



ANTIBACTERIAL ACTIVITY OF GARLIC EXTRACT (*ALLIUM SATIVUM*) AGAINST *STAPHYLOCOCCUS AUREUS* *IN VITRO*

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ABSTRACT

The study was conducted to investigate the inhibitory effect of alcoholic extract of Garlic on the inhibition of growth of *Staphylococcus aureus*, which were isolated from skin infections *in vitro*. It was drawn garlic using ethanol 95%, where the percentage of extraction of 48% of the weight of dry powder has attended. A concentration gradient of the alcoholic extract (10-100mg /ml) was prepared and its effective was tested by agar diffusion method using bacteria *Staphylococcus aureus* compared with ethylene glycol. Results showed that the diameters of the inhibition of the growth of bacteria increased with increasing of the concentration of alcoholic extract. Lower and medium efficiency were detected for concentrations of 10-20 and 40-60mg/ml respectively. Whereas the concentrations of 80- 100 mg / ml was highly effective against the growth of bacteria *Staphylococcus aureus*.

KEY WORDS: Garlic (*Allium sativum*), *Staphylococcus aureus*.

INTRODUCTION

Historically, garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Louis Pasteur was the first described the antibacterial effect of onion and garlic juices against both Gram-positive and Gram-negative bacteria (Whitemore and Naidu, 2000). From the published research articles it is clear that the raw juice of garlic was effective against many common pathogenic bacteria (Kumar and Sharma, 1982), and against the strains that have become resistant to antibiotics (Jezowa *et al.*, 1966) and even toxin production by some pathogenic strains prevented by garlic (Dewitt *et al.*, 1979). Louis Pasteur was the first describe the antibacterial effect of onion and garlic juices (Srinivasan *et al.*, 2001). Garlic is a strong antibacterial agent against both Gram-positive and Gram-negative bacteria such as *E. coli*, *Salmonella spp.*, *Streptococcus spp.*, *Staphylococcus aureus*, *Klebsiella spp.*, *Proteus mirabilis*, *Shigella senteriae*, *Pseudomonas aeruginosa* and *Helicobacter pylori*, (Indu *et al.*, 2006), also it's effective even against those strains that have become resistant to antibiotics (Ross Z. *et al.*, 2001). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization source to obtain a variety of drugs (Santos *et al.*, 1995). Allicin, is the active ingredient of *A. sativum* has been reported to have a range of potential targets. Firstly, inhibiting the acetyl CoA forming system lead to inhibit DNA and protein synthesis, secondly inhibiting RNA synthesis as a primary target (Cutler, 2004). The ability of bacteria to develop resistance to antibiotics in addition to the side effects of some of these antibiotics (Cheke *et al.*, 2005). The purposes of this study are to detect the *in vitro* activity

of Garlic extract in the growth inhibition of *Staphylococcus aureus* isolated from skin diseases, so this study carried out to:-

1. Prepare alcoholic extraction of Garlic in ethyl alcohol 95%.
2. Detection of inhibitory effects of different concentrations of extract on the growth of *Staphylococcus aureus* by using agar well diffusion method .

MATERIALS & METHODS

Culture media

Are prepared according to the producing companies instructions and sterilized in autoclave at 121 C under pressure of 15 PSI after incubation at 37 C for 24 hrs, used for culture and diagnosis of bacteria used in this study (Forbes *et al.*, 2007).

Preparation of plant

Garlic were collected from the local market and authenticated as *Allium sativum* (University of Anbar college of Science).

Extraction methods

Garlic was skinned and sliced; 50 g sliced Garlic pieces were crushed in awarding blender for 1minute, and then soaked in 450 ml ethanol 95%. It was naturally extracted for 3 months at room temperature; the mixture was separated in test tubes by centrifugation 3000 rpm, the filtrate was dried in oven 37 C for 24 hrs. The final product was stored in freezer at (-20) C (Krell *et al.*, 1996).

Culture preparation

Bacteria were activated by re culturing on nutrient agar and kept in the incubator for 24 hrs at 37 °C, then transferred was sterilize tubes containing heart infusion broth , then placed in the incubator for 24-72 hrs at 37 °C . Total bacterial count was estimated by using spectrophotometer, the percentages of light transmittance

was 27% at a wave length of 580 nanometer, while the light transmittance was 100% for nutrient broth used to prepare the bacteria (Jassim, 2003).

Preparation of standard dilutions of Garlic extract

The dilution were prepared by using ethylene glycol which is inert solvent against microorganism (Charlas *et al.*, 1969) and by using serial concentrations from 10-100 mg from the extract, then dilute it with ethylene glycol and the volume was completed to 2 ml to get the final concentrations from 1-10% .

Garlic extracts activity test well diffusion method screening

Screening of the anti-bacterial activity was performed by well diffusion technique (Saeed *et al.*, 2005). The Mueller-Hinton agar plates were seeded with 0.1 ml of the standardized inoculums of bacteria. The inoculums were spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37 C for 20 minutes. A standard crack border of 9 mm diameter was used to cut uniform wells on the surface of the plates, and 0.1 ml of each concentration was introduced in the well with ethylene glycol as a control. The inoculated plates were incubated at 37 C for 24 hrs and zone of inhibition diameter was measured to using nearest millimeter (mm).

RESULTS

1-Identification of bacteria

- a. The bacteria grow well on mannitol salt agar.
- b. Microscopic examination:- Gram-positive , spherical in shape .
- c. Biochemical tests were confirmed the identification of *Staphylococcus aureus* , catalase and gelatinase + ve, oxidase (– ve) , blood agar (B-haemolysis , and production of local golden pigment).

2- The inhibitory effect of Garlic extract

The sensitivity of the previously mentioned bacteria gradually increased with the increment of concentration of extract. The zone of the inhibition was 9 mm was recorded for the concentration of 10 mg/ml, and 23 mm was for the concentration 100 mg/ml. The concentrations 10-20 mg/ml were rather low active in preventing the growth of *Staphylococcus aureus* , the concentrations 40-60 mg/ml were moderate active, while the concentrations 80-100mg/ml were highly active compared to ethylene glycol as a control as shown in Table 1, and Fig. 1. There was a proportional relation between the concentrations of extract and the diameters of inhibition zones of the growth of *Staphylococcus aureus*.



FIGURE 1: The inhibitory effect of different concentration of Garlic extract on the growth of *Staphylococcus aureus*.

TABLE 1: effect of different concentration of garlic extract on the growth of *Staphylococcus aureus* measured by the diameter of zone of inhibition (mm) In vitro inhibitory

Conc.(mg/ml)	100	80	60	40	20	10
Zone of inhibition						
Garlic extract	23	21	18	15	13	9

DISCUSSION

In this study, the Garlic possessed anti-bacterial effect against *Staphylococcus aureus* , and the sensitivity of the bacteria was gradually increased with the increasing of extract concentrations (Table 1) Bacterial drug resistance is a world problem , a high number of bacterial species have become resistant to anti-bacterial drugs (Garau *et al.*, 1994). Thus, there is a need to evaluate the efficacy of plant chemicals concerning with the growth of bacteria by extracts of plants to be used, with dichloromethane extraction (Laenger *et al.*, 1996), maceration and soxhlet fluid extraction with hexane (Vilegs *et al.*, 1997). These preparations are unavailable to person for self-medication, with these consideration the activity of Garlic extract on the growth of *Staphylococcus aureus* were studied. Garlic

has been known to have anti-bacterial, anti-fungal, and anti-viral activity (Bakri *et al.*, 2005). The present results are in fair correlation with the study above and the study carried out by Reuter *et al.*, 1996 in which Garlic has been reported to inhibit the growth of *Staphylococcus* and many other species. In another study crude juice of Garlic has been found to be high active against *E. coli* and *Salmonella typhi* (Abdon *et al.*, 1972). Sasaki *et al.*, 1999 found the Garlic activity against methicillin-resistant *Staphylococcus aureus* and *candida albicans*. Garlic extract possesses anti-bacterial activity against *H. pylori* at moderate concentration, thus it has protective effect against stomach ulcer (Satiawane *et al.*, 2005). This observation needs many studies and investigations. Allicin is biologically active compound responsible for the anti-

microbial properties of Garlic. The inhibitory effect of Garlic on the growth of *Staphylococcus aureus* in this study is due to the important allicin compound in the Garlic extract. Pure allicin was effective against many clinical isolates of *Aspergillus in vitro* study (Shadkchan *et al.*, 2004).

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