

ANTIBACTERIAL ACTIVITY OF GARLIC EXTRACT ON
BACTERIA ISOLATED FROM TEETH
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Abstract

Background: Allium vegetables, particularly garlic (*Allium sativum*) have been used for centuries to combat infectious diseases. It exhibits a broad antibiotic spectrum against many types of bacteria, and can be used for formulation of newer broad spectrum antibacterial substances.

Objective: This study aimed to explore the antimicrobial activity of aqueous garlic extract on different types of bacteria which cause mouth sore and dental caries.

Methods: The study was carried out in College of Medicine / University of Diyala. A total of 200 teeth swabs were collected from students of the college. The isolated bacteria were identified according to standard bacteriological methods and their susceptibility to different antibiotics was tested according to Kirby-Bauer method. Antimicrobial activity of different concentration of aqueous garlic extract was measured by MBC (Minimal Bactericidal Concentration) according to agar-dilution method.

The results: Our study revealed that 187 (93.5%) were *Streptococcus mutans* (*S. mutans*), and 144 (72%) were *Staphylococcus aureus* (*S. aureus*). The aqueous garlic extract was effective as antibacterial agent against *S. aureus* and *S. mutans* which were resistant to different antimicrobial agents. The averages of MBCs of extract against *S. aureus* and *S. mutans* were 2 mg/mL and 4 mg/mL respectively.

Conclusion: Garlic extract has antibacterial activity in low concentration on *S. aureus* and *S. mutans*. Garlic extract is safe and inexpensive remedy that can be used as a better alternative especially in developing countries.

Key word: Garlic extract; teeth microorganism; *S. aureus*; *S. mutans*.

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تأثير مستخلص الثوم كمضاد للبكتريا المعزولة من الأسنان

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الخلاصة

استخدم الثوم ومنذ عدة قرون لمكافحة الأمراض المعدية إذ يمتلك طيفاً واسعاً ضد أنواع عديدة من البكتيريا، ويمكن استخدامه لإنتاج مضادات بكتيرية جديدة. هدفت هذه الدراسة إلى الكشف عن تأثير مستخلص الثوم المائي المضاد للبكتريا على أنواع مختلفة من البكتريا المسببة لتسوس الأسنان والتهاب الفم. وقد أجريت الدراسة في كلية الطب / جامعة ديالى حيث تم جمع 200 مسحة من أسنان طلبة الكلية، و شخّصت البكتريا المعزولة وفقاً للمعايير البكتريولوجية القياسية. ثم اختبرت حساسيتها للمضادات الحيوية المختلفة حسب طريقة كيربي باور. كما قيست فعالية تراكيز مختلفة من مستخلص الثوم المائي المضادة للبكتريا عن طريق حساب التركيز القاتل الأدنى (MBC) وفقاً لطريقة تخفيف الأكار. أظهرت النتائج أن 187 عزلة (93.5%) كانت تعود لبكتريا *S. mutans*، و 144 (72%) كانت *S. aureus*. ووجد أن مستخلص الثوم المائي كان فعالاً ضد *S. aureus* و *S. mutans* المقاومة لمختلف أنواع المضادات الميكروبية. كان معدل التركيز القاتل الأدنى MBC للمستخلص ضد بكتريا *S. aureus* و *S. mutans* 2 ملغم/ مل و 4 ملغم/ مل على التوالي. ويمكن أن نخلص إلى أن مستخلص الثوم كان له تأثيراً فعالاً كمضاد بكتيري كما إنه علاج آمن وغير مكلف ويمكن استخدامه كبديل أفضل وخاصة في البلدان النامية.

كلمات الدالة : مستخلص الثوم ؛ ميكروبات الأسنان ؛ بكتريا *S. mutans* ؛ بكتريا *S. aureus*

Introduction

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (1).

Garlic (*Allium sativum* Linn.) is one of those plants that was greatly investigated over the years. It has been used for centuries to combat infectious diseases (2). Some of the earliest references of this medicinal plant are found on Sumerian clay tablets dating from 2600-2100 BC. (3). The early Egyptians to treat diarrhea, the ancient Greeks used it to treat intestinal and extra-intestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever (4). The antimicrobial activity of garlic was first described by Louis Pasteur and it was used as an antiseptic to prevent gas gangrene during World War II (5).

Allium vegetables, particularly garlic (*Allium sativum* Linn.) exhibit a broad antibiotic spectrum against both Gram-positive and Gram-negative bacteria (6) and can be used for formulation of newer spectrum antibacterial substances (7).

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From the published researches articles, It is clear that the raw juice of garlic was effective against many common pathogenic bacteria and against that have become resistant to antibiotic (8) and even toxin production by some pathogenic strains prevented by garlic (9).

Therapeutic effect of garlic is possible because of its oil- and water-soluble organosulfur compounds. Thiosulfates (eg. Allicin) play an important role in the antibiotic activity of garlic. Feldberg, *et al* (10) showed that allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action.

Tooth decay, a progressive destruction of enamel, dentine and cementum, mediated by microbial activity at the tooth surface (11), is not only excruciating but, economically debilitating. About 20 billion dollars is spent on restoring and replacing carious teeth in the United States only, annually (12). Plant extracts formulations have therefore become a better alternative, especially in developing nations. Moreover, most antimicrobial agents that are currently in use have been rendered ineffective by the wide occurrence of multiple drug resistant strains of microbes (13).

The aim of this study is to identify aqueous garlic extract as a common and cheap herbal remedy for the treatment of mouth sore and dental caries, especially in developing countries.

1. Methods:

1.1. Bacterial isolates:

This study was done in College of Medicine/ Diyala University. Two hundred teeth swabs were collected from students of the college during the period between March-April, 2012. Swabs were cultured on blood agar (under aerobic and anaerobic conditions), MacConkey's agar, and chocolate agar (under CO₂) for 24 hrs. at 37°C. The isolates were further identified according to standard bacteriological methods.

1.2. Preparation of aqueous garlic extract:

Dried, grinded garlic was collected from local markets of Baquba City in May, 2012. Four grams of garlic were suspended in 100 ml of distilled water, pooled for two days and mixed by electric magnetic stirrer. The mixture was centrifuged; the supernatant was collected, and then filtered. The filtrate was used as stock solution with concentration of 40 mg/ml.

1.3. Inoculum preparation:

Four ml of nutrient broth was taken into a screw capped tube and pure colonies of freshly cultured bacteria was added, the broth was incubated at 37°C for about 3 hrs to produce a bacterial suspension with turbidity compared to 0.5 MacFarland solution.

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1.4. Antimicrobial susceptibilities:

The *in vitro* antimicrobial susceptibility test to different antibiotics (Amoxicillin, Cefotaxime, Cephalexin, Ciprofloxacin, Erythromycin, and Penicillin G) was performed by disc diffusion method according to Bauer and Kirby (14) which was accepted by NCCLS (15). Different concentration of aqueous garlic extract (1, 2, 4, 8, 12, 16, 20) mg/ml were obtained from the original stock solution of garlic extract and tested on the resistant cultures of isolated bacteria according to Sforzin (16).

Twenty bacterial isolates were inoculated in Mueller-Hinton agar plates supplemented with different concentrations of garlic extract. Negative control plates with no bacterial inoculation were simultaneously maintained.

MBC of the extract was determined according to agar dilution technique (17). The averages of MBCs of each species were calculated.

2. Result and discussion:

The results in table (1) revealed that among 200 teeth swabs investigated, 187 (93.5%) were *Streptococcus mutans* and 144 (72%) were *Staphylococcus aureus*. Actually, the predominance of *S. mutans* is not unusual since similar results have been reported (12,18). The presence of *S. aureus* in high percentage (72%) is different from other study (12). The prevalence of *S. aureus* may be due to their role in formation of cavities, since they have the ability to convert sugars into acids such as lactic acid through the glycolytic process of fermentation (19). Also, their present as a colonizer of upper respiratory tract, especially anterior nares, in 20-70% of adult individuals (13,20,21) may implicated in this prevalence. Regarding the antimicrobial susceptibility patterns of bacterial isolates, it was found that (59.9, 49.7, and 29.9) % of *S. mutans* were resistant to cephalexin, erythromycin, and ciprofloxacin, respectively. Table (1). The results in the same table also revealed that (59.7, 40.3, 20) % of *S. aureus* were resistant to amoxicillin, erythromycin, and cephalexin, respectively.

Table (1): the percentage of the bacterial isolates resistant to different antibiotics.

Antibiotic	<i>S. aureus</i>	<i>S. mutans</i>
	No. (%)	No. (%)
Cephalexin	29 (20)	112 (59.9)
Cefotaxime	0 (0)	0 (0)
Erythromycin	58 (40.3)	93 (49.7)
Amoxicillin	86 (59.7)	0 (0)
Penicillin G	0 (0)	0 (0)
Ciprofloxacin	14 (9.7)	56 (29.9)

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These results are not unusual since they agree with previous studies (13, 22).

The widespread use of antimicrobials has been implicated in the development of resistant strains.

The results of this investigation revealed that different concentrations of aqueous garlic extract were effective as antibacterial agent against *S. aureus* and *S. mutans* and the averages of MBCs of aqueous garlic extract were 2 mg/ml and 4 mg/ml, respectively. Table (2).

This result is very interesting, because these bacteria have the ability to develop multi-drug resistant strains. However, this concentration level may vary as different authors have stated. This might be due to the garlic species variation in different country, the processing difference on the garlic species and the inoculums' densities.

Table (2): the averages of MBCs of aqueous garlic extract

Bacterial species	Average of MBCs mg/ml
<i>S. aureus</i>	2
<i>S. mutans</i>	4

Previous researchers have described the antibacterial activity of garlic against multi-drug resistant (MDR) *S. mutans* (23), as well as methicillin-resistant *S. aureus* (24). The bactericidal effect of garlic might be due to the structural characteristics of organisms which play a role in the bacterial susceptibility to garlic constituents (25) particularly the lipid content of the membrane which have an effect on the permeability of allicin and other garlic constituents.

In conclusion garlic extract is a safe agent could be used in treatment of dental caries and mouth sore. However, further studies on the garlic/antibiotic synergistic effects are recommended.

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