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Antimicrobial Effect of *Allium sativum* (Garlic) against E. *coli* and S. *aureus* Isolated from Cow's Mastits Milk

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ABSTRACT

To determine the antimicrobial effect of garlic (Allium sativum juice), two bacterial strains was tested. In this work were *Escherichia coli, Staphylococcus aureus,* both obtained from cow's mastitis milk in the region of Tiaret, Algeria. To investigate the antimicrobial properties of garlic juice we used two different methods (Disc diffusion or aromatogram method and the diffusion method). The antibacterial effect of garlic extract against E. *coli* and S. *aureus* was significant; the diameters of the inhibition zones were 23 mm and 30 mm in the disc diffusion method and 20 mm and 30 mm in the well diffusion method. In this study, it was proved that garlic juice plays a very important role in the fight against the growth of the bacteria tested (E. *coli* and S. *aureus*) and can therefore replace by its plant nature and its biological property the use of chemical synthesis antibiotics that always had unwanted side effects on the body such as allergies and antimicrobial resistance.
KEYWORDS*:* Garlic juice, Antibacterial effect, Escherichia coli, Staphylococcus aureus.

INTRODUCTION

The Mediterranean region is very rich of many variety of medicinal plants used in many civilizations before. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century [1]. Garlic (Allium sativum) was used to fight infectious diseases over several years and is one of those plants that were seriously investigated for their antibacterial properties [2]. Pasteur (1958) described the antimicrobial properties of garlic first, and since then, many researches had demonstrated its effectiveness and broad spectrum antimicrobial activity against many species of bacteria, viruses, parasites, protozoan and fungi [3]. Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Pseudomonas, Shigella, Salmonella, Proteus, and Helicobacter pylori [4]. Its antibacterial activity is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin. Allicin is considered to be the most potent antibacterial agent in crushed garlic extracts, but it can be unstable, breaking down within 16 h at 23°C [5]. In this study we analyzed the effect of fresh garlic juice on Staphylococcus *aureus* and E *coli* isolated from milk of cows with clinical mastitis.

MATERIALS AND METHOD

Garlic (Allium sativum) used in the present study was purchased from the local market. Bulbs were peeled and washed from the foreign particles. Bulbs were pressed and the juice was kept in sterile bottle under refrigerated condition until use.
To assess antibacterial activity at different concentrations, fresh and pure garlic juice was used in decreasing doses: 100%, 75%, 50%, and 25%. (Distilled water was used as diluents).
The test microorganisms (Staphylococcus *aureus* and E. *coli*) used in this study was obtained from cow's clinical mastitis milk. The cows were raised in Tiaret, at the west of Algeria.
About 18 hour broth culture of the test bacteria isolate was suspended into sterile nutrient broth. It was standardized by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0 × 106 CFU/ml.

Antibacterial activity test:

Fresh and pure garlic juice was used in decreasing doses: 100%, 75%, 50%, and 25%. (Using distilled water for dilution) to assess antibacterial activity at different concentrations.

In our study, the evaluation of the antibacterial effect was studied by several methods, which are in the following order:

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Inoculums preparation:

The antibacterial tests must be carried out from the young cultures of (18 to 24 hours) in exponential growth phase. By taking 2 or 3 identical colonies in 5 ml of sterile physiological water, the mixture was sacked by a vortex for a few seconds.

Standardization of the suspension from 106 to 107 CFU/ml was performed using a spectrophotometer set at a wavelength of 625 nm. According to McFarland, an optical density between 0.08 and 0.1 corresponds to a concentration of 107 to 108 germs / ml.

Disc diffusion method (aromatogram):

The aromatogram is based on a technique in medical bacteriology, called antibiogram or method by diffusion in agar medium or disk method. The principle of this method is based on the diffusion of antimicrobial compounds in a solid medium (Muller Hinton), by the appearance of a zone of inhibition around the disks after a certain time of contact between the aromatic extract (juice) and the effect of the antimicrobial product on the target is assessed by measuring the zone of inhibition. Aseptically the Muller Hinton (MH) agar culture medium was poured in into dishes at the rate of 15 ml per dish and allowed to cool and solidify on the bench. The bacterial culture suspension of about (106 CFU / ml) was prepared from an exponential growth phase culture and is then plated on the surface of the agar medium (MH) using a swab.

Deposit of records

Using a sterile clip, successively take four Wattman paper discs and arrange them on the surface of the inoculated agar, each inoculated with a precise amount (10 µl) of garlic juice (with one of the concentrations The Petri dishes are then closed and incubated at 37 ° C / 24h.

The well diffusion method

The principle of this method is based on the radial diffusion of the antibacterial substances of the aromatic extract studied from a well giving a zone of clear and easily measurable inhibition, it consist on cutting a vertical circular hole in the agar and pouring a quantity of the aromatic extract with a known concentration.

The seeding of the inoculums prepared beforehand is carried out by swabbing on the surface of the (MH) agar medium cast in dishes. After 15 minutes, four wells were cut using a Pasteur pipette (the 6 mm thick end). The bottom of the wells is sealed with a drop of (MH) agar to limit the diffusion of the juice under the agar. Then, 20 µl of the juice is inoculated into each well (where each one receives a determined concentration). The dishes were then closed and incubated at 37 ° C. for 24h.

The results were read by measuring the diameter of the inhibition area around each disc using a scale (mm). The results are expressed by the diameter of the zone of inhibition and can be symbolized by signs based on the sensitivity of the strains to the aromatic extract (garlic juice).

Not sensitive (-) or resistant: diameter < to 8mm; Sensitive (+): diameter between 9 and 14 mm; Very sensitive (++): diameter between 15 to 19 mm; Extremely sensitive (+++): diameter > to 20.

RESULTS AND DISCUSSION

The results obtained in the experiments are shown in Tables 1 and 2. Fresh garlic juice showed the highest antibacterial activity. The most biologically active compounds in garlic found is allicin (diallyl thiosulfinate or diallyldisulfide), and the most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 and 30 mg/g in fresh and dry garlic, respectively [6].

According to Iwalokun et al. (2004) [7], the antibacterial effect of garlic extract against several multi-resistant bacterial strains, including E. *coli* and S. *aureus* was significant, the diameters of the inhibition zones were 21.5 mm and 24.6 mm, respectively. These are slightly lower compared to our results which are moderately of the order of 23 mm and 30 mm.

The results obtained by Srinivasan et al. (2009) proved the effect of garlic juice at different concentrations (100%, 50%, 25% and 10%), on E. *coli* and S. *aureus* with inhibition zone diameters corresponding respectively 33 mm, 16 mm, 8 mm, and 0 mm, those results are in concordance with those obtained in this work. However, it should be noted that the effectiveness of this juice is directly proportional to its concentration [8].

The antibacterial activity of other garlic varieties (Ophioscordon and Sativum) was tested on pathogenic species and reported inhibition zone diameter in an average of 19mm [9]. By comparing the results obtained by the micro-atmosphere method with those obtained by other methods (disk diffusion and diffusion), the vapors of garlic juice don't have effective antibacterial action than that manifested by direct contact between micro-organisms.

It was reported that the antibacterial effect of garlic juice according to Pibiri et al. (2003) [10] who demonstrated a bactericidal effect manifested by the absence of colony development because of active substances such as allicin and other hydrophilic derivatives responsible for the anti-bacterial action [11].

Table N°01: Inhibition zone diameter *E. coli* and *S. aureus* in the disc diffusion method.

Juice concentration (%)	Inhibition zone diameter (mm)	
	E. *coli*	S. *aureus*
100	23	30
75	14,5	17
50	0	0
25	0	0

Table N°2: Inhibition zone diameter *E. coli* and *S. aureus* in the well diffusion method.

Juice concentration (%)	Inhibition zone diameter (mm)	
	E. coli	*S. aureus*
100	20	30
75	15,8	21
50	2,5	0
25	0	0

CONCLUSION

The research for new purely natural antimicrobial substances is the main concern of most researchers at present. For this reason we were interested in evaluating the antibacterial effect of garlic, which is a food and medicinal plant at the same time. Our study was based on the extraction of garlic juice by a simple pressing technique in order to demonstrate its antibacterial effect against E. *coli* and S. *aureus*. In this study, it was proved that garlic juice plays a very important role in the fight against the growth of the bacteria tested (E. *coli* and S. *aureus*) and can therefore replace by its plant nature and its biological property the use of chemical synthesis antibiotics that always had unwanted side effects on the body such as allergies and antimicrobial resistance.

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