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Research Article

SCREENING OF ESSENTIAL OIL OF *ALLIUM SATIVUM* FOR ANTIBACTERIAL EFFECTS AGAINST *BACILLUS SUBTILIS*

Fariba Najafi[1]., Reza Tahvilian[2]., Mohammad Mahdi Zangeneh[1,3]*., Akram Zangeneh[1,4]., Rohallah Moradi[1,5]

[1]Department of Dermatology, School of Medicine, Kermanshah University of Medical Science, Kermanshah, Iran
[2]Research pharmaceutical center, School of pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
[4]Microbiology section, Pathobiology & Basic sciences department, Veterinary faculty, Razi University, Kermanshah, Iran
[5]Department of Chemistry, Kermanshah Center of Payame Noor University, Kermanshah, Iran

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ABSTRACT

Augmenting bacterial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is an instantaneous and steady need for novel antibacterial compounds from plants. As we know, there is no documented proof on antibacterial effects of *Allium sativum* (*A. sativum*) in west of Iran. The aim of the current study was evaluation antibacterial activities of essential oil of *A. sativum* against *Bacillus subtilis*(*B.subtilis*) in west of Iran (in Kermanshah). The antibacterial properties of *A. sativum* essential oil was evaluated by macro-dilution method in Mueller-Hinton broth medium, agar disk and well diffusion methods. The results illustrated that the essential oil of *A. sativum* have inhibited the growth of *B. subtilis* and destroyed it. Also, by increasing the concentration of the *A. sativum* essential oil, the inhibition zones increased. We believe that the article provide support to the antibacterial effects of the essential oil. In fact, the results indicate that the essential oil of *A. sativum* can be useful as medicinal or preservative composition. Fractionation and characterization of active molecules will be the future work to investigate.

INTRODUCTION

Plants have been screened for their potential uses as other remedies for the treatment of several infectious diseases (Beaglehole *et al*., 2004). These plants contain medicinal effects which make them potent to treat or prevent diseases (Sofowora, 1982). Most plant extracts have been shown to possess antimicrobial agents active on bacteria in vitro. Some medicinal plants used in traditional Iranian medicine are effectual in treating several diseases caused by bacterial and oxidative stress (Chan *et al*., 2008). An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils could be extracted from different parts like leaves, stems, flowers, and roots (Alizadeh, 2013; Sepahvand *et al*., 2014; Topçu *et al*., 2013). Interest in essential oil of plants with antibacterial activities has revived as a result of current problems associated with the use of antibiotics (Elliot *et al*., 1986). Essential oils are efficacious on a wide range of Gram-negative and positive bacteria (Burt, 2004; Burt *et al*., 2005).

A. sativum (Allium genus and Amaryllidaceae family) commonly known as garlic. *A. sativum* is originally from Asia but it is also cultivated in China, North Africa, Europe and America (PDR, 2000). *A. sativum* is an endemic and resistance species in dry and sub-dry forests in mountainous regions of Western Iran. *A. sativum* is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. A wide range of microorganisms have been shown to be sensitive to *A. sativum*. *A. sativum* has been shown to be antiviral and antifungal, as well as possessing both antitumor and antithrombotic effects (Cavallito *et al*., 1945;

*Corresponding author: **Mohammad Mahdi Zangeneh***
Department of Dermatology, School of Medicine, Kermanshah University of Medical Science, Kermanshah, Iran

Ross *et al*., 2000). The allicin derivative products (diallyl disulfide, diallyltrisulfide) found in garlic essential oils have shown good antimicrobial and antioxidant activities (Kim *et al*., 2004; Tsao& Yin 2001; Amagase *et al*., 2001).

To our knowledge antibacterial of Iranian *A.sativum* have not been studied so far in west of Iran (in Kermanshah). This prompted us to study the possible antibacterial properties of essential oil of Iranian *A. sativum*.

MATERIALS AND METHODS

***Plant sample collection*:** In the empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

***Essential oil extraction*:** Essential oil from fresh, clean, weighed aerial part *A. sativum* extracted by hydro-steam distillation using the Clevenger apparatus were collected and stored in sterile vials. Briefly, 100 to 150 g of plant was introduced in the distillation flask (1L), which was conjuncted to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oil was liberated from the plant material and vaporized into hot steam. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system in order to compress the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was purged through anhydrous Na_2SO_4 to dry the yielded essential oil. Then, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the oil were done using dimethyl sulfoxide (DMSO).

***Source of microorganisms*:** Bacterium specie namely *B. subtilis* (ATCC No. 21332) was procured from Iranian Research Organization for Science and Technology as lyophilized. Bacterium strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10^8cfu/ml using Muller Hinton broth.

***Culture media*:** Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

***Evaluation of antimicrobial activities*:** Agar disk and well diffusion methods were used as screen tests to evaluate antibacterial property of *A. sativum* based on standard protocol. The solution of the *A. sativum* was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk and well in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. DMSO was used as negative control whereas Cephalexin was used as positive control in case of *B. subtilis*. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In this case, after incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter (CLSI, 2006).

***Statistical Analysis*:** Antibacterial effect was determined by One way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.05$.

RESULTS

***Agar disk diffusion test*:** About *A. sativum*, the widest zone was seen in 0.062 g/ml concentration (The value of growth inhibition zone was 14 mm in this dilution). There was no inhibition zone in *B. subtilis* due to 0.002 and 0.003 g/ml concentrations. No inhibition zone was observed due DMSO. Growth inhibition zones due to different dilutions are listed in figure 1.

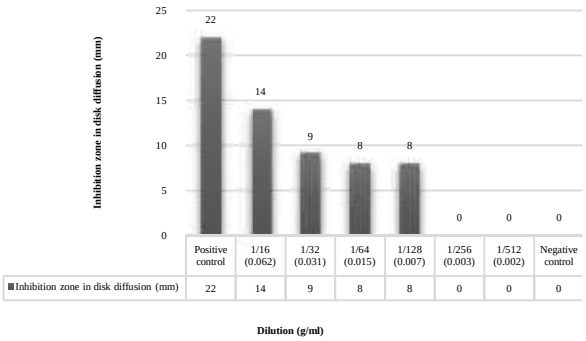

Figure 1 The diameters of growth inhibition zones in agar disk diffusion different dilutions of *A. sativum*.

***Agar well diffusion test*:** In regard to *A. sativum*, the widest zone was seen in 0.062 g/ml concentration (The diameter of growth inhibition zone was 10 mm in this dilution). No inhibition zone was observed due to DMSO. The data are discoverable in figure 2.

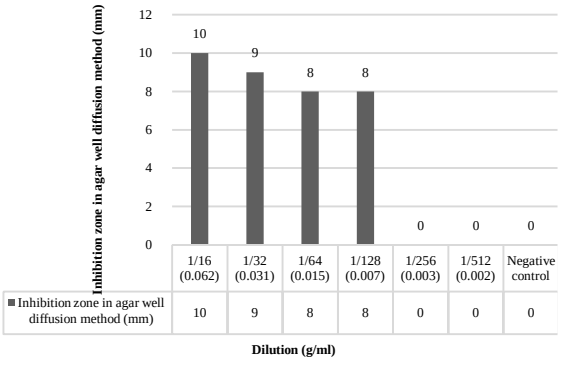

Figure 2 The diameters of growth inhibition zones in agar well diffusion t different dilutions of *A. sativum*.

MIC and MBC determination: The values of MIC and MBC are 0.003 g/ml and 0.015, respectively.

DISCUSSION

Antibiotics drug used in the treatment and prevention of bacterial infections. But, some antibiotics have been associated with many of harmful side effects ranging from chronic to acute depending on the type of antibiotic used, the microbes targeted, and the individual patient (Slama *et al*., 2005; Nychas, 1995). Cefalexin or cephalexin, is an antibiotic that can treat a number of bacterial infections. It kills gram-positive and some gram-negative bacteria by disrupting the growth of the bacterial cell wall. But, this antibiotic like other antibiotics have different side effects. Current side effects of cephalexin include stomach upset, diarrhea and allergic (Cephalexin, 2014). Almost all of human nutrition depends on plants, either directly through foods consumed by people, or indirectly as feed for animals or the flavoring of foods. The use of plant compounds to cure infections is an old practice in a large part of the world. Interest in plants with antimicrobial activities has revived as a result of common problems associated with the use of antibiotics (Abu-shanab *et al*., 2006). Also, because of their safety and low cost as well as their impact on a wide range of microbes in traditional medicine uses plants. Medicinal plants may have the ability to treat bacterial resistance to several types of antibiotics. The type and level of antibacterial properties exhibited by any plant material depends on many factors, including the plant part, soil conditions, harvest time, drying method, storage conditions, and post-harvest processing (Hassawi & Hassawi, 2006). The antibacterial activities of essential oils extracted from a large number of plants have been evaluated and reviewed (Koutsaviti *et al*., 2011; Stefanello *et al*., 2011), and the mechanisms that enable the natural ingredients of herbs and spices to resist bacteria have been discussed (Montanari *et al*., 2012). The results show that these mechanisms vary greatly depending on the components of the essential oil (Holley& Patel, 2005; Reichling *et al*., 2009). *A. sativum* is well known plant in Iran and several parts of this plant have long been used in traditional medicines of Iran. It is also used as a spice and food additive (Singh *et al*., 2009; Eja *et al*., 2007). It contains many substances which studies have shown to act together to prevent different diseases such as hypertension, fungal, viral, and parasitic diseases. *A. sativum* contains some sulphur-containing compounds such as allicin, ajoene, diallylsulphide, dithin, allyl methyl sulfide, Sallylcysteine, enzymes as well as some non sulphur containing compounds including vitamin B, proteins, minerals, saponins and flavonoids (Lanzotti *et al*., 2012; Azimi *et al*., 2011). Sulphur-containing compounds are thought to be the major compounds responsible for the antimicrobial effect of *A. sativum*. The main compound that is offered to be responsible for antibacterial effect of *A. sativum* is volatile allyl methyl sulfide as a lead compound of volatile *A. sativum* metabolites (Becker *et al*., 2012).

The results indicated that *A. sativum* essential oil with 0.003 g/ml concentration has prevented from the growth *B. subtilis*, also in 0.015 g/ml concentration has destroyed it. Also, the activities of essential oil of *A. sativum* were considerably dependent upon concentration. Thus, the research represents the antibacterial activities of the medical plant on *B. subtilis*. There are similarities and differences between these results and the resembling studies. It was shown in a study that Gram-negative diarrheagenic pathogens from stool samples were very sensitive to *A. sativum* (Eja *et al*., 2007). In other study indicated *A. Sativum* prevented the growth of the pathogenic bacteria, though with varying degrees of susceptibility. *Staphylococcus aureus* was more susceptible to the toxic effects of *A. sativum* than its gram negative counterparts (EL-mahmood, 2009). In contrast reported that *A. sativum* did not display any in vitro inhibition on the growth of test bacteria including *S.aureus* (Onyeagba *et al*., 2004). In other study revealed that samples of *A. sativum* showed no bacteriostatic or fungistatic properties on the microorganisms tested, including *Escherichia coli*, *Pseudomonas aeruginosa* and *S. aureus* (Packer& Luz, 2007). An in vitro study on the properties of *A. sativum* against *B. subtilis*, *P.aeruginosa*, *E. coli*, *S. aureus* and *Klebsiella pneumonia* showed that the most susceptible for gram-positive bacteria to the extract was the *B. subtilis* (Ibezym& Gladys, 2015). Also, in this study indicated that there were no inhibitory effects from all concentrations of *A. sativum* on the growth of *S. aureus*, *B. subtilis* and *P. aeruginosa*. In other study, revealed that *A. sativum* had less MIC for gram-positive than gram-negative organisms (more effective on *B. subtilis* and *S. aureus*). In this study demonstrated that there was maximum inhibitory zone for *B. subtilis* (Durairaj *et al*., 2009).

From the study it can be concluded that the essential oil of *A. sativum* possess antibacterial properties. In fact essential oil of *A. Sativum* have inhibited the growth of *B. subtilis* and destroyed it. Also, by increasing the concentration of the essential oil, the inhibition zone in creased. The results determined that in tested bacterium, there was a considerable discrepancy in terms of sensitivity to *A. sativum* essential oil. In other words, the most sensitivity was observed in disk diffusion method. Our results support the use of the plant in traditional medicine and offer that essential oil of *A. sativum* possess good antibacterial properties. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Additional *in vivo* studies and clinical trials would be needed to justify.

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Authors' Contribution

The core idea of this work came from Mohammad Mahdi Zangeneh and Akram Zangeneh, also the experiments, evaluation and Statistical Analysis of antimicrobial activities done by Fariba Najafi, Reza Tahvilian, Mohammad Mahdi Zangeneh, Akram Zangeneh, Rohallah Moradi.

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