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Research Article

In Vitro Antibacterial Efficacy of Essential Oil of *Allium sativum* Against *Staphylococcus aureus*

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ABSTRACT
Increasing 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 activities of *Allium sativum* (*A. sativum*) essential oil in west of Iran. The aim of the current study was assessment antibacterial effects of *A. sativum* against *Staphylococcus aureus* (*S. aureus*) in west of Iran (in Kermanshah). The antibacterial effects of *A. sativum* essential oil was evaluated by macro-dilution method in Mueller-Hinton broth medium, agar disk and well diffusion methods. The results indicated that the essential oil of *A. sativum* have inhibited the growth of *S. aureus* 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. Our finding shows the fact that the essential oil of *A. sativum* can be useful as medicinal or preservatives composition.

Keywords: *Allium sativum*, Essential oil, Antibacterial activity.

INTRODUCTION

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[1,2]. Cefalexin or cephalexin, is an antibiotic that can remedy a number of bacterial infections. It destroys gram-positive and some gram-negative bacteria by disrupting the growth of the bacterial cell wall. However, this antibiotic like other antibiotics have many side effects. Common side effects of cephalexin include stomach upset, diarrhea and allergic[3]. Plants have been screened for their potential uses as other remedies for the treatment of different infectious diseases[4]. These plants contain medicinal properties which make them potent to improve or prevent diseases[5]. Most plant extracts have been shown to possess antimicrobial agents active against bacteria in vitro. Some medicinal plants used in traditional Iranian medicine are impressive in treating different diseases caused by bacterial and oxidative stress[6]. An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils could be extracted from various parts like leaves, stems, flowers, and roots[7-9]. In recent years, they have applications as anesthetic, deodorant, diaphoretic, disinfectant, expectorant, antiseptic, fumigant, inhalant, insect repellant, vermifuge, bronchitis, arthritis, cancer, diabetes, diarrhea, diphtheria, dysentery, mastitis, encephalitis, enteritis, erysipelas, fever, inflammation, pharygnitis, laryngitis, and wounds. Interest in essential oil of plants with antibacterial effects has revived as a result of current problems associated with the use of antibiotics[10]. Essential oils are effective on a wide range of Gram-negative and positive bacteria[11,12]. *A. sativum*, commonly known as garlic, is a species in the Allium genus and Amaryllidaceae family. *A. sativum* is originally from Asia but it is also cultivated in China, North Africa, Europe and America. The *A. sativum* is a bulb growing to 25-70 cm with hermaphrodite flowers[13]. *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[14,15]. It contains many substances which provides antimicrobial activity. *A. sativum* contains

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some sulphur-containing compounds such as allicin, ajoene, diallylsulphide, dithin, allyl methyl sulfide, Sallylcysteine, enzymes as well as some non sulphurcontaining compounds including vitamin B, proteins, minerals, saponins and flavonoids[16,17]. Based on knowledge of authors, there is a very little data about antibacterial effects of *A. sativum* essential oil collected from Kermanshah province, west of Iran. The aim of the current study was assessment of antibacterial activities of the essential oil on common pathogen (*S. aureus*) with broth macro-dilution, agar disk and well diffusion methods.

MATERIAL 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 *S. aureus* (ATCC No. 25923) 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 10^8 cfu/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 *S. aureus*. 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[18].

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 24 mm in this dilution). There was no inhibition zone in *S. aureus* 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.

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 12 mm in this dilution). No inhibition zone was observed due to DMSO. The data are discoverable in figure 2.

MIC and MBC determination

In the examined bacterium, MIC and MBC values were the same and equal to 0.015 g/ml concentration.

DISCUSSION

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 treat infections is an old practice in a large part of the world. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics[19]. Also, because of their safety and low cost as well as their impact on a large number of microbes in traditional medicine uses plants. Medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics. The type and level of antibacterial effects exhibited by any plant material depends on many factors, including the plant part, geographical source, soil conditions, harvest time,

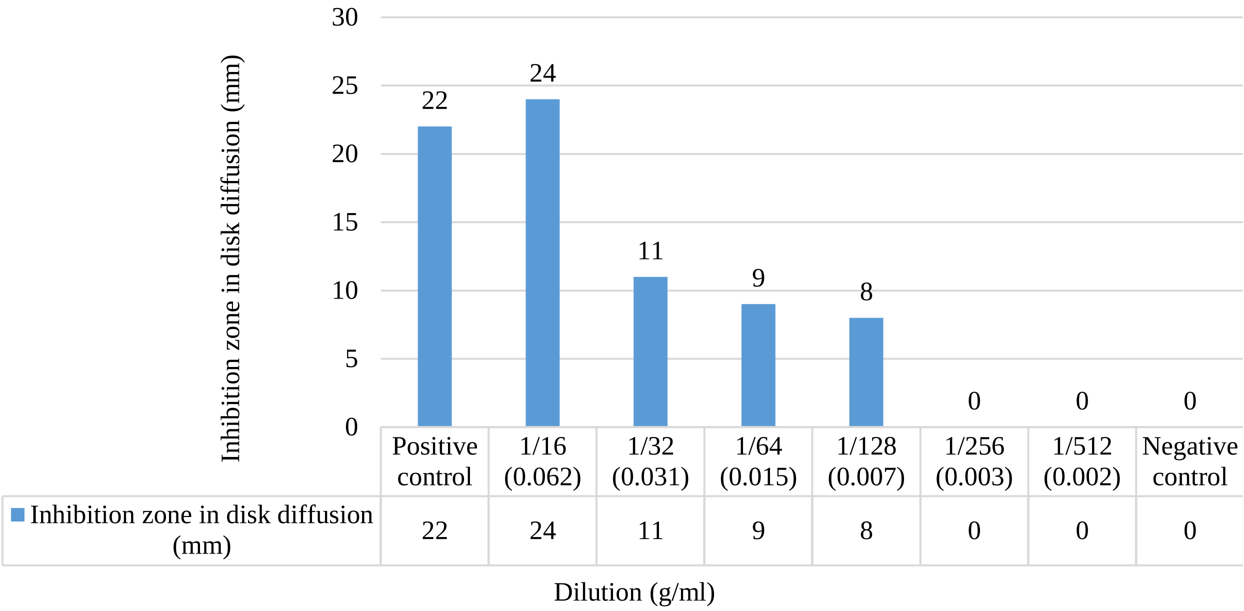

Figure 1: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *A. sativum*.

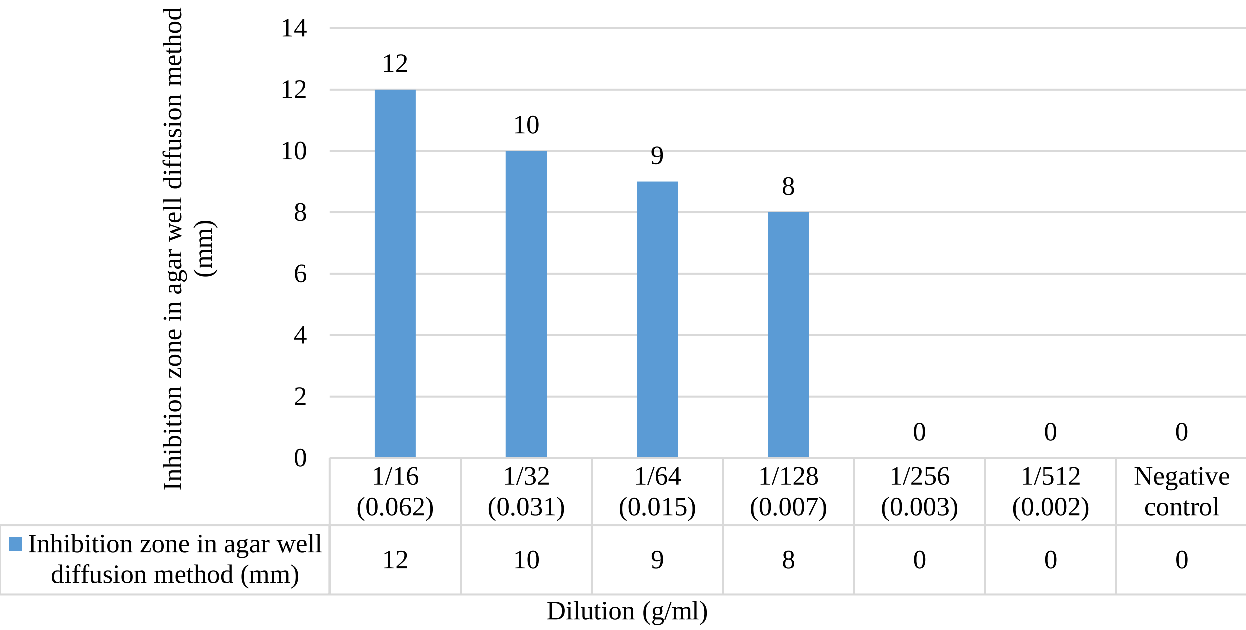

Figure 2: The diameters of growth inhibition zones in agar well diffusion test in different dilutions of *A. sativum*.

moisture content, drying method, storage conditions, and post-harvest processing[20]. The antimicrobial effects of essential oils extracted from a large number of plants have been evaluated and reviewed[21,22], and the mechanisms that enable the natural ingredients of herbs and spices to resist microbes have been discussed[23]. The results show that these mechanisms vary greatly depending on the components of the essential oil[24,25]. *A. sativum* is well known plant in Iran and different parts of this plant have long been used in traditional medicines of Iran. It is also used as a spice and food additive[26,27]. It contains many substances which studies have shown to act together to prevent different maladies such as hypertension, cancer fungal, viral, and parasitic diseases[16-17]. Sulphur-containing compounds are thought to be the major compounds responsible for the antimicrobial effect of *A. sativum*. The main compound that is suggested to be responsible for antibacterial effect of *A. sativum* is volatile allyl methyl sulfide as a lead compound of volatile *A. sativum* metabolites[28]. The antibacterial results showed that the essential oil of *A. sativum* inhibited the bacterium and the activities were considerably dependent upon concentration. Also, the results indicated that *A. sativum* essential oil with 0.015 g/ml concentration has prevented from the growth *S. aureus*, also in this concentration has destroyed *S. aureus*, actually MIC and MBC are equal for the bacterium. Thus, the research represents the antibacterial activities of the medical plant on *S. aureus*. There are Similarities and differences between these results and the resembling studies. An *in vitro* study on the properties of aqueous and ethanolic extracts of *A. sativum* against *Escherichia coli* and *salmonella typhi* showed that the aqueous extract had little or no inhibition while the ethanolic extract had a higher inhibitory activity[29]. It was shown in another study that Gram-negative diarrheagenic pathogens (E. coli, Shigella sp, Salmonella sp, and Pro. mirabilis) from stool samples were very sensitive to *A. sativum*[27]. In a study indicated Aqueous, ethanol and chloroform extracts of *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[30]. In contrast reported that crude extracts of *A. sativum* did not display any in vitro inhibition on the growth of test bacteria including Staphylococcus spp[31]. Many studies report that *A. sativum* essential oil act more on Gram-positive than Gram-negative bacteria[32]. In a study shown that there is no general rule with respect to the Gram sensitivity because many controversies exist in the different published works. The Gram-negative bacteria *Campylobacter jejuni* has been characterized as particularly sensitive to the action of essential oil of *A. sativum*[33]. In other study revealed that samples of *A. sativum* essential oil showed no bacteriostatic or fungistatic properties on the microorganisms tested, including *E. coli*, *P. aeruginosa* and *S. aureus*[34]. From the study it can be concluded that essential oil of *A. sativum* have inhibited the growth of *S. aureus* and killed it. Also, by increasing the concentration of the essential oil, the inhibition zones enhanced. The results determined that in tested bacterium, there was a remarkable variation in terms of sensitivity to *A. sativum* essential oil. In other words, the most sensitivity was observed in agar disk diffusion method. Our results support the use of the plant in traditional medicine and suggest that essential oil of *A. sativum* possess good antibacterial effects. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Also, further assessment is irrevocable on potential of the plant as an antibacterial agent in topical or oral applications.

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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, Mohammad Mahdi Zangeneh, Reza Tahvilian, Akram Zangeneh, Hossein Amiri, Nassim Amiri, Rohallah Moradi

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