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# Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*)

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**Abstract**

Antimicrobial activity of different concentrations (50, 100, 200, 300 and 500 ml/l) of essential oil extracts of three type of onions (green, yellow and red) and garlic against two bacteria, *Staphylococcus aureus*, *Salmomella* Enteritidis, and three fungi, *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*, was investigated. The essential oil (EO) extracts of these *Allium* plants (garlic and onions) exhibited marked antibacterial activity, with garlic showing the highest inhibition and green onion the lowest. Comparatively, 50 and 100 ml/l concentrations of onions extracts were less inhibitory than 200, 300 and 500 ml/l concentrations. However, with garlic extract, high inhibitory activity was observed for all tested concentrations. *S. aureus* showed less sensitivity towards EO extracts inhibition, however *S.* Enteritidis was strongly inhibited by red onion and garlic extracts. The fungus *F. oxysporum* showed the lowest sensitivity towards EO extracts, whereas *A. niger* and *P. cyclopium* were significantly inhibited particularly at low concentrations. Conclusively, where seasoning is desired, essential oil extracts of onions and garlic can be used as natural antimicrobial additives for incorporating in various food products.
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*Keywords:* Essential oils; Inhibition; *Allium cepa*; *Allium sativum*; Bacteria; Fungi

## 1. Introduction

Onion and garlic may be among the first cultivated crops in the world due to their long storage time and portability. They could be dried and preserved for several months. At the present time, the *Allium* family has over 500 members, each differing in appearance, color and taste, but close in biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess antibacterial and antifungal activities, and contain the powerful sulfur and other numerous phenolic compounds which arouse great interest (Rivlin, 2001; Griffiths, Trueman, Crowther, Thomas, & Smith, 2002). Onions and garlic are composed mainly of water (85–90 g/100 g and 60–70 g/100 g fresh weight, respectively) and the most significant components, medicinally, are the organosulfur-containing compounds. However, garlic contains nearly three times as much sulfur-containing compounds as onions (11–35 mg/100 g fresh weight) (Lawson, 1996). The mature, intact *Alliums* contain mainly cysteine sulfoxides, and when tissues are chopped, the enzyme allinase is released, converting the cysteine sulfoxides into the thiosulfinates. These compounds are reactive, volatile, odor producing and lachrymatory (Block, Naganathan, Putman, & Zhao, 1992). In addition to their nutritional effects, the antibacterial and antifungal activities against a variety of Gram-negative and Gram-positive were, and continue to be extensively investigated (Whitemore & Naidu, 2000). Han, Lawson, Han, and Han (1995) reported that the antibiotic activity of 1 mg of allicin, which is a (+)-*S*-methyl-L-cysteine sulfoxide, has been equated to that of 15 IU of penicillin. Recent investigations have also demonstrated an inhibitory effect by aqueous extracts on numerous bacterial and fungal species (Sivam, Lampe, Ulness, Swanzy, & Potter, 1997; Phay et al., 1999; Hsieh, Mau, & Huang, 2001; Ward, Fasitsas, & Katz, 2002). During the last 50 last years, protection of food from spoilers and pathogens aroused great interest and was achieved by various physical and

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chemical methods. Among these numerous and abundant naturally occurring compounds, essential oil extracts have been considered as natural preservatives or food additives, and can be used as additional methods of controlling pathogens (Naidu, 2000). The aim of this investigation was to study the effect of essential oils extracts of various onion types and garlic on two major bacterial pathogens, and three fungal species usually causing rotting of *Allium* crops during their storage.

## 2. Materials and methods

### 2.1. Onions and garlic

Three type of onions (*Allium cepa*), green onion (var. Blanc), yellow (var. Jaune d'Espagne) and red (var. Rouge Amposta), and garlic (*Allium sativum*) (var. Cristo), were selected for this investigation. Onions and garlic were cultivated in Mascara region, and are free of any pre-harvest chemical treatment (organic products). Onions and garlic samples freshly harvested were sorted for uniformity and absence of defects and stored at 4°C prior analyses.

### 2.2. Microbial strains

The microorganisms, maintained on Nutrient Agar (Merck, Darmstadt, Germany), were supplied by the microbiology laboratory of the university. The bacteria were selected because they are frequently reported in food spoilage, while the selected fungi are commonly encountered in onions and responsible for bulb diseases. Two species of bacteria, *Staphylococcus aureus* (ATCC 11522) and *Salmonella* Enteritidis (ATCC 13076) and three species of fungi, *Aspergillus niger* (ATCC 10575), *Penicillum cyclopium* (ATTC 26165) and *Fusarium oxysporum* (ATCC 11850), were used in this study.

### 2.3. EO extraction

Essential oils were extracted by steam distillation, and all operations were carried out at room temperature. Samples (200 g) were chopped in small pieces, homogenized in 200 ml distilled water using a domestic blender (model MX-X61-W, National, Japan) during 1 min at medium speed, then homogenate was macerated during 1 h. The essential oils were extracted by steam distillation using a vertical steam distillation unit. A flask (2 l) containing the homogenate was heated during 3 h and the vapor condensed and separated throughout an auto-oil/water separator. Then, to obtain a final yield of extraction (ratio final volume of extract/weight of fresh plant) of 0.5, the volume of EO extract was adjusted to 100 ml with sterile distilled water, thus obtaining the crude essential oil extracts used for antimicrobial tests. Each EO extraction was running in duplicate.

### 2.4. Preparation of inoculum

Bacteria inocula were prepared by growing cells in Brain–Heart Infusion broth (Merck) for 24 h at 37°C. These cell suspensions were diluted with peptone water (Institut Pasteur, Algiers, Algeria) to provide initial cell counts of about $10^5$–$10^6$ CFU/ml. An aliquot of 1 ml is used for antimicrobial test. Fungi were cultured on YGCA medium (Merck), and a mycelia mass of 5 mm of diameter was used for antifungal test.

### 2.5. Antibacterial activity test

The antibacterial activity of the extracts was carried out by disc diffusion test (Kim, Marshall, & Wei, 1995). The concentrations tested were 50, 100, 200, 300 and 500 ml/l. Appropriate volume of EO extracts were added to sterile water (vol/vol) to obtain desired concentrations cited above. The Petri dishes containing Potato Dextrose Agar (PDA) medium (Merck) were used for antibacterial test. An aliquot of 1 ml was evenly spread on agar using a glass rod spreader. The Petri dishes were left at room temperature for 1 h to allow agar surface to dry. Sterile filters paper (Wathman No. 1, diameter 5 mm) were impregnated with EO extracts of different concentrations and placed on the culture medium (PDA). For control, discs were impregnated with sterile water. After 30 min, plates were turned upside down and incubated at 37°C for 48 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antimicrobial activity. Five discs per plate and three plates were used, and each test was run in triplicate.

### 2.6. Antifungal activity test

The antifungal activity was carried out in vitro, in Petri dish containing Yeast Glucose Chloramphenicol Agar (YGCA, Merck) (Lattenzio, De Cicco, Di Venere, Lima, & Salerno, 1994). The concentrations tested were the same as described above, but EO extracts were added to YGCA medium. For control tests, sterile water (50, 100, 200, 300 and 500 ml/l, vol/vol) was added to YGCA medium. Then the fungi were inoculated immediately after preparation of the Petri dishes by placing in the center of each plate a 5 mm diameter of the mycelial mass of the cultivated test fungi, cut with a sterile cork borer from the periphery of growing cultures on YGCA plates prepared as described above. The Petri dishes were incubated in dark at 21°C and the diameter of the mycelial growth was measured. The incubation was stopped when the mycelial mass of control Petri dishes had almost filled the Petri dish (ca. 12–13 days).

Diameter of the growth mass was determined by averaging the radial growth of the mycelial mass in two orthogonal directions. Each test was run in triplicate.

### 2.7. Statistical analysis

All experiments were conducted in triplicate and tests were duplicated (two extractions). Experiment was conducted twice, and data were averaged and analysed statistically by determination of least significant difference (LSD at $\rho < 0.05$) using XLStat. Pro® statistical software (XLStat, Paris, France).

## 3. Results and discussion

### 3.1. Antibacterial activity of EO extracts

Onions and garlic EO extracts exhibited different inhibition levels against *S. aureus* and *S.* Enteritidis as shown in Tables 1 and 2. In the dose response study, the inhibition zone increased with increasing concentration of extracts. Low concentrations (50 and 100 ml/l) inhibited weakly the development of bacteria; however *S.* Enteritidis was more sensitive than *S. aureus*. At high concentrations (200, 300 and 500 ml/l), EO extracts exhibited marked inhibition activity against bacteria, and inhibition of EO extract of garlic was strongest than those of onions EO extracts. Comparatively, *S. aureus* was less sensitive to the inhibitory activity of the onions and garlic extracts than *S.* Enteritidis which was more inhibited at same concentrations of EO extracts. The inhibitory activity of essential oils extracts of onions or other *Allium* plants was not extensively reported except garlic extract which was widely investigated. On the other hand, *S. aureus* was extensively studied and its sensitivity to essential oils extracts was widely discussed. Kyung, Kim, Park and Kim (2002) reported that allicin of garlic extract showed strong antibacterial activity against *S. aureus* at 150 ml/l concentration. This antibacterial activity was enhanced and highest when garlic extract was heated for 45 min at 121°C. The antibacterial activity of other and close, chemically, cysteine sulfoxides (S-methyl-L-CS and methyl methane-CS) of cabbage also was markedly observed, particularly concentrations of 10, 20 and 50 mg/l (Kyung, Han, & Fleming, 1997). Alrozeky and Nakahara (2002) reported weak antibacterial activity of extracts from some edible plants commonly consumed in Asia. Combined extracts of corni fructus, cinnamon and Chinese chive (1:6:6, vol/vol/vol) exhibited low inhibitory effect against this bacteria than other combined ratios and against other bacterial species (Hsieh et al., 2001). Although the paper disk assay is a practical approach to study potential antibacterial compounds, using the size of inhibition zone to indicate relative antibacterial activity of the essential oils is not adequate. The zone must be affected by the solubility and rate of diffusion in agar medium or its volatilization; and thus the results could be affected.

Table 1
Antibacterial activity of essential oil extracts of *Allium* plants against *Staphylococcus aureus* after 48 h

| | EO concentration (ml/l) | | | | |
|---|---|---|---|---|---|
| | 50 | 100 | 200 | 300 | 500 |
| Green onion | 5.6±0.2 | 6.3±0.7 | 6.8±1.1 | 7.6±0.5 | 8.6±0.5 |
| Yellow onion | 6.1±0.7 | 7±0.3 | 7.1±1.4 | 7.3±1.8 | 7.5±0.4 |
| Red onion | 5.9±0.3 | 7.3±0.2 | 7.6±0.5 | 8.2±0.3 | 8.8±0.7 |
| Garlic | 6.3±0.4 | 7.6±0.8 | 7.9±0.9 | 8.5±0.7 | 9.3±0.2 |

Zone of inhibition is expressed in mm.

Table 2
Antibacterial activity of essential oil extracts of *Allium* plants against *Salmonella* Enteritidis after 48 h

| | EO Concentration (ml/l) | | | | |
|---|---|---|---|---|---|
| | 50 | 100 | 200 | 300 | 500 |
| Green onion | 7.1±0.7 | 7.3±0.1 | 8.1±0.7 | 8.8±1.7 | 8.9±0.9 |
| Yellow onion | 7.1±0.2 | 8.1±0.7 | 7.5±0.4 | 7.6±0.5 | 9.8±0.4 |
| Red onion | 7.8±1.9 | 9.1±0.7 | 9.3±0.2 | 10.3±0.2 | 11.1±0.4 |
| Garlic | 8.3±0.5 | 9.3±0.1 | 9.9±0.5 | 11.3±1.0 | 13.1±0.2 |

Zone of inhibition is expressed in mm.

### 3.2. Antifungal activity of EO extracts

Antifungal activity of EO extracts on *A. niger*, *P. cyclopium* and *F. oxysporum* are shown in Figs. 1–3. In dose response study, *A. niger* was less inhibited by low concentrations (50 and 100 ml/l) of EO of green and yellow onions. However, higher concentrations exhibited marked inhibition. On the other hand, all concentrations of red and garlic EO showed strong inhibitory effect against *A. niger* and developments were close despite low concentrations. Statistically, no significant difference was observed between control and concentrations 50 and 100 ml/l of EO extracts of green and yellow onions. Nevertheless, concentrations 200, 300 and 500 ml/l of green and yellow onions, and all concentrations of red onion and garlic were significantly different. The sensitivity of *P. cyclopium* to EO extracts of onions and garlic was close to those of *A. niger*. However, EO extract of red onion showed less inhibitory effect against *P. cyclopium* at low concentrations (50 and 100 ml/l) (Fig. 2). On the other hand, similar and strong inhibitory effect of EO extract of garlic on *P. cyclopium* was observed and development was very low compared to control or other EO extracts. Statistical analysis showed that 50 and 100 ml/l concentrations of green,

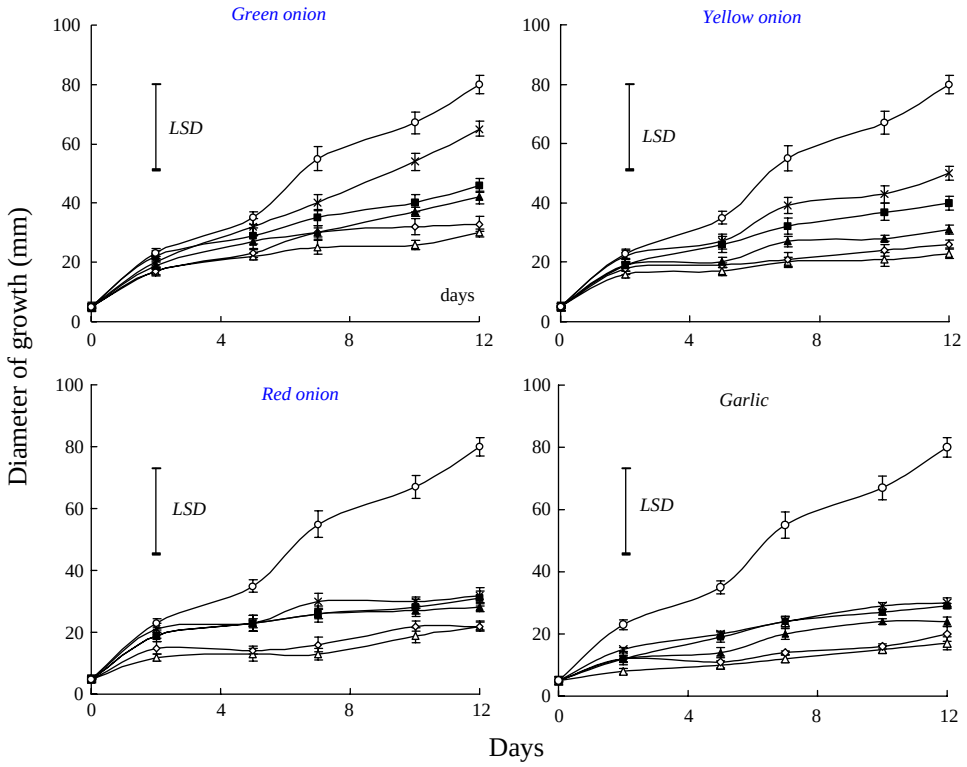


Fig. 1. Effect of EO extracts of onion and garlic on growth of *A. niger* (○ Control, × 50, ■ 100, ▲ 200, ◇ 300 and △ 500 ml/l concentrations) (LSD at $\rho < 0.05$).

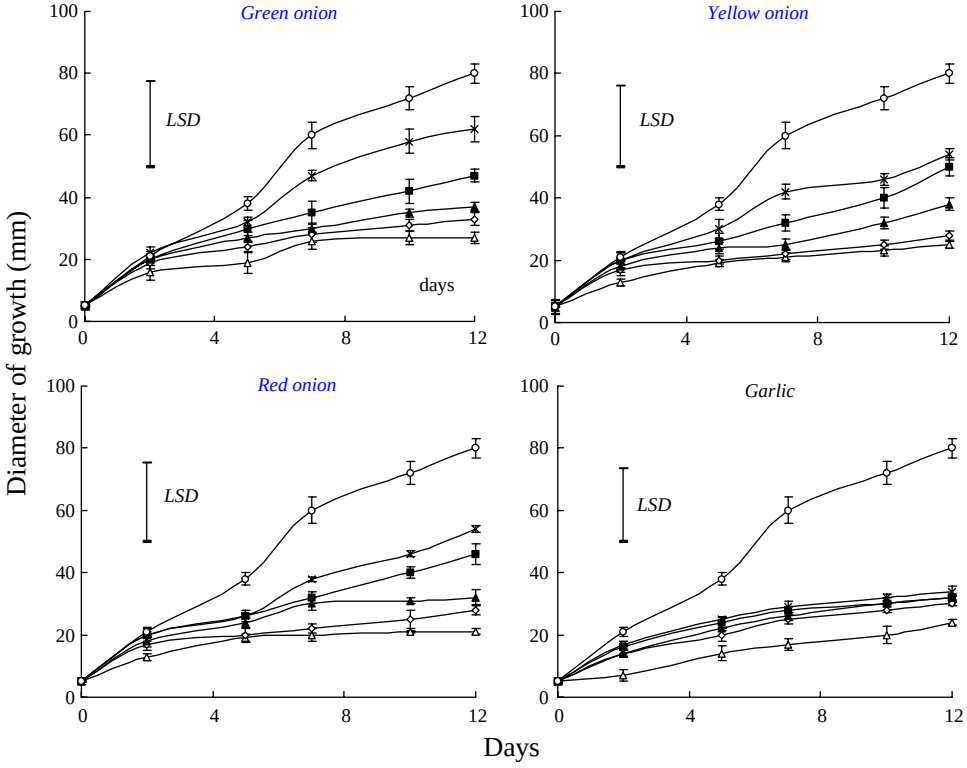


Fig. 2. Effect of EO extracts of onion and garlic on growth of *F. oxysporum* (○ Control, × 50, ■ 100, ▲ 200, ◇ 300 and △ 500 ml/l concentrations) (LSD at $\rho < 0.05$).

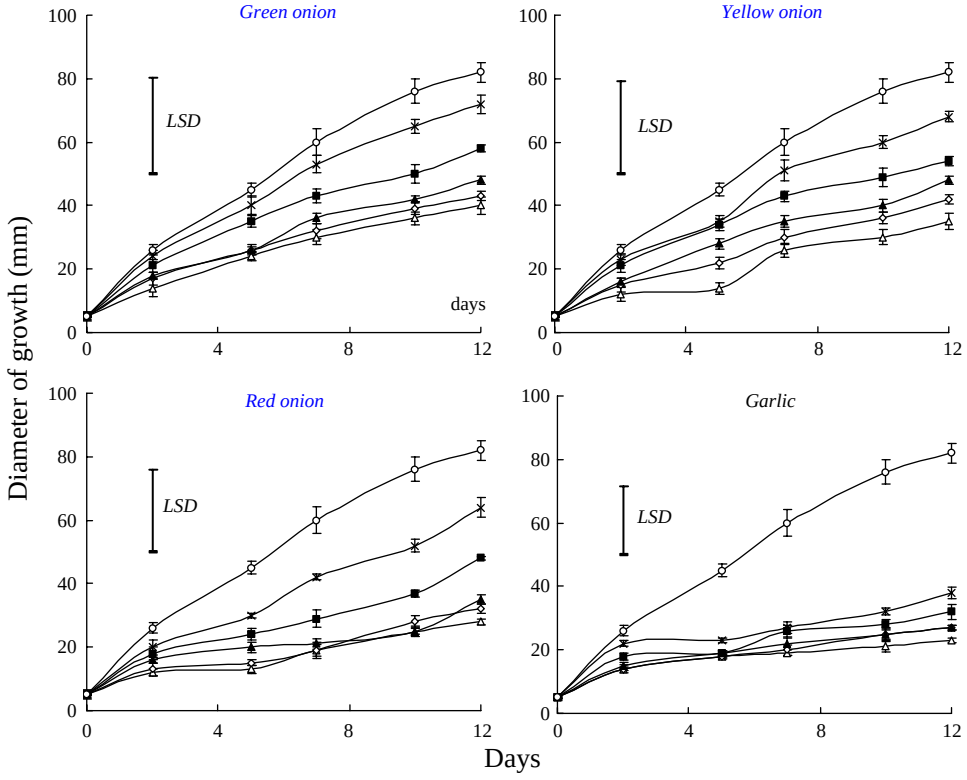


Fig. 3. Effect of EO extracts of onions and garlic on growth of *P. cyclopium* (○ Control, × 50, ■ 100, ▲ 200, ◇ 300 and △ 500 ml/l concentrations) (LSD at $\rho < 0.05$).

yellow and red onions were not significantly different. On the other hand, concentrations 200, 300 and 500 ml/l of EO Onions extracts, and all concentrations of EO extracts of garlic were significantly different. *F. oxysporum* showed the lowest sensitivity to green, yellow and red EO extracts except 300 and 500 ml/l concentrations of red onion which inhibited markedly its development (Fig. 3). On the other hand, similar strong inhibitory activity of EO extracts of garlic was noted. The concentrations of 50, 100 and 200 ml/l of green and yellow onions were not significantly different. However, concentrations 300 and 500 ml/l were significantly different. The similar highly significant difference was observed between control and EO extracts of garlic against *F. oxysporum*. The inhibitory activity of EO extracts of *Allium* plants against mould was reported by numerous authors; however, essential oils are in general more effective inhibitors of fungi than of bacteria (Zaika, 1988). Antifungal activity of seven *Allium* plants was reported by Yin and Tsao (1997). These authors observed that garlic showed highest antifungal activity against three *Aspergillus* species tested. Fistulosin, an antifungal compound isolated from roots of welsh onion exhibited marked antifungal activities against several fungal species particularly *P. roqueforti* and *A. oryzae* which showed high sensitivity (Phay et al., 1999). Hsieh et al. (2001) noted high sensitivity of *A. niger* to the combined extract of corni fructus, cinnamon and Chinese chive (1:6:6, vol/vol/vol).

Finally, it is concluded from the results of this investigation that essential oils extracts of common onions and garlic were found to inhibit bacterial and mould growth. However, the effectiveness of this inhibition was strongly related to the type of onions or garlic extracts used. The results also showed that a significant ($\rho < 0.05$) effect was obtained when concentrations of essential oils extracts were above 10%. On the other hand, it will also be of interest to examine the different compounds of the extracts and their antimicrobial activities against a wide range of a commonly food contaminating bacteria and fungi to maintain their qualities. However, despite the fact that *Allium* plants could be used as a potential source for inhibiting, to date unfortunately, the relative biochemical instability of the alliicin, thiosulfonates and related compounds, and the strong odor seem to have limited their use as practical food additive or preservative.

## References

Alrozeky, N. S., & Nakahara, K. (2002). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology*, *80*, 223–230.

Block, E., Naganathan, S., Putman, D., & Zhao, S. H. (1992). *Allium* chemistry: HPLC analysis of thiosulfonates from onion, garlic, wild garlic, leek, scallion, shallot, elephant garlic, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *Journal of Agriculture and Food Chemistry*, *40*, 2418–2430.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions—a global benefits to health. *Phytotherapy Research*, *16*, 603–615.

Han, J., Lawson, L., Han, G., & Han, P. (1995). A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. *Annals of Biochemistry*, *225*, 157–160.

Hsieh, P. C., Mau, J. L., & Huang, S. H. (2001). Antimicrobial effect of various combination of plant extracts. *Food Microbiology*, *18*, 35–43.

Kim, J., Marshall, M. R., & Wei, C. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agriculture and Food Chemistry*, *43*, 2839–2845.

Kyung, K. H., Han, D. C., & Fleming, H. P. (1997). Antibacterial activity of heated cabbage juice, S-methyl-L-cysteine sulfoxide and methyl methanethiosulfonate. *Journal of Food Science*, *62*, 406–409.

Kyung, K. H., Kim, M. H., Park, M. S., & Kim, Y. E. (2002). Alliinase-independent inhibition of Staphylococcus aureus B33 by heated garlic. *Journal of Food Science*, *67*, 780–785.

Lattenzio, V., De Cicco, V., Di Venere, D., Lima, G., & Salerno, M. (1994). Antifungal activity of phenolics against fungi commonly encountered during storage. *Italian Journal of Food Science*, *1*, 23–30.

Lawson, L. D. (1996). Garlic: a review of its medicinal effects and indicated active compounds. In L. D. Lawson, & R. Bauer (Eds.), *Phytomedicines of Europe: Their chemistry and biological activity* (pp. 176–209). Washington DC: ASC Press.

Naidu, A. S. (2000). Natural food antimicrobial systems. Boca Raton, FL: CRC Press.

Phay, N., Higashiyama, T., Tsuji, M., Matsuura, H., Fukushi, Y., Yokota, A., & Tomita, F. (1999). An antifungal compound from roots of welsh onion. *Phytochemistry*, *52*, 271–274.

Rivlin, R. S. (2001). Historical perspective on the use of garlic. *Journal of Nutrition*, *131*, 951S–954S.

Sivam, G. P., Lampe, J. W., Ulness, B., Swanzy, S. R., & Potter, J. D. (1997). *Helicobacter pylori*—in vitro susceptibility to garlic (*Allium sativum*) extract. *Nutrition and Cancerology*, *27*, 118–121.

Ward, P. M., Fasitsas, S., & Katz, S. E. (2002). Inhibition, resistance development, and increased antibiotic and antimicrobial resistance caused by neutraceuticals. *Journal of Food Protection*, *65*, 528–533.

Whitemore, B. B., & Naidu, A. S. (2000). Thiosulfinates. In A. S. Naidu (Ed.), *Natural food antimicrobial systems* (pp. 265–380). Boca Raton, FL: CRC Press.

Yin, M. C., & Tsao, S. M. (1997). Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. *International Journal of Food Microbiology*, *49*, 49–56.

Zaika, L. L. (1988). Spices and herbs: Their antimicrobial activity and its determination. *Journal of Food Safety*, *9*, 97–118.