

## CHARACTERIZATION OF SHALLOT, AN ANTIMICROBIAL EXTRACT OF ALLIUM ASCALONICUM

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### ABSTRACT

**Objective:** The objective of this study was characterization of antimicrobial extract of shallot in terms of its stability at different pH, Heat, enzymes and detergents and also determination of its MIC and shelf life.

**Methodology:** Active fraction was determined by column chromatography and agar diffusion test. The amount of carbohydrate and protein in different forms of shallot extract were estimated. Stability of antimicrobial activity of shallot extract at different pH and temperature, solubility in different solvent, determination of shelf life and susceptibility to enzymes and detergents were evaluated.

**Results:** Shallot extract was active against microbes at pH 4-8. Relative activities of shallot extract at temperature -7 to 121°C were 88 to 100 %. Extract of shallot only was soluble in dimethyl sulphoxide, dimethyl formamide and water. The enzymes and detergents used in this study had no effect on antimicrobial activity on water extract of shallot. Relative antimicrobial activity at incubation times of one hour to 6 months were 94 to 100 %.

**Conclusion:** In this study antimicrobial properties of shallot were investigated for discovery of a new antibiotic. Based on this the antimicrobial compound can be an effective medicine for treatment of dermatomycosis and other infectious diseases.

**KEY WORDS:** *Allium ascalonicum*, antimicrobial, MIC, characterization.

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### INTRODUCTION

Due to emergence of resistance among microorganisms to antibiotics, investigation for novel antimicrobial agents has always been one of the major preoccupations of medical society. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since late 19<sup>th</sup> century have documented the antimicrobial properties of some spices, herbs, and their components.<sup>1,2</sup> Studies in the past decade confirm that the growth of both gram positive and gram

negative foodborne bacteria, yeasts and molds can be inhibited by garlic, onion, cinammon, cloves, thyme, sage, and other spices. Effects of the presence of these spices/ herbs can be seen in food products such as pickles, bread, rice and meat products. The fat, protein, water, and salt content of food, influence the microbial resistance. Thus, it is observed that higher levels of spices are necessary to inhibit growth in food than in culture media.<sup>1</sup>

Present study is first report of the characterization of antimicrobial extract of *Allium ascalonicum* (shallot). Shallot is a member of family Liliaceae. The genus *Allium* consists of more than three hundred different species<sup>3</sup>, but only two species, *Allium sativum* (garlic) and *Allium cepa* (onion), are well-known remedies in the prevention and treatment of different diseases.<sup>4,5</sup>

Shallot is a native of Palestine and is cultivated in USA and some European countries. It is commonly used as a folk-lore medicine, and used to cure earache, fever, antidote for snake venom and also as an aphrodisiac.<sup>6</sup> In our last study the antimicrobial activity of shallot extract had been investigated. Most of the fungi were relatively more sensitive than bacteria to extract of shallot. No obvious differences between the sensitivity of gram negative and gram positive bacteria, yeasts and filamentous fungi, were observed.<sup>7</sup> Although, these findings are in contrast with the results reported by Dankert et al<sup>8</sup> who found that crude juice of shallot has no activity against gram negative bacteria. The objective of this study was characterization of antimicrobial extract of shallot in terms of its stability at different pH, Heat, enzymes and detergents and also determination of its MIC and shelf life.

## METHODOLOGY

*Extraction of antimicrobial water extract from shallot:* The water extracts of underground bulbs of shallot was prepared by suspending 200g bulbs of shallot in 100ml distilled water and homogenized in mixer. After five hours, the extracts were filtered through whatman number one filter paper. The filtrate of extract was dried at 50°C in oven, redissolved in water and used for column chromatography.

*Fractionation of shallot extract by silica gel column chromatography:* For establishment of column chromatography, suspension of 10 gram silica gel (60-120 mesh) in 100ml of distilled water and ethanol (50:50) was prepared and it was slowly poured and packed in a glass column. The diameter and height of the tube were 2cm and 35cm respectively. A small piece of glass wool was used in the bottom of the tube to support the silica. 2 ml of water extract of shallot (500mg ml<sup>-1</sup>) was used for loading the column. As soon as the sample passes through the column, the particular solute was removed by amount of solvent (distilled water and ethanol) from the column.<sup>9</sup> The flow rate was 1 ml per 30 minutes. 40 fractions of one ml were collected in small glass tubes. An agar diffusion assay was used for evaluating antimicrobial activity of all fractions against *Bacillus cereus*.

*Determination of minimal inhibitory concentration by E test:* The E test has been proved to be remarkably flexible method for determining MIC of a wide range of antimicrobial agents.<sup>10</sup> Microbial suspensions of freshly grown cultures were prepared in sterile saline and adjusted to a density of 10<sup>6</sup> spore /cell ml<sup>-1</sup>, corresponding to 68 to 82 % transmittance at 530 nm.<sup>11</sup> The plates, Sabouraud Dextrose Agar, Hi Media, (SDA) for fungi and Mueller Hinton Agar, Hi Media (MHA) for bacteria were inoculated by dipping a sterile cotton swab into the cell suspension and streaking it across the surface of the agar in three directions. The plates were dried at ambient temperature for 15 minutes before applying the discs. Eight sterile discs (diameter 6 mm) were kept on the agar surface in a line. The water extract of shallot was serially diluted in water; and 10 µl of each dilution was separately used to impregnate the disc. The plates were incubated for 48 hours at 28° C for *A. niger* and *Trichophyton rubrum* and 18 hours at 37° C for others. The MIC values were read as the antimicrobial concentration at the point where dense colonial growth intersected the disc, ignoring sparse subsurface hyphae at the margins. Microcolonies within the eclipse were ignored.<sup>12</sup> The test was performed in quadruplicate for each culture.

*Stability of antimicrobial activity of water extract of shallot at different pH:* Antimicrobial activity of water extract of shallot was evaluated at different pH values, by diffusion test. The pH of the extract was varied, using the following buffers: acetate buffer (pH4.0, 5.0) phosphate buffer (pH6.0, 7.0, 8.0), tris - hydrochloride buffer (9.0) and carbonate – bicarbonate buffer (pH 10.0).<sup>13</sup> The pH stability of the extract was evaluated by agar diffusion testing on MHA media against *Bacillus cereus*. 0.5 ml of extract (200 mg ml<sup>-1</sup>) was added in the tubes containing 0.5 ml of different buffers.<sup>13</sup> After 1hour incubation at 37°C, 20 ml was impregnate in blank disc on MHA.

*Heat stability of antimicrobial activity of water extract of shallot:* The effect of temperature on extract of shallot in liquid form was determined by agar diffusion test. 20 mg ml<sup>-1</sup> concentration of shallot extract was prepared and divided into 5 tubes. The tubes were incubated at -7°C for 24 h, 4° C for 24 h, 22° C for 24 h, 100° C for 30 minutes. and 121° C for 20 minutes. Then 10 ml of each tube was impregnated in blank discs on MHA that previously seeded with *Bacillus cereus*.

*Susceptibility of water extract of shallot to enzymes and detergents:* Susceptibility to denaturation by detergents and enzymes was studied by treating the shallot extract with cetrimide, tween-20, tween-80, sodium dodecyl sulphate, triton x-100 and sodium lauryl sulphate as detergents and trypsin, pepsin, lipase, and amylase as enzymes. One ml of detergents was added to shallot extract at concentrations of 10<sup>-2</sup> M of detergent ml<sup>-1</sup> shallot extract containing 200 mg dried

Table-I: Susceptibility of microbes to water extract of shallot determined by E test

| Bacteria and Fungi  | MIC (µg/ml) |
|---------------------|-------------|
| <i>S. aureus</i>    | 75          |
| <i>B. subtilis</i>  | 38          |
| <i>T. rubrum</i>    | 156.2       |
| <i>E. coli</i>      | 156.2       |
| <i>A. niger</i>     | 62.5        |
| <i>C. humicolus</i> | 156.2       |
| <i>S. typhi</i>     | 78.1        |

extract ml<sup>-1</sup> distilled water. These preparations were incubated at 30°C for six hour and tested for antimicrobial activity.<sup>14</sup>The diffusion test was used as described previously. A water solution of extract of shallot was used as control.

*Determination of shelf life of shallot extract:* The effect of incubation time on antimicrobial activity of water extract of shallot was determined by keeping the extract at 4° C in different tubes, for 1 hour, 24 hour, one week, one month, two month, three month and six month. After specified incubation period, the antimicrobial activity of the extract was tested by disc diffusion against *B. subtilis*. All experiments mentioned above were performed in four folds.

## RESULTS

*Minimal inhibitory concentration of water extract of shallot by E test:* Susceptibility of seven microorganisms to water extract of shallot was determined by E test. All microorganisms tested were sensitive to the shallot extracts. *B. subtilis* was more sensitive to extract than others (MIC 38µg ml<sup>-1</sup>) (Table-I).

*Antimicrobial activity and purity of silica gel column fractions:* All the fractions obtained by column were tested by disk diffusion assay. The fractions 12-15 showed more activity than others. TLC was performed for checking the

Table-II: Stability of antimicrobial activity of shallot extract at different pH

| pH | Inhibition zone of extract (mm) | Relative activity (%) |
|----|---------------------------------|-----------------------|
| 4  | 15                              | 88                    |
| 5  | 15                              | 88                    |
| 6  | 17                              | 100                   |
| 7  | 17                              | 100                   |
| 8  | 14                              | 82                    |
| 9  | 10                              | 58                    |
| 10 | 8.0                             | 47                    |
| 11 | 8.0                             | 47                    |

\* Inhibition zone of different original buffers for pH 4-9 was zero and for pH10-11 was 8.0.

\* Inhibition zone for original extract (pH 6.5) was 17.

Table-III: Effect of temperature on antimicrobial activity of shallot extract

| Temperature C° | Inhibition zone of extract (mm) | Relative activity (%) |
|----------------|---------------------------------|-----------------------|
| 0.0            | 13                              | 100                   |
| 4.0            | 13                              | 100                   |
| 20             | 13                              | 100                   |
| 40             | 13                              | 100                   |
| 60             | 13                              | 100                   |
| 80             | 13                              | 100                   |
| 100            | 13                              | 100                   |
| 121            | 13                              | 100                   |

purity of bioactive fractions. All the four antimicrobial active fractions showed single spot on TLC plate at similar locations (Rf was 0.80), which were developed by iodine. No spots were detected by ninhydrin.

*Stability at different pH:* Antimicrobial activity of extract of shallot was stable at different pH ranging from 4 to 8. But at high alkaline pH (9-11) the stability was reduced. The diameter of inhibition zone at pH 4 was 15 mm, at pH 7 was 17mm and at pH 11 was 8 (Table-II).

*Stability of shallot extract at different temperatures:* The extract of shallot was stable at different temperatures. There was no difference between diameters of inhibition zones caused by autoclaved and frozen (15mm) water extract of shallot (Table-III).

*Susceptibility to enzymes and detergents:* The enzymes and detergents used in this study had no effect on antimicrobial activity on water extract of shallot. There was no significant difference between inhibition zone of control (water extract of shallot) and extract treated with enzymes and detergents (Table-IV).

*Determination of shelf life of shallot extract:* The antimicrobial activity of the shallot extract was stable at 4° c for 1 h till 6 months (Table-V).

## DISCUSSION

To our knowledge there have been few reports detailing the investigation of antimicrobial compound in shallot bulbs. Wang and Ng (2002) isolated an anti-fungal peptide from bulbs of

Table-IV: Inhibition of growth of *Bacillus subtilis* by extract of shallot treated with enzymes and detergents

| Detergent and enzymes   | Inhibition zone | Relative activity (%) |
|-------------------------|-----------------|-----------------------|
| Cetramide               | 10              | 83                    |
| tween-20                | 12              | 100                   |
| tween-80                | 11              | 91                    |
| sodium dodecyl sulphate | 12              | 100                   |
| triton x-100            | 11              | 91                    |
| sodium lauryl sulphate  | 12              | 100                   |
| trypsin                 | 12              | 100                   |
| pepsin                  | 12              | 100                   |
| lipase                  | 11              | 91                    |
| amylase                 | 12              | 100                   |
| water (control)         | 12              | 100                   |

shallot. This peptide inhibited mycelial growth in the fungus *Botrytis cinerea* but not in the fungi *Mycosphaerella arachidicola* and *Fusarium oxysporum*.<sup>15</sup>

Dankert et al<sup>8</sup> have evaluated the antimicrobial activity of garlic, onion and shallot using crude juices of bulbs of these plants. Fattorusso et al<sup>16</sup> also have done an extensive phytochemical analysis on the polar extracts from bulbs of shallot. These researchers isolated two new furostanol saponins but there is no antimicrobial investigation in their report. Mo et al<sup>17</sup> have isolated a new mannose-binding lectin from shallot bulbs by affinity chromatography on an

Table-V: Determination of shelf life of shallot extract at 4° C by diffusion test against *B subtilis*

| Incubation time | Inhibition zone (mm) | Relative antimicrobial activity (%) |
|-----------------|----------------------|-------------------------------------|
| 1 h             | 17                   | 100                                 |
| 24 h            | 17                   | 100                                 |
| 1 week          | 16                   | 94                                  |
| 1 month         | 16                   | 94                                  |
| 2 months        | 16                   | 94                                  |
| 3 months        | 16                   | 94                                  |
| 6 months        | 16                   | 94                                  |

immobilized D-mannose column. In this investigation there was no antimicrobial study done on the new lectin.

Regarding great antimicrobial activity of shallot extract against wide spectrum of pathogenic and non-pathogenic microorganisms, water was a good solvent that isolated a maximum amount of antimicrobial compound from fresh shallot bulbs.

Antimicrobial compound of water extract of shallot was very heat stable and active over a wide range of pH. Antimicrobial principle of shallot was resistance to and active under both acid pH and basic pH. Different temperatures did not show significant effect on antimicrobial activity of shallot extract. These results increase the chance of utilization of shallot extract as a natural preservative at different temperatures from -7C - 121C. However garlic has been reported for using as a natural preservative in camel meat<sup>18</sup>, but the instability of garlic products is a problem in this way.

Proteolytic enzymes such as trypsin and pepsin did not destroy the antimicrobial activity of shallot extract. These results prove that the active fraction of this plant is not a protein. Treatment with lipase also did not cause any loss in antimicrobial activity, probably because of the absence of lipid moieties in the principle of antimicrobial agent. It seems that shallot extract can be used as a prophylactic antimicrobial agent.

In conclusion the shallot extract is a powerful antimicrobial agent and is stable at different pH, temperatures, enzymes and detergents. It suggested using this extract as a food preservative and local ointment for skin infections.

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