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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITIES OF A MEDICINAL PLANT

Mohammad Mahdi Zangeneh[1*]., Akram Zangeneh[2]., Reza Tahvilian[3]., Rohalah Moradi[4]., Majid Hajialiani[4]., Marzieh Moradi[4]., Samira Simanizad[5] and Parya Chamani[4]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
[2]Department of Microbiology Section, Pathobiology & Basic Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
[3]Research Pharmaceutical Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4]Department of Chemistry, Payame Noor University, Tehran, Iran
[5]Department of Chemistry, Islamic Azad University, Kermanshah, Iran

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ABSTRACT

Enhancing bacterial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is aninstantaneous and steady need for novel antimicrobial compounds from plants.*Cucurbitamoschata*(*C. moschata*) seeds is a native plant in Iran, which the plant has been used as an antioxidant, antiparasitic, antifungal, antiviral, and antiflammatory agent in Iran. Effect of *C. moschata*seeds oil on growth of the common pathogen *Bacillus subtilis* ATCC No. 21332 (*B. subtilis*) in macro-dilution Mueller-Hinton broth agar disk and agar well diffusion is described. The results of agar disk and agar well diffusion tests indicated *C. moschata*seeds have prevented the growth of *B. subtilis*and destroyed it. Also in many of samples, by increasing the concentration of *C. moschata* seeds, the inhibition zone increased.Partially, the widest zone of inhibition in agar diffusion (17mm) and agar well (10mm) occurred at 0.125 g/ml *C. moschata* seeds. Minimum inhibitory and bactericidal concentrations were 0.125 g/ml *C. moschata* seeds oil. There was no inhibition at 0.003, 0.007 g/ml or in dimethyl sulfoxide (DMSO). We find discrepancies in *B. subtilis* sensitivity to *C. moschata*seeds oil with greater inhibition indisk diffusion tests. Thus, the present research demonstrates the antibacterial properties of the ethnomedical plant on *B. subtilis*, offering to use as antibacterial supplement towards the development of new therapeutic agent.

INTRODUCTION

Overuse of antibiotics is linked to emergence and dissemination of multi-drug resistant strains of bacteria. *Bacillus subtilis* as a gram positive bacterium has been the major cause of serious disease recently. This bacterium as other bacteria is becoming resistance to appointed type of antibiotics (such as Cephalexin), so it has become a great concern for finding a favorable substitution(such as plants and their derivatives) for preventing, control andtreatment it[1-5].

Down the ages plants have evoked interest as sources of innate products[6]. They have been screened for their potential uses as alternative remedies for the treatment of different infectious diseases[7-10]. Some medicinal plants used in traditional Iranian medicine are effective in treating diverse ailments caused by bacterial and oxidative stress[11-14]. A plant oil is a substance or an active with favorable effects that is removed from the tissue of a plant, to be used for a specific purpose[15, 16]. The antibacterial activities of oil have been identified for many years, and their rudiment have found applications as naturally occurring antibacterial agents in the field of pharmacology, pharmaceutical botany, medical and clinical microbiology, food maintenance, etc[17-19]. The original benefit of plant oils is that they do not increase the antibiotic resistance because they have a significant role in the defense system of the plant to bacterial diseases due to their intrinsic antioxidative and antibacterial properties[20-22]. Herbal oil have antibacterial activity on a wide number of bacteria such as*Staphylococcus aureus*, *B. subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, and most of these compounds have phenolic groups in their structure[23-25]. In Iranian medicine, plant oils in the form of infusion, decoction, tincture or herbal oil are consumed by the population for the treatment of diseases including bacterial diseases[21, 22]. In western states of Iran, a plant with the scientific name of *Cucurbitamoschata*(*C. moschata*) has traditional medical usage. *C. moschata*is the member of

flowering plants family called *Cucurbitaceae*. The genus is concentrated in central Asia with only a few species in central Europe and North America[26]. *C. moschata*have been used since ancient times in traditional medicines to treat eczema, wounds, ulcers, cancer and microbial disease. Different extracts of the plant are traditionally used in treating bacterial diseases[27]. Probably, the antibacterial activities of the plant are related to its phenolic, flavonoid, and flavone compounds. These components are adjoin to the bacterial outer membrane proteins, deactivate the matrix metalloproteinase and stop growth of bacteria or destroyed bacteria[28-30]. We report effects of *C. moschata*seeds oil on *B. subtilis*in broth macro-dilution, agar well and disk diffusion in west of Iran (in Kermanshah).

MATERIALS AND METHODS

Lyophilized *Bacillus subtilis* ATCC No. 21332 (*B. subtilis*) was furnished by The Iranian Research Organization of Science and Technology. The strain was activated on Tryptic Soy broth kept at 37°C for 18 h. 60 µl broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was adjusted to obtain a final concentration of 10^8cfu/ml in Muller Hinton broth. Mueller-Hinton Agar growth media was knotted according to 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.

The crushed *C. moschata* seeds were dried in an oven at 105^{o}C to sweep any moisture presence at different interval of time. A 10 g of dried *C. moschata*seeds was placed onto a thimble and the thimble was put into the sohxlet extractor. N-hexane solvent was casted into three-neck- round bottom flask that is joined with the extractor and flask along with the condenser on the top to eschew any solvent losses. Thelump assembly was then placed on the temperature controller heater to put out the required temperature. The temperature was measured by a thermometer that was inserted in one of the necks of the round bottom flask. After specified interval of the time the tryout was stopped and the trapped oil in the solvent was separated. The mixture of solvent and oil was separated using rotary evaporator under vacuum at temperature of 65^{o}C. The oil obtained after evaporation was weighed.

Agar disk and agar well diffusion were applied as screen tests to assessment antibacterial properties ofoil of *C. moschata* seeds based on standard protocol. 1g/ml *C. moschata* seeds oil was 6 fold diluted (v/v) and 60 µl of each dilution was poured on each disk and well. After 24h incubation diameters of growth inhibition zones were measured. Dimethyl sulfoxide(DMSO)and Cephalexin were used as negative and positive controls, respectively. Minimum inhibitory concentration (MIC) is the lowest concentration which inhibits bacterial growth despite removing or preventing growth of bacteria. Minimum bactericidal concentration (MBC) is the lowest concentration of an agent which causes death to test bacteria calculated by 60 µl in 6 dilutions on agar plates. After incubation the lowest concentration which discontinues growth is the MBC. For MIC the macrobroth dilution method was used[31].

RESULTS

In agar disk diffusion the widest zone 17 mmoccurred at 0.125 g/ml *C. moschata* seeds oil. There was no inhibition in 0.007, 0.003, and DMSO. Inhibition zones are shown inTable 1.In Agar well diffusion the widest zone 10 mm again was in 0.125 g/ml *C. moschata*seeds and no inhibition occurred in 0.015, 0.007, 0.003, and DMSO as shown in Table 2. MIC and MBC were 0.125 g/ml *C. moschata* seeds oil.

Table 1 Inhibition (mm) in agar disk diffusion at different dilutions of oil of*C. moschata* seeds.

Dilution(g/ml)	Inhibition zone in disk diffusion(mm)
Microorganism	*B. subtilis*
Positive control	19
1/8 (0.125)	17
1/16 (0.062)	10
1/32 (0.031)	9
1/64 (0.015)	8
1/128 (0.007)	0
1/256 (0.003)	0
Negative control	0

Table 2 Inhibition (mm) in agar well diffusion test in different dilutions of oil of *C. moschata* seeds.

Dilution(g/ml)	Inhibition zone in well diffusion(mm)
Microorganism	*B. subtilis*
1/8 (0.125)	10
1/16 (0.062)	9
1/32 (0.031)	8
1/64 (0.015)	0
1/128 (0.007)	0
1/256 (0.003)	0
Negative control	0

DISCUSSION

Antibiotics are types of antibacterial drugs used in the remedy and inhibition of bacterial infections. They may either assassinate or prevent the growth of bacteria. Since the detection of these antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would cause to the presumptive eradication of infectious diseases. But overuse of antibiotics has become the main factor for the dissemination of multi-drug resistant strains of several groups of microorganisms[32]. Because of their security and low cost as well as their effect on a great number of bacteria without any side effect, medicinal plants may have the potency to treat bacterial resistance to different types of antibiotics[33-35]. The antibacterial activities of plant extracts from a wide range of plants have been appraised and reviewed[36, 37], and the mechanisms that enable the natural components of herbs and spices to resist bacteria have been considered[38]. Plant extracts-derived products have a major variety of phytochemicals compounds. The results showed that these mechanisms of antibacterial activities of plants extracts vary greatly depending on the components of the plant[39, 40]. *C. moschata* have long been used in Asian countries as a medicinal plant for the treatment of diseases; it has been applied for treating different inflammatory and bacterial diseases[26, 27].

As the figures showed, the inhabitation zone have been increased when the oil amount has increased. The results defined that in tested bacterium, there was a considerable discrepancy in terms of sensitivity to*C. moschata* seeds. In agar disk diffusion test, no inhibitory effect of oil of the plant against the*B. subtilis* in 0.003 and 0.007 g/mlconcentration but in agar well diffusion test, no inhibitory effect of *C. moschata*seeds in 0.003, 0.007 and 0.015 g/ml concentrations. *C. moschata*seeds in 0.125 g/ml concentration has prevented from the growth of the bacterium and has destroyed bacterium.

Thus, the research represents the antibacterial effects of the medical herb on *B. subtilis*.

There are correspondences between this result and the similar studies. In a study, demonstrated to Gram-positive bacteria were very sensitive to oils antibacterial properties because of absence of lipopolysaccharide in theircell wall which may inhibit antibacterial compounds reach the cytoplasmic membrane of gram-positive bacteria[41]. In other study indicated that *C. moschata*seeds have strong antibacterial propertie[42].

CONCLUSION

From this study it can be concluded that in many of samples, by increasing the concentration of the oil of *C. moschata* seeds, the inhibition zone increased. Also, *C. moschata*seeds have inhibited the growth of *B. subtilis* and eradicated it in 0.125 g/ml concentration. The results indicated that in tested bacterium, there was a considerable difference in terms of sensitivity to oil of *C. moschata*seeds and the most sensitivity was observed in agar disk diffusion method. In fact, these results demonstrated *C. moschata*seeds have antibacterial activity. It can be used as antibacterial supplement in the developing countries towards the development of new remedial agent. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of the plant as an antibacterial agent in topical or oral applications.

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