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# Microbial Ecology of Dental Plaques of Jordanian Patients and Inhibitory Effects of *Allium sativum* and *Allium cepa* L. Extracts

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The present research aimed to study the inhibitory effects of garlic (*Allium sativum*) and onion (*Allium cepa* L.) extracts on microorganism isolated from dental caries plaques of Jordanian patients. Dental caries plaques were collected in a private clinic then processed and worked upon in a university laboratory. Dental plaques from 91 patients were obtained and grown on plates following standard protocols. A total of 116 isolates were grown. *E. coli, Lactobacillus acidophilus, Candida albicans, Streptococcus mitis, Actinomyces naeslandii, Enterococcus faecalis* and *Streptococcus oralis* were decreasingly the most frequent organisms. Standard and different dilutions of garlic and onion extracts were tested against those isolated microorganisms. Median values for zones of inhibitions were calculated and blotted. Garlic extracts consistently showed significantly higher inhibitory effects than onion extracts. This study concludes that Garlic extracts do have significant inhibitory effects against microorganisms associated with dental caries and further studies are needed to explore potential therapeutic uses for such extracts. It also showed that microorganisms associated with dental caries of Jordanian patients are similar to those encountered in other different areas of the world.

**Key words:** Garlic, onion, plant, extract, *Allium sativum*, *Allium cepa* L.

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## INTRODUCTION

Dental caries is a very common problem that affects all age groups. It is a process in which the enamel and the dentine, are demineralized by acids produced by bacterial fermentation of carbohydrates (Soet and Graaff, 1998). In real life, it is the most common infectious disease affecting human beings (Balakrishnan *et al*., 2000). Mutans streptococci (*Streptococcus mutans and Streptococcus sobrinus*) are the most common organisms that are associated with dental caries (Hanada, 2000). *Lactobacilli* are also common important players in the pathogenesis of dental caries (Soet and Graaff, 1998). Nikawa *et al.* (1998) have also shown that *Candida albicans* colonizing the oral cavity can also cause dental caries. Many studies have been conducted trying to modulate the interactions of different factors that facilitate the effect of above and other organisms in the pathogenesis of dental caries (Balakrishnan *et al*., 2000; Hanada, 2000; Marsh, 1999; Michalek *et al*., 2001; National Institutes of Health (US) 2001; Simmonds *et al*., 2000)

Many other studies have focused on studying the microbial ecology of dental plaques trying to find measurable risk factors and establish suggested mechanisms for dental caries (Bowden, 1997; Marsh and Bradshaw, 1995; Marsh, 1994).

There has been a renewal of interest in antimicrobial activity of medical plants including *Allium* species. Garlic extracts have been studied extensively for different purposes including inhibitory effects against a variety of microorganisms. McNulty *et al.* (2001) have tested garlic oil against *Helicobacter pylori* in dyspeptic patients. Sasaki *et al.* (1999) tested successfully garlic powder made from fresh garlic pulps against *Escherichia coli* O-157, methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella enteritidis* and *Candida albicans*. Many studies have shown variable to significant effects of garlic and garlic-derived extracts on common cold (Josling, 2001), fungi, (Wang and Ng, 2001), herpes viruses, *Mycobacterium tuberculosis* (Uchida *et al*., 1975) and parasites (Lun *et al*., 1994).

Onion extracts have also been studied, but less extensively than garlic (Farbman *et al*., 1993). It showed bactericidal effects on oral *Streptococcus mutans* and *Streptococcus sobrinus* (Kim, 1997). Zohri *et al.* (1995) demonstrated inhibitory effects of onion oil against four gram positive bacteria, four gram negative bacteria and dermatophytic fungi. Recently, Mantawy (2001) showed that exposure of snails (the intermediate host of *Schistosoma mansoni*) to water containing either garlic or onion caused snail toxicity.

## MATERIALS AND METHODS

**Patients and samples:** A total of 91 samples were collected from patients attending a private dental clinic in Irbid city in northern Jordan who are suffering from dental caries plaques on their teeth. This work was done between July 2003 and September 2003. Specimens were transported to our laboratory with thioglycolate transport medium and grown on blood agar, nutrient agar, MacConkey agar, chocolate agar and sabouraud dextrose agar (Difco) at 37°C.

**Preparations of aqueous garlic extract and aqueous onion extract:** Fresh peeled garlic cloves and onion (100 g) were chopped separately into small pieces and were homogenized at high speed in 200 mL of sterile distilled water in a blender for 5 to 7 min. The homogenate was filtered through a gauze cloth. The filtrate was passed through 0.45 Millipore filter and was immediately used. The plant materials used in this study were purchased from local markets in Jordan

**Susceptibility testing:** The clinical isolates were identified and approved by biochemical tests. Five mm diameter filter paper discs were impregnated with 50 μg of the tested plant and placed in duplicates onto Muller-Hinton agar, the surface was then spread with 0.2 mL of microorganism culture (ca. $10^8$ cells/mL) and the plates were incubated for 24 h at 37°C for bacteria and 48 h at 28°C for *Candida albicans*. Experiments were carried out in duplicates and the results (mean of three experiments) were recorded by measuring the zones of inhibition surrounding the discs. Control discs were used and contained sterile transport broth medium only. Dilutions of extracts used were 1:2, 1:4, 1:8 and 1:16.

**Calculations:** The median values for zones of inhibitions for both garlic and onion extracts at different dilutions for different isolated microorganisms were calculated and blotted (Fig. 1 and 2).

## RESULTS AND DISCUSSION

The present study trying to find out the microorganisms and their frequency in association with dental caries in Jordanian patients. A total of 116 isolates were grown from 91 patients with dental caries (Table 1). *E. coli, Lactobacillus acidophilus, Candida albicans, Streptococcus mitis, Actinomyces naeslandii, Enterococcus faecalis* and *Streptococcus oralis* were decreasingly the most frequent organisms. These results are more or less consistent with already accumulated

Table 1: Frequency and percentage of microorganisms isolated from patients with dental caries

| Microorganism | Number of isolated strains | Percentage |
|---|---|---|
| *E. coli* | 14 | 12 |
| *Lactobacillus acidophilus* | 12 | 10 |
| *Candida albicans* | 11 | 9 |
| *Streptococcus mitis* | 11 | 9 |
| *Actinomyces naeslandii* | 10 | 9 |
| *Enterococcus faecalis* | 10 | 9 |
| *Streptococcus oralis* | 10 | 9 |
| *Enterobacter* spp. | 7 | 6 |
| *Streptococcus mutans* | 6 | 5 |
| *Actinomyces viscosus* | 4 | 3 |
| *Lactobacillus casei* | 3 | 3 |
| *Streptococcus anginosus* | 3 | 3 |
| *Veillonella* spp. | 3 | 3 |
| *Haemophilus* spp. | 2 | 2 |
| *Klebsiella* spp. | 2 | 2 |
| *Neisseria* spp. | 2 | 2 |
| *Pseudomonas aeruginosa* | 2 | 2 |
| *Staphylococcus huminis* | 2 | 2 |
| *Staphylococcus simulans* | 1 | 1 |
| *Streptococcus sanguis* | 1 | 1 |

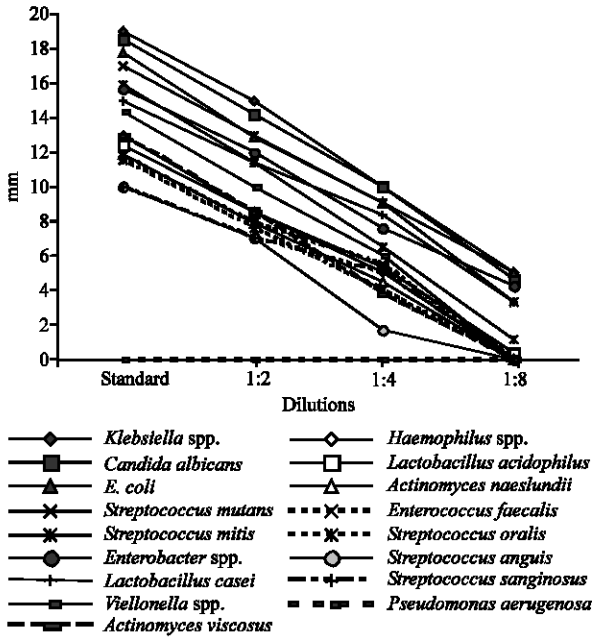


Fig. 1: Median values of zones of inhibitions measured at different garlic (*Allium sativum*) extracts against mentioned microorganisms

literature regarding pathogens associated with dental caries. These results show that garlic extracts have significantly higher inhibitory effects than onion extracts even though most microorganisms showed same patterns of inhibition to both garlic and onion extracts. (apart from *Candida albicans, Streptococcus mitis* and *Veillonella* spp.) (Fig. 1 and 2). In both onion and garlic groups, *Klebsiella* and *Pseudomonas aeruginosa*

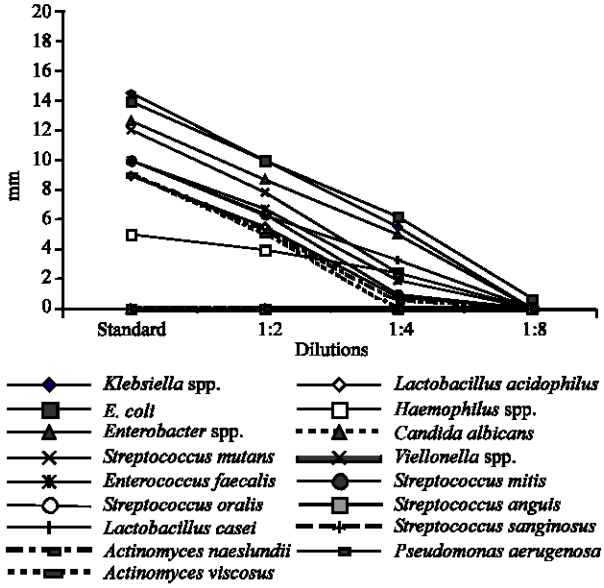


Fig. 2: Median values of zones of inhibitions measured at different onion (*Allium cepa* L.) extracts against mentioned microorganisms

isolates were the least and most resistant organisms, respectively. However, both microorganisms were of no statistical significance in ecology of dental caries. *Klebsiella* species, *E. coli, Streptococcus mutans* and *Enterobacter* species were consistently the least resistant organisms for both extracts. On the other end, *Pseudomonas aeruginosa, Streptococcus sanguis* and *Streptococcus anginosus* were consistently the most resistant organisms for both garlic and onion extracts. It is interesting that *E. coli* which is the most common microorganism associated with dental caries, is also very sensitive to both onion and garlic extracts. The weaker antimicrobial activity of onion extract might be possibly explained by the presence of component that are less active than allicin which is found in garlic. Toxicity of garlic has been reported by several studies and garlic-sensitive patients have shown positive reactions to allyl propyl disulfide, allyl mercaptan and allicin.

## CONCLUSIONS

Garlic extracts with different dilutions were of significant inhibitory effects against microorganisms associated with dental caries which might predict potential beneficial future uses of garlic extracts. On the contrary, onion extracts showed significantly weaker inhibitory effects against those microorganisms. Microorganisms associated with dental caries of

Jordanian patients are similar in types and frequency to those encountered in other different areas of the world. Based on the *in vitro* results of this study and on the known organic chemistry of garlic, future studies should focus on finding the concentration of the active constituents that can be tolerated by humans in order to try using them for topical treatment. Other studies may investigate the effect of human intestinal proteolytic enzymes on the active components of *Allium* species

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