

## In Vitro Antibacterial Properties of Garlic Extract against Some pathogenic bacteria isolated from burn unit

Iqbal Azeez Ameen \* Hind Abdallah Salih \* Ali Taher Abaas \*\*

\* Biology department – Science College – Thi-Qar University

\*\* Branch of clinical sciences and Laboratory - College of Pharmacy - Thi-Qar University

### Abstract

The antibacterial effects of aqueous garlic extract and Crude Juice against 38 Gram-positive and Gram-negative bacterial isolates, from burn unit were studied. The bacteria isolates were including *Staphylococcus* spp. , *Serratia* spp. , *Pseudomonas* spp., *Enterobacter* spp., *Acinetobacter*. Spp. and *Ochrobactrum anthropi*. were studied. Antibacterial activity of aqueous garlic extract and Crude Juice by cup plate method was characterized by inhibition zones of diameter (14-34) mm for gram-positive bacteria and (14-32) mm for gram-negative bacteria respectively. The results of the study of garlic extract both types of aqueous extract and crude juice in the degree of impact inhibition as results showed that Crude Juice had a greater effect than aqueous extract effect.

**Key words:**-antibacterial effect, garlic and susceptibility

### مختبريا الخصائص المضادة للبكتيريا لمستخلص الثوم على بعض انواع البكتيريا المرضية المعزولة من وحدة الحروق

علي طاهر عباس \*\*

هند عبدالله صالح \*

اقبال عزيز امين \*

\*قسم علوم الحياة – كلية العلوم – جامعة ذي قار

\*\* فرع العلوم المخبرية والسريرية – كلية الصيدلة – جامعة ذي قار

### الخلاصة

التأثيرات ضد بكتيريا لمستخلص الثوم المائي والكحولي ضد ٣٨ عزلة بكتيرية سالبة وموجبة لصبغة جرام من وحدة الحروق تضمنت *Staphylococcus* spp. , *Serratia* spp. *Pseudomonas* spp., *Enterobacter* spp., *Acinetobacter*. Spp. and *Ochrobactrum anthropi*. اظهرت نتائج الدراسة أن نشاط المضاد البكتيري لمستخلص الثوم المائي والكحولي حدد بطريقة الحفر وكانت تتميز بوجود مناطق تثبيط قطرها ( ١٤-٣٤ ) ملم للبكتيريا الموجبة لصبغة جرام و ( ١٤ - ٣٢ ) ملم للبكتيريا السالبة لصبغة جرام على التوالي . يتضح من نتائج الدراسة خلال استخلاص الثوم لكلا النوعين من المستخلص المائي والعصارة لهما درجة تأثير تثبيطي كما أظهرت النتائج أن العصارة كان لها تأثير أكبر من تأثير مستخلص المائي.

**الكلمات المفتاحية:** .: تأثير المضاد البكتيري ، الثوم والحساسية

## Introduction

*Allium sativum*, commonly known as garlic, is a plant belonging to the family of Liliaceae, which is native to central Asia and nowadays can be found throughout the world. Over the last centuries, various species of garlic have been used as spice or condiment for flavoring food. In herbal medicine, garlic has been prescribed for treating different kinds of diseases (Haciseferogullari *et al.*, 2005). This plant is thought to be regulating the blood sugar and protecting the cardiovascular system. It also bears antibacterial, anticarcinogen, antioxidant and anti-inflammatory properties (Iwalokun *et al.*, 2004). In addition, antibacterial effects of garlic on various types of bacteria have been reported in some studies (Sivam, 2001; Jazani, *et al.*, 2007). Immediately following wounding, whether it is caused by a severe burn, trauma or surgery, the affected area is at risk of colonization by a variety of micro-organisms, which proliferate within the damaged tissues and establish an infection (Rafla, and Tredget, 2011). These microorganisms may come from the host's skin, respiratory or gastrointestinal flora (Posluszny, *et al.*, 2011). Alternatively, they may reach the wound through contact with health care workers or come from environmental sources (Rafla, and Tredget, 2011; Posluszny, *et al.*, 2011). Microorganisms proliferating within the tissue may gain access to the bloodstream, the result of which can be sepsis, multiorgan failure and death (Church, *et al.*, 2006). Such infections are responsible for ~75% of mortality in burn patients (Rafla, and Tredget, 2011). The two most common bacteria causing burn wound infections are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. (Rafla, and Tredget, 2011; Posluszny, *et al.*, 2011). This broad spectrum of activity has been attributed to the over 100 phytotherapeutic sulfur compounds present in varying concentrations in garlic. They include allicin and thiosulfonates, which are formed by crushing-induced metabolic action of the enzyme alliinase (a cysteine sulfoxidylase) on the odorless amino acid alliin (Lawson, *et al.*, 1991).

## Materials and methods

Study design and setting: a cross sectional study had been conducted in burn unit in Al-Hussein hospital at Thi-qar, one of the southern province in Iraq for the period from 1<sup>st</sup> of November 2014 to the end of June 2015.

**1.Sampling** : one hundred swabs were collected from the skin of patients in burn unit in Al-Hussein hospital at Thi-qar.

**2. Cultural media** : swabs were cultured on ; Blood agar, MacConkey agar and Nutrient agar, which were prepared according to the manufacture companies, and incubated at 37°C for (24 - 48) hours.

**3. Isolation and identification** : Purification of bacterial growth colonies yield pure isolates of bacteria and subsequently their cultural, morphological, microscopically and biochemical characteristics had been studied according to (Retty, *et al.*, 2007; Steven, *et al.*, 2001, Finegold, ; Koneman, *et al.*, 1992 and Martin, 1982).

For identification of isolates the following kits were used:

API Staph kit (BioMeriux) for staphylococci identification

API 20E kit (BioMeriux) for Gram -ve bacilli identification

**4. Antimicrobial Sensitivity tests** : Susceptibility for the studied isolates were investigated according to (Stocks, and Ridgway, 1987) by using Muller - Hinton agar and the results were interpreted as recommended by (CLSI, 2009) and the following antibiotics discs were used : Ceftriaxone, Cefepime, Imipenem, Meropenem, Tetracyclines and Ciprofloxacin.

## **5- Preparing and extraction of garlic**

### **5-1 Aqueous extract**

According to Masoudi (2001), the preparation of the aqueous extract of garlic, garlic cloves removed and cut into small pieces. A weight of 40 g garlic mix with water by (1: 2) (weight: volume) and mixed by electric mixer for 15 minutes and leave the solution for 30 minutes a piece of cloth used to separate large particulates, and centrifuged at 300 r / min for 10 minutes, then save in sterile bottles.

### **5-2Crude Juice Preparation**

Mixing 10 g of chopped garlic cloves with 25 ml of sterile distilled water and mixing in an electric mixer and used directly in the test (Reuter, *et al.*, 1996).

## **6-Estimation of antibacterial activity of garlic extract**

### **Cup plate method**

The antibacterial activity of the extracts was determined by using the agar well diffusion technique. Muller – Hinton agar plates were needed with 0.1 ml of overnight culture, allowed incubating for 24hrs. cup were made in Petri plates using sterile cork borer (0.85 cm) and 50 µl of each extract was added into each well

( Agarwal, 1976 ). Then bacterial plates were incubated at 37° C 24hrs. Each test compound has got two bores for which zone of inhibition diameter and mean values were determined. Antibacterial activity was determined by measurement of zone of inhibition around each well in plate using zone reader.

### **Results and Discussion**

Bacterial growth had been observed in 38 cultures (38%) out of 100 swabs and samples which were collected from burn unit ( Table 1 ).

The pure culture were divided into two groups depending on Gram stain, accordingly 17 Gram positive isolates and 21GramNegative isolates were identified . The most prevalent genus among Gram +ve bacteria was *Staphylococcus* spp. ( 11) isolates ( Table 1 ) , The reason for this the rule of bacteria such as bacteria possess many surface antigens and enzymes that enable them to penetrate the body's tissues. This is one of the bacteria and pathogens that cause opportunistic infections because of the natural presence in or on the digestive and genital tract and nose and skin holding the bodies of her ( Landman, 2001) . while the most prevalent genus among Gram -ve was *Pseudomonas* spp. ( 10 isolates ) had been found( Table 1).Susceptibility tests for some antibiotics showed different results depending on the genus of bacteria and type of antibiotics used For *Pseudomonas* spp. the resistance was highly significant against 4 antibiotics,(Cefepime, Ceftriaxone, Meropenem and Imipenem) The resistance was 100%(Table 2) .while the resistance of Tetracycline and Ciprofloxacin antibiotics was 90%(Table 2) which show consistency with a study that had been done in ( Nwobu, and Oguntibeju, 2004). In the ratio resistant bacteria *Pseudomonas* spp. to these antibiotics. The rest of all bacterial species have shown high resistance to antibiotics used(Table 2) .Such a high frequency and nature of antibiotic resistance may be due to over usage of antibiotics as well as no availability and high cost of preferred antibiotics of choice ( Edwards, and Greenwood, 2003). Increasingly bacteria are becoming multiple antibiotic resistant, leaving little or no effective systemic treatment option ( Neely, and Holder,1999) . The bacteria *Ochrobactrum anthropi* did not show any resistance to antibiotics Ciprofloxacin , Imipenem and Meropenem. Where the inhibition zone of these bacteria 27 mm , 29 mm and 19 mm respectively (Table 2)The reason for inhibition is due to the scarcity of this type of bacteria.Seen from the

results of the study during the garlic extract both types of Aqueous extract and Crude Juice showed that Crude Juice had a greater effect of aqueous extract effect (Table 3)..The Crude Juice his effect "inhibiting" for the growth of most bacterial species. This explains on the basis of what belongs the garlic of active substances, as it has several types of antibiotics most prominent the first and second Alistanin( Allistatin I &II ) ( Chakravarty, 1979). The antimicrobial activity of garlic has been attributed to the presence of thiosulfinate materials (e.g: allicin) in which their complete removal render garlic to be ineffective against microorganisms (Hughes, and Lawson, 1991).Table 3 summarizes the result of the preliminary investigation of Aqueous extract and Crude Juice . The extract exhibited growth inhibition of both gram-positive and gram negative bacteria on Muller Hinton agar with inhibition zone diameter of 14-34 mm for gram-positive bacteria and 14-32 mm for gram-negative bacteria. which show inconsistency with a study that had been done in ( Iwalokun, *et al.*, 2004 ) . Varied intensity of aqueous extract of garlic impact in the growth of the bacteria used in this study where we note in the [Table 3 ]. The minimum inhibitory concentration has reached 14 mm in two types *Staph.* Spp. and *Pseudomonas.* spp. and the highest inhibitor concentration was 30 mm in kind *Staph.* spp. The differences in the susceptibility of each test organism to the garlic extract may be directly related to the presence of secondary plant metabolites (thiosulfates) and its concentration, in addition to the ability of microorganism to resist the antimicrobial agent that may occur because of multiple uses of antimicrobial agents in medicine ( Chen, and Chang, 1985).The aqueous extract given inhibition "for all isolates have been the reason is due to the ability of water to pull many of the active ingredients (alkaloids, saponins, sterols, and Turaaterpinoadat) from plant sources without other solvents, these compounds characterized by high impact on the inhibition of microbiology and particular bacteria ( Grimshow, 1976 ).[Table 3] shows the efficiency of the fresh juice on the inhibition of bacterial species studied where the minimum inhibitory concentration (16) mm in bacteria *Pseudomonas* spp. The highest inhibitory concentration34 mm in bacteria. Allicin, the active ingredient of garlic, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target ( Eja, *et al.*, 2007 )The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids ( Griffiths, *et*

al., 2002 ). Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity ( Jombo, et al., 2011 ; Aboaba, and Efuwape, 2001 ).

The garlic cutting and crushed in during the preparation of fresh juice increases the contact surfaces and then edit the active compounds ( Dankert, 1979 ). For this reason, we note that fresh juice gave effective against microbial more of the aqueous extract and antibiotics used.

Table 1- Bacterial isolates from burn unit and number of isolates and percentage

Bacterial isolates	Numbers of Isolate	Percentage
<i>Staphylococcus</i> spp.	11	28.94
<i>Serratia</i> spp.	6	15.78
<i>Pseudomonas</i> spp.	10	26.31
<i>Enterobacter</i> spp.	8	21.05
<i>Acinetobacter</i> spp.	2	5.26
<i>Ochrobacterum anthtopi</i>	1	2.63
TOTAL	38	100%

Table 2 The effect of antibiotics on some pathogenic bacteria isolated from burn unit.

Antibiotics	Types of bacteria					
	<i>Pseudomonas sp.</i>	<i>Enterobacter Spp.</i>	<i>Serratia spp.</i>	<i>Acintobacter Spp.</i>	<i>Staphylococcus spp.</i>	<i>Ochrobacterum anthropi</i>
Ciprofloxacin CIP	9 90%	5 62.5%	2 33.3%	2 100%	8 72.7%	S(27)
Ceftriaxone CTR	10 100%	8 100%	5 83.3%	2 100%	11 100%	1 100%
Cefepime FEP	10 100%	8 100%	6 100%	2 100%	10 90.9%	1 100%
Imipenem IMP	10 100%	6 75%	3 50%	2 100%	10 90.9%	S(29)
Meropenem MEM	10 100%	7 87.5%	3 50%	2 100%	10 90.9%	S(19)
Tetracyclines TE	9 90%	8 100%	4 66.6%	2 100%	8 72.7%	1 100%

Table 3 Antibacterial activity of Garlic extracts against some pathogenic bacteria isolated from burn unit

Extractions	Types of bacteria					
	<i>P. sp.</i>	<i>Entero. Sp.</i>	<i>S. sp.</i>	<i>Och. Sp.</i>	<i>Actino. Sp.</i>	<i>Staph. Sp.</i>
Aqueous extract	14 26	15 26	16 25	24	16 20	14 30
Crude Juice	16 30	18 30	19 32	30	20 22	19 34

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