showed inhibitory effect on tumor in all doses and concentration dependently. The percentage of blocking was more than 20%, P<0.01, (Figure 4). The IC50 of aqueous extract in DMSO was 0.715 mg/ml (CL, 95%; 0.471- 1.19). These data indicated that methanolic extract effect was more effective than the aqueous extract against *A. tumefaciens*-induced tumor.

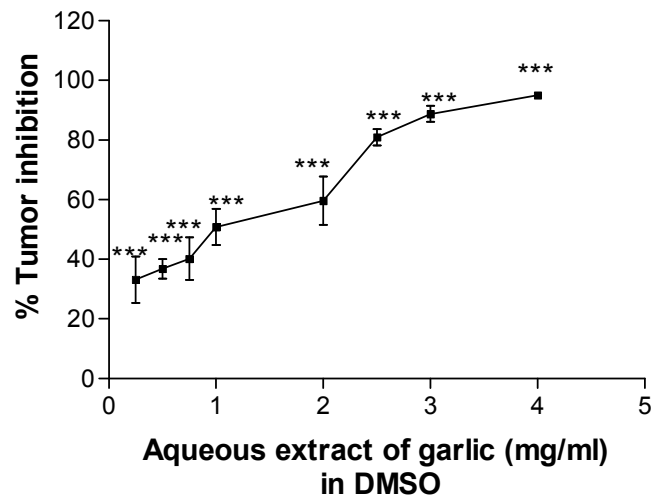


Fig 4. The effect of different concentrations of aqueous extract of garlic in DMSO on *A. tumefaciens*-induced tumor

Values are the mean ± S.D. n= 16. ***P < 0.001, compared to control, Tukey–Kramer

Discussion

It is demonstrated that many human cancers is caused by environmental factors such as smoking and dietary factors (24-27). Although surgery has considerably diminished the death of cancer, other cure like radiotherapy and chemotherapy have a few effect in cancer mortality. Hence prevention of cancer is more important than treatment and utilization of food and medicinal plants containing anticancer constituent can reduce the risk of cancer.

A. tumefaciens caused a neoplastic illness in some plants called crown gall. This bacterium carries the Ti plasmid that induced the transformation of normal plant cells into tumor cells (28). Potato disc bioassay is a simple, inexpensive and sensitive method to evaluate the anticancer effect of substances by inhibition of the formation of tumor on potato disc induced by *A. tumefaciens* (22).

It is shown that the potato disc bioassay results are in agreement with the 3PS assessment leukemia test in mice (28, 29). In this study the antitumor effect of the methanolic and aqueous extract of garlic was tested using potato disc assay. Both extracts of garlic revealed antitumor activities in potato disc bioassay. Garlic contains lots of organosulfur compounds such as ajoene, diallyl sulfide, diallyl disulfide and diallyl trisulfide (lipid soluble) and S-allylcysteine, S-allylmercaptocysteine (water soluble) (30).

Some studies indicated the antiproliferative and antitumor activity of water and lipid soluble organosulfur compounds (30-32). This effect depends on lipid and water solubility of these substances because of their difference in absorption, metabolism and polarity in aqueous and alcoholic solvents (32). It was shown that lipid soluble sulfur compounds are more potent than water-soluble substances (30) and this compound mostly present in methanolic extract, so the effect of garlic methanolic extract on tumors was more than the aqueous extract. Our results are in consistent with other studies that showed the anticarcinogenic effect of garlic in vivo and in vitro tests (33, 34).

It is concluded that the methanolic and aqueous extract of garlic demonstrated antitumor activity against *A. tumefaciens* induced tumor in potato disc assay. Reagarding to the antitumor effects of garlic, this plant may be utilized as a therapeutic agent to prevent the incidence of cancer in the future and these data confirm the folkloric idea about the benefits of garlic against cancer.

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