ANTIBACTERIAL EFFECT OF LYOPHILIZED POWDER OF SHALLOTS (ALLIUM ASCALONICUM) AGAINST NOSOCOMIAL INFECTIONS

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Abstract: Objective - To evaluate the effect of lyophilized powder of shallot on the growth of bacteria involved in nosocomial infections. The results of the antibacterial activity assay of lyophilized powder of shallot indicated that this activity was strong. The most sensitive and resistant microorganisms against lyophilized powder of shallot were Staphylococcus aureus and Pseudomonas aeruginosa, respectively. There were significant differences in MIC values between different types of the inhibiting growth microbes (p=0.001). MIC values in microdilution assay were 3.18 ± 0.6757 μg/mL. MIC values are found to be lower for Staphylococcus aureus and highest for Pseudomonas aeruginosa. The effectiveness of Klebsiella and Proteus MIC concentrations was higher than other bacteria. There were statistically significant differences between effective of concentrations MIC Staphylococcus with Proteus, Klebsiella and Pseudomonas (p<0.05). It is of interest that lyophilized powder of shallot has shown most sensitivity to Staphylococcus aureus, since it is still one of the five most common causes of hospital-acquired infections and it also occurs in vivo, the use lyophilized powder of shallot with differences MIC values can be candidate for inhibition growth of such nosocomial infections.

Key words: Antibacterial, Infections, Nosocomial, Shallots

OBJECTIVE

Nosocomial infection is one of the most prevalent problems in the hospital (1), which is major causes of morbidity and mortality in hospital patients (2). Several kinds of microorganisms are involved in nosocomial infections and about ninety percent of these infections are caused by bacteria. Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Proteus spp., and Pseudomonas aeruginosa are among the most common causative agents of nosocomial infection (3). Different antibiotic treatments were used in order to eradicate these infections. Most bacteria that cause nosocomial infections have become drug resistant using indiscriminate of antibiotics (4, 5). Resistance to antibiotics has caused a lot of problems in the treatment of infections. The process of finding a new drug such as traditional medicinal plants for antibiotic resistant bacteria can be useful in treatment of infections. Medicinal plants have played a key role in world health. Herbal medicines have been important sources of products in developing countries for treatment of common infections (6). Shallots is one of the most important species in the pharmaceutical industry of Iran (7). A few study showed that the shallots has antioxidant properties and inhibits the growth of microorganisms (8, 9). There are a few studies indicate that the lyophilized

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powder of shallots can inhibition growth of bacteria involved in nosocomial infections. Study of Mahmoudabadi et al showed that the shallots oil and all four major diallyl sulfides inhibit food-borne pathogenic bacteria (10). The aim of this study was to evaluate the effect of lyophilized powder of shallots on the growth of bacteria involved in nosocomial infections including Proteus, Klebsiella, Staphylococcus, Escherichia coli and Pseudomonas.

**SETTING**

This study was done in the hospitals of Ardabil city, Iran, 2012-14. The study was approved by Ardabil University of Medical Sciences. At the beginning of study, lyophilized powder of shallots was prepared.

**DESIGN**

In a Quasi- experimental study in hospitals of Ardabil, Iran, blood samples were collected from all patients with septicemia (13) and were cultured in blood agar, EMB agar, and MacConkey agar etc. Selective culture media was used for the isolation of bacteria as pure culture. These samples were cultured in Nutrient agar after incubation at 37°C for 24 h and then the colonies were identified by growing bacteria. Then lyophilized powder of shallots, antibacterial activity was examined on them.

**PLANT MATERIAL**

**THE BULBS SHALLOTS (ALLIUM ASCALONICUM) WERE COLLECTED FROM HAMADAN CITY SOUTHWEST OF IRAN, IN SPRING**

**EXTRACTION AND ISOLATION OF LYOPHILIZED POWDER OF SHALLOTS**

About 100 g of underground root of shallots (Allium ascalonicum) was washed thoroughly in water and properly skin was peeled. Then clean bulbs were chopped into small pieces. About 400 ml of pure water in two steps (every time 200 ml) is added to content mill. The mixture was homogenized using ultrasonic homogenizer for 10 minutes with 200 watts and then broken cell wall with ultrasound waves. Therefore, content of plant cells were placed in the water environment. The mixtures after pass of filter with a clean cloth were placed in a 50 mL Falcon tubes and centrifuged at 4000 rpm for 10 minutes. Solutions were collected in 250 ml glass bottles and store at the -80°C freezer space. The next day, after preparing the freeze-drier (Christ Germany), bottles containing of aqueous extract of frozen were out and placed into glass of freeze dryer. Water of extract was evaporated and white powder of soluble in water remained at the bottom of the bottle. Powder prepared was subsequently kept at -20°C for examination.

**DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION (MIC) OF LYOPHILIZED POWDER OF SHALLOTS**

**ANTIMICROBIAL PREPARATION (LYOPHILIZED POWDER OF SHALLOTS AND MUeller HINTON LIQuID CULTURE)**

**INTERVENTION**

Blood samples were taken from hospital patients and expansion provided was prepared depending on the type of bacteria. Samples were incubated for 24 hours 37°C. Bacterial suspensions were prepared using standard solution.

Half McFarland: About 4ml of physiological saline was poured into the test tube and picked up from colonies grown on 24 hours in media. Then, suspensions were prepared based on standard solution of half McFarland. About 0.1ml of suspensions and 0.9 ml of physiological saline were into micro tubes, respectively. The concentration of 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 mg of lyophilized powder of shallots were weighed using with a 0.001 sensitive scale and were mixed with 5 ml of Mueller Hinton solution into cap test tubes. Therefore, with slow motion and rotation were stirred and filtered and then their sterilized were poured into another tube.

Determination of minimal inhibitory concentration (MIC)

MIC is the lowest concentration which inhibits bacterial growth or it is the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. Microplate used for determination of MIC. About 0.1 ml of the concentrations of 10 to 30 shallots suspensions prepared was poured into each of the vertical columns. The amounts of 0.05 ml bacterial suspensions were poured into uniform wells. Sterile sampler separate used for each well. MIC was repeated for 3 times for all samples and mean values were calculated.

For positive and negative control; 0.1 ml of liquid culture plus 0.05 ml one of the strains was poured to the wells each as a positive control, and 0.1ml of last concentration of antimicrobial were
poured to the wells each as a negative control. The microplates were incubated for 16 h at 37°C for controlling of bacterial growth. MIC values were read as the antibacterial concentration at the point where dense colonial growth intersected the disc. The result of bacteria species MIC were compared with MIC of reference isolate of bacteria.

STATISTICAL METHODS

Data were analyzed using ANOVA and Tukey tests.

RESULTS

The results of the antibacterial activity assays of lyophilized powder of shallots indicated that this activity was strong. Comparisons of mean concentrations of MIC types of bacteria were given in figure 1. All tested isolates were sensitive to the lyophilized powder of shallots with different MIC values. The most sensitive and resistant microorganisms against lyophilized powder of shallots were Staphylococcus aureus and Pseudomonas aeruginosa, respectively. The comparison effectiveness of MIC concentration types of microorganisms against lyophilized powder of shallots (Allium ascalonicum) is presented in Table 1. There were significant differences in MIC values between types of the inhibiting growth microorganisms (p<0.001). MIC values in microdilution assay were 3.18 – 7.57 μg/mL and MIC values are found to be lowest for Staphylococcus aureus and highest for Pseudomonas aeruginosa. The MIC values of lyophilized powder of shallots concentration was for Staphylococcus aureus, E. coli, Klebsiella, Proteus and Pseudomonas strains were 3.18, 3.29, 3.60, 7.33 and 7.57 μg/mL, respectively. MIC values are expressed as the median values (μg/mL) for each antibacterial agent. The values are the mean of at least three determinations. The median was calculated only on the basis of the strains susceptible, as judged by MIC; those with reduced susceptibility or resistant to lyophilized powder of shallots were excluded. The effectiveness of MIC concentrations on Klebsiella and Proteus was higher than other bacteria. There were statistically significant differences between effective of concentrations MIC Staphylococcus with Proteus, Klebsiella and Pseudomonas (table 2) (p<0.05). According to table 2 the growth of all tested microorganisms were stopped by shallots extract but Staphylococcus aureus was the most sensitive one.

The effectiveness differences MIC of lyophilized powder of shallots for bacteria tested are presented in Table 3. The most MIC among strains of Staphylococcus aureus strains, E. coli, Klebsiella, Proteus, Pseudomonas were 3 and 3.5, 3.5, 7 and 8, respectively that represents resistant of Pseudomonas against lyophilized powder of shallots.

DISCUSSION

Our study showed that the most sensitive and resistant microorganisms against lyophilized powder of shallots were Staphylococcus aureus and Pseudomonas aeruginosa, respectively. Shallots is widely consumed as a component of the diet of many populations, particularly in Asian diets. It is widely believed to be beneficial to health and even curative potential against a range of debilitating conditions and diseases (11). Shallots has impotent nutritional properties and can inhibit growth of pathogenic bacteria in food (12). Several researches showed that the shallots extract has anti-bacterial, anti-fungal, and antiprotozoan activities (10, 13-15). Our study showed that the lyophilized powder of shallots has antimicrobial properties against bacteria isolated in hospital infections and inhibit the growth of all microorganism strains. In consistence our finding, Yin et al showed that the shallots significantly inhibits nosocomial bacteria such as Staphylococcus aureus (16) and Rahbar et al showed that shallots extract has antimicrobial property against E. Coli and S. aureus (17). Aminet et al evaluated antifungal and antibacterial effects of shallots, garlic and onion extracts. In the study of them, three types of shallots extract were used, fresh, dried and autoclaved extracts, which showed that S. aureus species were more sensitive to shallots extract than other bacteria (18). In other study by Dankert et al., antimicrobial effects of shallots, garlic and onion were assessed on 5 gram-negative, 3 gram-positive bacteria and 2 yeast species (19). In our study lyophilized powder of shallots showed growth inhibitory effect on all mentioned microorganisms, while S. aureus and E. coli strains were sensitive. In the present study, various concentrations of the fresh lyophilized powder of shallots inhibit the growth of bacteria species. Possible mechanisms of antibacterial shallots are related to its antioxidant effects (20) and other compounds including compounds containing of Sulphides, e.g. dialyl sulfide, dialyl monosulfide, dialyl disulfide, Diallyl trisulfide, and diallyl tetrasulfide (21). The in vitro and in vivo
antioxidant activities of these diallyl polysulphides have been observed (22, 23). Thus, these sulphide agents may contribute to the antioxidant activity observed in shallots. Our previous works (24, 25) and other studies (26, 27) have found that these diallyl polysulphides possessed antibacterial activity against several nosocomial pathogenic bacteria and fungi. According to the fact that the shallots extracts were inhibiting growth all bacteria involved in nosocomial infection. The most important result of this study is to show that fresh lyophilized powder of shallots is effective against all microorganism tested and most sensitive was Staphylococcus aureus. We can obtain some effective antibacterial agents with minimal side effects from lyophilized powder of shallots for nosocomial infection treatment, but in vivo studies for evaluation of pharmacokinetic effects of lyophilized powder of shallots are required.

CONCLUSION

This study indicated that the antibacterial activity of lyophilized powder of shallots was strong and with differences MIC values has antibacterial activities and can inhibit growth of bacteria involved nosocomial infections. MIC values are found to be lowest for Staphylococcus aureus and highest for Pseudomonas aeruginosa. The most sensitive and resistant microorganisms against lyophilized powder of shallots were Staphylococcus aureus and Pseudomonas aeruginosa, respectively.

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References


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transcriptase-inhibiting activity from shallot bulbs.


**Table 1.** Comparison effective concentration of MIC between types of microbes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>SOS**</th>
<th>DF***</th>
<th>RMS*</th>
<th>F</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Values of MIC concentration</td>
<td>Between group</td>
<td>497.19</td>
<td>4</td>
<td>124.3</td>
<td>523.85</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Within group</td>
<td>32.98</td>
<td>139</td>
<td>0.237</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>530.18</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

**Root mean squares**, Sum of squares**, Degree of freedom***

**Table 2.** The mean effective concentration MIC of bacteria tested

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Staphylococcus aureus</th>
<th>Staphylococcus aureus</th>
<th>E. coli</th>
<th>Proteus</th>
<th>Pseudomonas</th>
<th>Klebsiella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective of MIC $</td>
<td>3.18</td>
<td>3.29</td>
<td>7.33</td>
<td>7.57</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus</td>
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<td></td>
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<tr>
<td>Pseudomonas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Based on Post Hoc test ($p<0.05$) $S$=concentration

**Table 3.** In vitro activity lyophilized powder of shallots against of bacteria spp.

<table>
<thead>
<tr>
<th>Type of bacterial</th>
<th>Effectiveness of MIC concentration</th>
<th>3</th>
<th>3.5</th>
<th>4</th>
<th>4.5</th>
<th>6</th>
<th>7</th>
<th>7.5</th>
<th>8</th>
<th>8.5</th>
<th>Number and percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>28 (66.7)</td>
<td>13 (31)</td>
<td>1 (2.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42 (100)</td>
</tr>
<tr>
<td>E. coli</td>
<td>18 (46.2)</td>
<td>19 (48.7)</td>
<td>2 (5.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39 (100)</td>
</tr>
<tr>
<td>Proteus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11 (25.5)</td>
<td>5 (25)</td>
<td>4 (20.4)</td>
<td>0</td>
<td>0</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 (21.1)</td>
<td>1 (5.3)</td>
<td>3 (15.8)</td>
<td>15 (15.8)</td>
<td>8</td>
<td>19 (100)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>4 (17.4)</td>
<td>11 (47.8)</td>
<td>7 (30.4)</td>
<td>1 (4.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
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