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Research Article

ETHNOMEDICINAL PLANT: ANTIBACTERIAL EFFECTS OF ESSENTIAL OIL OFALLIUM SATIVUMAGAINST PSEUDOMONAS AERUGINOSA (PTCC NO. 1707) IN WEST OF IRAN

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ABSTRACT

Allium sativum(AS) is a native plant in Iran, which the plant has been used as an antioxidant, antiparasitic, antifungal, antiviral, and antiflammatory agent in Iran. Based on knowledge of authors, in comparison to many other pharmaceutical-industrial plants, there is a very little data about antibacterial activities of AS essential oil collected from Kermanshah province, west of Iran. Hence, the aim of the current study was evaluation of antibacterial effects of the essential oil on common pathogen (Pseudomonas aeruginosa (PA) (PTCC No. 1707)) in west of Iran (In Kermanshah).The antibacterial activities of AS essential oil were evaluated by macro-dilution method in Mueller-Hinton broth medium, agar disk and agar well diffusion methods. The results revealed that by increasing the concentration of the essential oil, the inhibition zone augmented. Also, the essential oil of AS have excluded the growth of PA and destroyed it. The results defined that in tested bacterium, there was a considerable discrepancy in terms of sensitivity to AS essential oil. Our finding indicate the fact that the essential oil of AS can be useful as medicinal or preservatives composition. Additional *in vivo* studies and clinical trials would be needed to justify.

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INTRODUCTION

A disease is a specific maladaptive condition, which affects part or all of an organism. Infection is the incursion of an organism's body tissues by disease-causing agents such as virus, bacterium, and fungus. Infections disease due to bacterial species also stay a serious clinical difficulty. Antibiotics provide the primary basis for the treatment of bacterial infections. But overuse of antibiotics has become the main factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms (Nychas, 1995). Kanamycin is an aminoglycoside bactericidal antibiotic that used to treat a wide range of infections. Kanamycin is mainly efficacious against Gram-negative aerobic bacteria. Crucial side effects of Kanamycin include tinnitus, toxicity to kidneys, and allergic reactions to the drug (Garrod *et al*,. 1981).

Plants are invaluable resources useful in daily life as food additives, pharmaceuticals, or directly in medicine. The uses of plants in treatment of animal and human diseases have long been established. 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 properties which make them potent to treat or prevent infectious 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 efficient in treating different 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 various parts like leaves, stems, flowers, and roots. In recent years, interest in essential oils has been

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incremented for pharmacological studies which claim that the essential oil have useful effect for the control and inhibition of human and food-borne microorganism's growth (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 effectual on a wide variety of Gram-negative and positive bacteria (Burt, 2004; Burt *et al*., 2005).

AS, commonly known as garlic, is a species in the Allium genus and Amaryllidaceae family. It is an annual grassy herb with 25-70 cm high which grows in the Asia, China, North Africa, Europe and America (PDR, 2000). AS is an endemic and resistance species in mountainous regions of Western Iran. AS is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. It contains many substances which studies have shown to act together to prevent several maladies such as fungal, viral, and parasiticdiseases (Cavallito *et al*., 1945; Ross *et al*., 2000). AS contains some sulphur-containing compounds such as allicin, ajoene, diallylsulphide, dithin, allyl methyl sulfide, Sallylcysteine, diallyl disulfide, diallyltrisulfideenzymes as well as some non sulphur-containing compounds including, proteins, minerals, saponins and flavonoids (Lanzotti *et al*., 2012; Azimi*et al*., 2011).

The aim of the current study was evaluation antibacterial activities of AS against PAin west of Iran (in Kermanshah).

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 AS 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 in to 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 PA (PTCC No. 1707) 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.

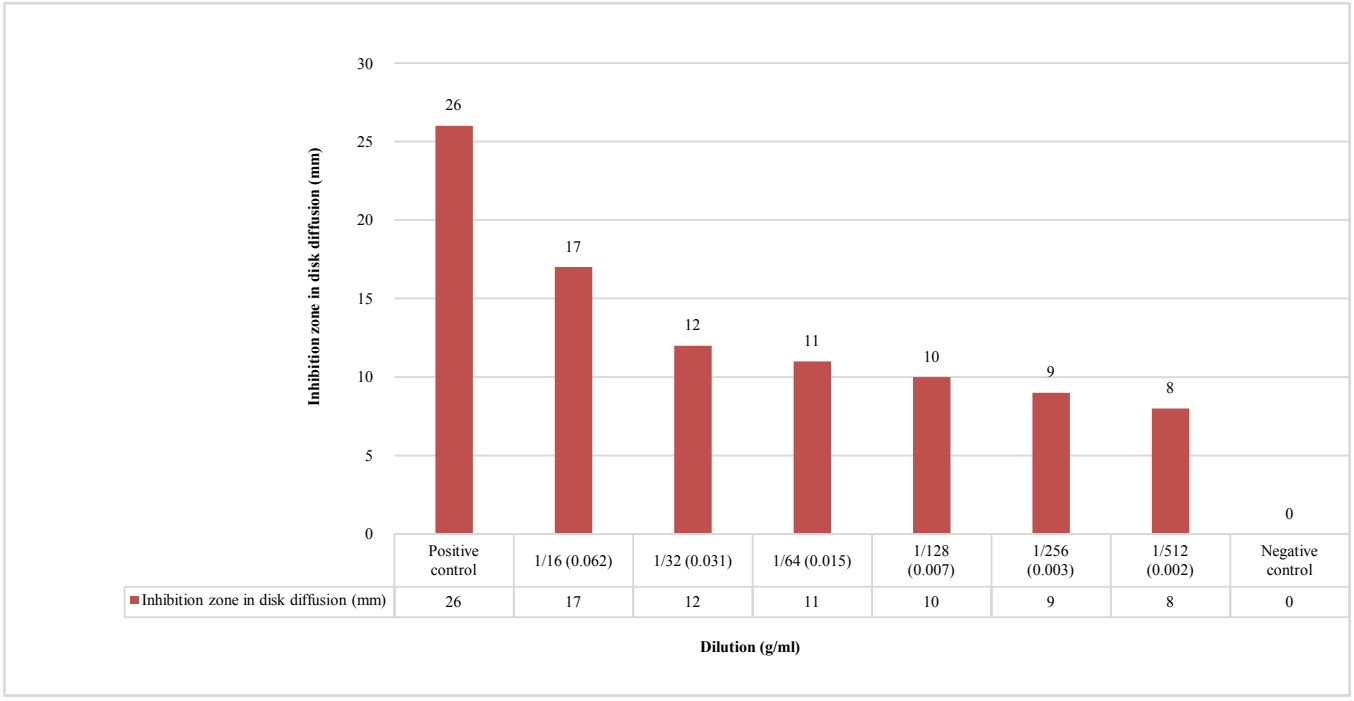

Figure 1 The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of AS.

Set plates were incubated overnight to ensure sterility before use.

Evaluation of antimicrobial activities

Agar disk and agar well diffusion were used as screen tests to evaluate antibacterial properties of AS based on standard protocol. The solution of the AS 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 kanamycin was used as positive control in case of PA. 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 effects were 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 AS, the widest zone was seen in 0.062 g/ml concentration (The value of growth inhibition zone was 17 mm in this dilution).

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 AS, the widest zone was seen in 0.062 g/ml concentration (The diameter of growth inhibition zone was 11 mm in this dilution). There was no inhibition zone in PA due to 0.002 and 0.003 g/ml concentrations. 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 0.031 g/mlcnocentration.

DISCUSSION

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 variety of microbesin traditional medicine uses plants. Medicinal plants may have the potency to treat bacterial resistance to different types of antibiotics. Different plants extract preparation methods have shown to exhibit a wide variety of antibacterial effects against clinical isolates of Gram negative and positive bacteria (Uchida *et al*., 1975; Gonzalez-Fandos *et al*., 1994).The antibacterial effects of essential oils extracted from a large number of plants have been appraised and reviewed (Koutsaviti *et al*., 2011;Stefanello*et al*., 2011), and the mechanisms that enable the natural ingredients of plants 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).AS 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 (Singh *et al*., 2009; Eja *et al*., 2007).

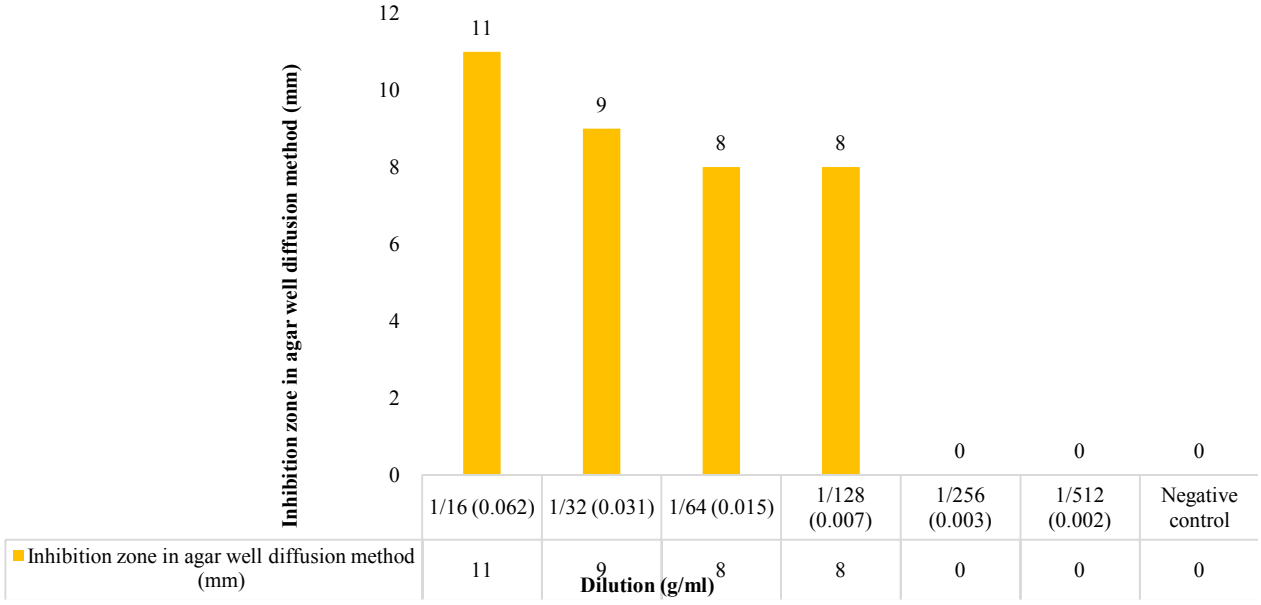

Figure 2 The diameters of growth inhibition zones in agar well diffusion test in different dilutions of AS.

It contains many substances which studies have shown to act together to prevent different diseases such as fungal, viral, and parasitic diseases. Sulphur-containing compounds of AS are thought to be the major compounds responsible for the antimicrobial effect of AS. The main compound that is offered to be responsible for antibacterial activities of AS is volatile allyl methyl sulfide as a lead compound of volatile AS metabolites (Becker *et al*., 2012).

The results indicated that AS essential oil with 0.031 g/ml concentration has prevented from the growth PA and has destroyed it. In a Study showed that MIC of ethanolic extracts of AS against PA was 67µg/ml (Karuppiah&Rajaram, 2012). In other study tested antibacterial activities of essential oil of ASonPA, their results yielded MICs vary between 16 µg/ml and 64 µg/ml (Tsao& Yin, 2001). In another study indicated that MICs of essential oil of ASwere against PA between 32 and 128 µg/ml concentrations (Khadri *et al*., 2010). Also, the results demonstrated that the activities of essential oil of AS were considerably dependent upon concentration. In fact, the inhibition zone were seen in 0.062, 0.031, 0.015, and 0.007 g/ml concentrations in agar disk and agar well diffusion methods. In a study indicated that low concentrations as low as 4 to 20 µg/ml of AS with agar disk diffusion method display a powerful antibacterial activities against PA (Ataee *et al*., 2016). In other study antibacterial activities of AS extract (5%, 10%, 20%, and 100%) against PA was shown. Also, in this study demonstrated that the inhibition zones of 5%, 10% and 20% concentrations were not significantly different from one another (Karuppiah&Rajaram, 2012). In a study revealed that samples of essential oil of AS showed no bacteriostatic or fungistatic effects on PA (Packer&Luz, 2007). In another study results reported the antibacterial effect of AS against PA in 67µg/ml with agar disk diffusion method (Zhou *et al*., 2014).
From the study it can be concluded that by increasing the concentration of the essential oil, the inhibition zone increased. Also, essential oil of AS have inhibited the growth of PA (PTCC No. 1707) and eradicated it in 0.031 g/ml concentration. The results indiated that in tested bacterium, there was a considerable difference in terms of sensitivity to AS essential oil. In other words, the most sensitivity was observed in agar disk diffusion method. This study confirmed the antibacterial effects *in vitro* towards PA of the essential oil of AS. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Also, further assessment is incumbent 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, Akram Zangeneh, Mohammad Saeed Zarei, Mohammad Reza Khedri, Erfan Bahrami, Masoud Shamohammadi.

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