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In vitro Antibacterial Evaluation of Fresh Garlic Juice (*Allium sativum* L.) Cultivated in Sudan

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Abstract: Garlic pulp is a very important spice with many therapeutic properties, well known all over the world. The Sudanese garlic has strong odour compared to other imported varieties and studies on it are scant. The *in vitro* antibacterial investigation using disc-diffusion method revealed that garlic has effective antibacterial activity against most tested bacteria and it was a competitor to the antibiotic (Chloramphenicol). However, its efficacy was much higher against the gram-positive bacteria. The highest susceptible gram-positive bacteria was *Staphylococcus epidermidis* (27.5±4.5mm), followed by *Staphylococcus saprophyticus* (20.5±0.5), *Staphylococcus aureus* (20.5±0.5mm), *Bacillus cereus* (18.5±0.5mm) and *Streptococcus pneumonia* (15.0±1.0mm), respectively. While, the most susceptible gram-negative bacteria was Escherichia coli (14.0±0.0mm) and *Shigella flexsneri* (14.0±1.0mm), followed by *Proteus vulgaris* (12.5±0.5mm), and *Klebsiella pneumonia* (10.0±1.0mm), respectively. Only *Pseudomonas aeruginosa* did not show any susceptibility toward garlic juice. The use of fresh garlic pulp in the daily dietary habits, particularly in developing countries, unhygienic areas and rural cantons, may lead to minimizing the probability of spread of bacterial diseases in these areas.

Keywords: Antibacterial; garlic; Allium sativumm; medicinal plant; Sudan.

1. Introduction

Plants have been the basis of medication since ancient times until the 19th century; the 20th century came with a new vision in pharmaceutical sciences and turned to pure and isolated chemicals, instead of plant extracts [1]. Penicillin, which discovered in 1928, and subsequently followed by the discovery of different classes of antibiotics has transformed medicine and achieved "Temporary" victory over microbial diseases. However, within few decades the antibiotic-resistant crises have strongly emerged [2]. As a result of this crisis, interest in medicinal plants has renewed again as an alternative source for new antimicrobial drugs, more natural, diverse and with minimal side effects [3]. Food improve health, this principle was well known to ancient civilization, it is still applied in east particularly China, but the western science has a limited knowledge about food and ignoring that foods have a therapeutic capacity [4]. Garlic is definitely one of these foods, the reason behind the importance of garlic as medicinal food is referred to the numerous investigations indicated that garlic has many biological and physiological benefits on the human body. Studies showed that garlic (*Allium sativum*) was found to have many pharmacological and bioactive benefits such as anti-oxidant, anti-cancer, anti-platelet aggregation, anti-microbial, anti-coagulant and fibrinolytic, anti-hypertensive, anti-diabetic, immunomodulating, hepatoprotective and prevention of cardiovascular diseases [5]. These biological and biomedical properties of garlic are attributed to its chemical constituents, which are mainly sulfur compounds, these compounds present in garlic are higher than any other Allium species, which are responsible for the distinctive garlic's pungent odor [6]. From the ethno-pharmacological point of view, Garlic variety of the current study is from Sudan, particularly Northern Sudan. Where bulbs of garlic are used traditionally as a spice, anti-septic, anti-hypertensive and to reduce blood pressure and cholesterol level [7]. It is important to mention that, in the Sudanese markets, there are two famous varieties of garlic, one exported from China, and the other is local, mostly cultivated in Northern Sudan, Chinese variety is easy peeling and bigger in size, while Sudanese variety is smaller but preferable for its strong odor. The current study aimed to evaluate the antibacterial activity the Sudanese garlic (*in vitro*), against varying bacterial isolates.

2. Materials and Methods

2.1. Plant Materials and Extraction

Garlic was purchased from local markets in Khartoum, Sudan. In this Study, the author used Sudanese variety. Fresh bulbs of the garlic were divided into cloves and peeled, and directly ground using machine grinder followed by mashing using mortar and pestle, and then squeezed, without adding any solvent or water, the garlic juice obtained was filtered using 4 layers of muslin cloth and the filtrate (juice) was used directly for the microbiological

evaluation after dipping sterile filter papers (6 mm), cut from Whatman No.1 filter paper. The pre-experimental examination showed that, the 6 mm filter paper (Whatman No.1) absorb approximately 15 µl.

2.2. Test Microorganisms

A varying of referenced microorganisms was used in this investigation, including Gram-negative and Gram-positive bacteria. The Gram-positive bacterial strains were *Bacillus cereus* ATCC® 10876™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus aureus* ATCC® 29213™, *Staphylococcus saprophyticus* ATCC® 43867™ and *Streptococcus pneumonia* ATCC® 49619™. The Gram-negative bacterial strains were *Escherichia coli* ATCC® 25922™, *Proteus vulgaris* ATCC® 6380™, *Klebsiella pneumonia* ATCC® 27736™, *Pseudomonas aeruginosa* ATCC® 9027™ and *Shigella flexsneri* ATCC® 12022™.

2.3. *In vitro* of Antimicrobial Testing

The antibacterial activity of fresh garlic juice was evaluated using Kirby-Bauer disc diffusion method as mentioned in [8] with minor modification. Prior to the experiments, bacterial isolates were sub-cultured overnight in Mueller-Hinton broth, then all cultured plates were transferred and kept in the refrigerator (4°C) until used within the same day, so we got fresh working bacterial strains at the exponential phase. Bottles containing 20 ml of Mueller-Hinton agar were autoclaved and poured hot on sterile Petri-dishes (90 mm in diameter) and left until solidified. Working bacterial strains were adjusted as McFarland standard, then 100 µl from each strain was loaded on a separate Mueller-Hinton plate and speeded using sterile cotton swap. Discs saturated with garlic juice (about 15 µl/disc) and as well discs saturated with 15 µl of Chloramphenicol (At concentration 5mg/ml) as antibacterial control was placed on the seeded agar plates previously prepared, and incubated at 37°C for 24 hours The *in vitro* antimicrobial activities were then determined by measuring the clear zone of inhibition to the nearest millimeter (mm) ± standard error of mean.

3. Results and Discussion

In the present study, the *in vitro* antibacterial evaluation of the Sudanese garlic showed that it has an efficient antibacterial capacity. As shown from Figures 1 and 2, and Table 1, garlic is highly effective against all tested gram-positive strains. The highest mean zone of inhibition recorded by *Staphylococcus epidermidis*(27.5±4.5), followed by *Staphylococcus saprophyticus* (20.5±0.5), *Staphylococcus aureus* (20.5±0.5), *Bacillus cereus* (18.5±0.5) and *Streptococcus pneumonia* (15.0±1.0), respectively. On the other side, the most susceptible gram-negative bacteria was Escherichia coli (14.0±0.0mm) and *Shigella flexsneri* (14.0±1.0mm), followed by *Proteus vulgaris* (12.5±0.5mm), and *Klebsiella pneumonia* (10.0±1.0mm), respectively (Figures 2 and 4, Table 2). Only *Pseudomonas aeruginosa* did not show any susceptibility toward garlic juice. Similar studies reported that the aqueous extract Garlic collected from Pakistan exhibited noticeable antibacterial activity against all tested bacteria higher than ethanol and methanol extracts, the susceptible bacteria were *S. epidermidis*, *S. aureus*, *B.subtilis*, *P. aeruginosa*, *K. pneumonia*, *S. typhi*, *E. coli* and *Shigella sp.*, respectively [9]. From that study, we observed that *P. aeruginosa* was susceptible to garlic and in our study it was not, because bacterial tolerance varies between strains, besides environmental conditions such as soil, water and temperature may lead to some variation in the bioactive constituents of the plant. Another investigations claimed that, different concentration of methanol and aqueous extracts of garlic pulp showed considerable and varied antibacterial activity against different clinical isolates of *Staphylococcus aureus* (Gram-positive), moreover the efficacy of garlic extract did not affect with the pH levels (from 6.9 to 7.4) [10, 11]. We intended in this study to use only fresh garlic and not extracted to avoid an indirect effect of the extraction processes. Garlic is rich in sulfur volatile compounds, a study conducted on black and fresh garlic succeeded to isolate 51 volatile compounds from garlic pulps, this study also showed that there were remarkable differences and variation on sulfur volatile profile between black and fresh garlic [12].

Figure-1. Representative photos showing the effect of garlic juice (G) compared with the chloramphenicol (Ch) against the gram-positive bacteria

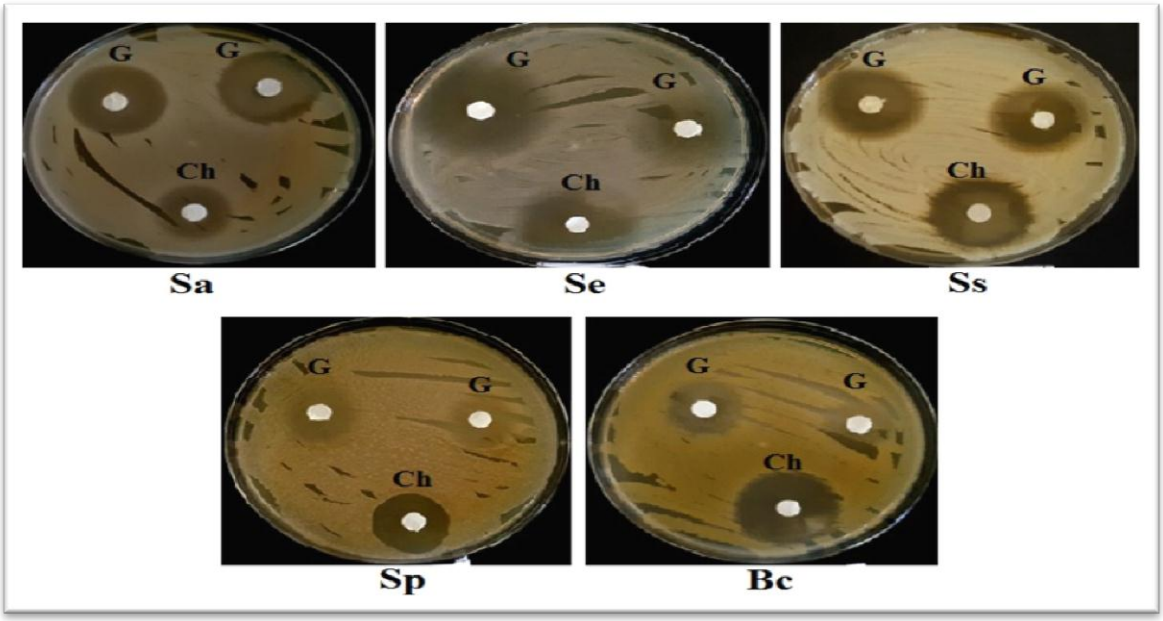
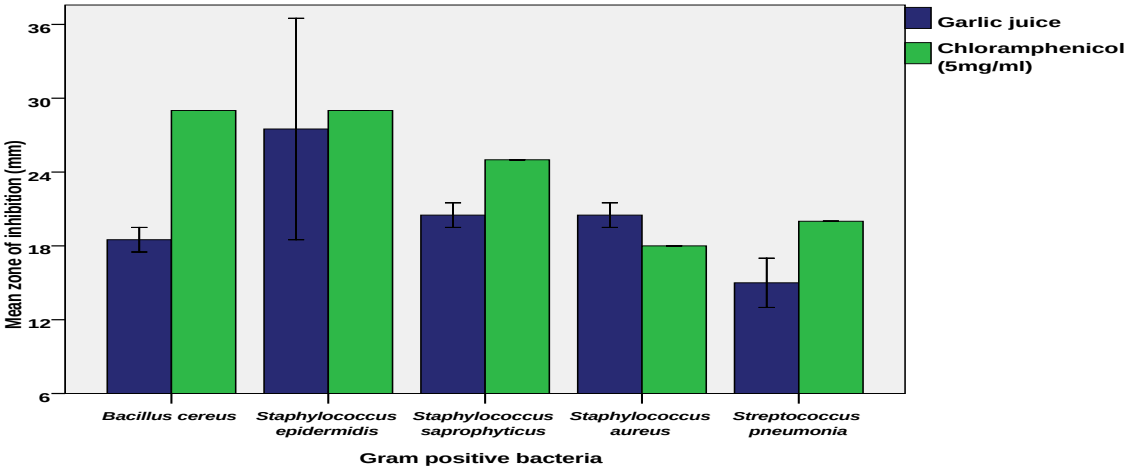


Table-1. Antibacterial activity of garlic juice against gram-positive bacteria

Tested	Zone of Inhibition (mm)				
	Bc	**Se**	**Ss**	**Sa**	**Sp**
Garlic* (15µl/disc)	18.5±0.5	27.5±4.5	20.5±0.5	20.5±0.5	15.0±1.0
Chloramphenicol** (15µl/disc)	29	29	25	18	20

Bc=Bacillus cereus ATCC® 10876™, Se=*Staphylococcus epidermidis* ATCC® 12228™, Ss=*Staphylococcus saprophyticus* ATCC® 43867™, Sa=*Staphylococcus aureus* ATCC® 29213™, and Sp=*Streptococcus pneumonia* ATCC® 49619™. *15 µl form the fresh filterate (juice) of garlic without additives. ** 15µl from chloramphenicol (5mg/ml). Zone diameter equal 6 mm= no inhibition.

Figure-2. Efficacy of garlic juice on gram-positive isolates compared with the antibiotic (Chloramphenicol)* *The paper disc diameter (6mm) was ommited.



Interestingly, the fresh juice of garlic exhibited higher effects on the gram-positive bacteria rather than the gram-negative ones (figures 1 and 3). This is definitely related to the nature of the garlic juice which is aqueous but containing considerable amount sulfur and volatile compounds [6]. Also, this can be explained by the fact that the structure of cell wall (the peptidoglycan layer) of the gram-positive bacteria allows hydrophobic molecules to penetrate into the bacterial cells and damage its internal structure, while the cell wall of the gram-negative is much complicated , where there is an outer membrane covering the cell wall (the peptidoglycan layer) which consists of a double layer of phospholipids, makes it more resistant to many natural extracts except some extracts with small hydrophilic compounds which are able to penetrate the outer membrane of the gram-negatives and the cell wall and subsequently destroy the cell [13]. Accordingly, fractionation and identification of the bioactive compounds from garlic may lead to explore some antibacterial compounds more effective on both of the gram-positive and gram-negative bacteria. Allicin was isolated from garlic and it was found responsible for its antibacterial efficacy, as it showed a considerable antibacterial effect against both of gram-positive and gram-negative bacteria [14].

Moreover, Lwalokum, *et al.* [15] cited that the aqueous extract of garlic exhibited good antibacterial activity against 133 multi-drug resistant isolates including gram-positive and gram-negative bacteria, and also revealed noticeable antifungal activity against 10 clinical isolates of *Candida spp*. Mnayer, *et al.* [16] mentioned that the essential oils extracted from garlic pulp recorded high antibacterial activity against food-borne pathogens, including two gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*), and three gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli* and *Campylobacter jejuni*).

Figure-3. Representative photos showing the effect of garlic juice (G) compared with the chloramphenicol (Ch) against the gram-negative bacteria

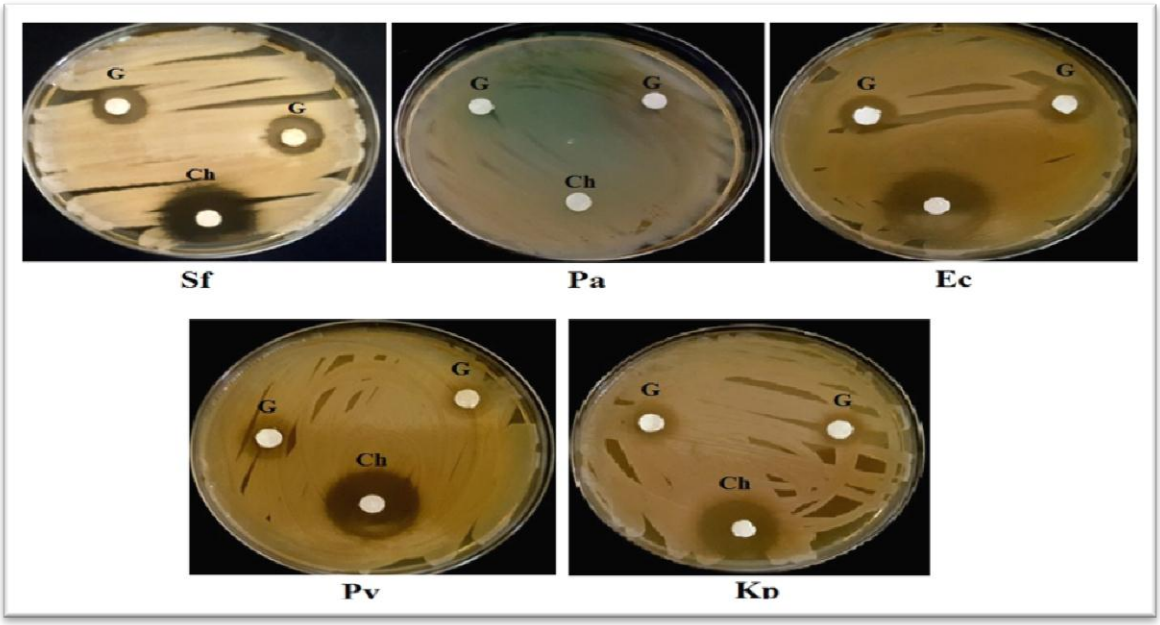
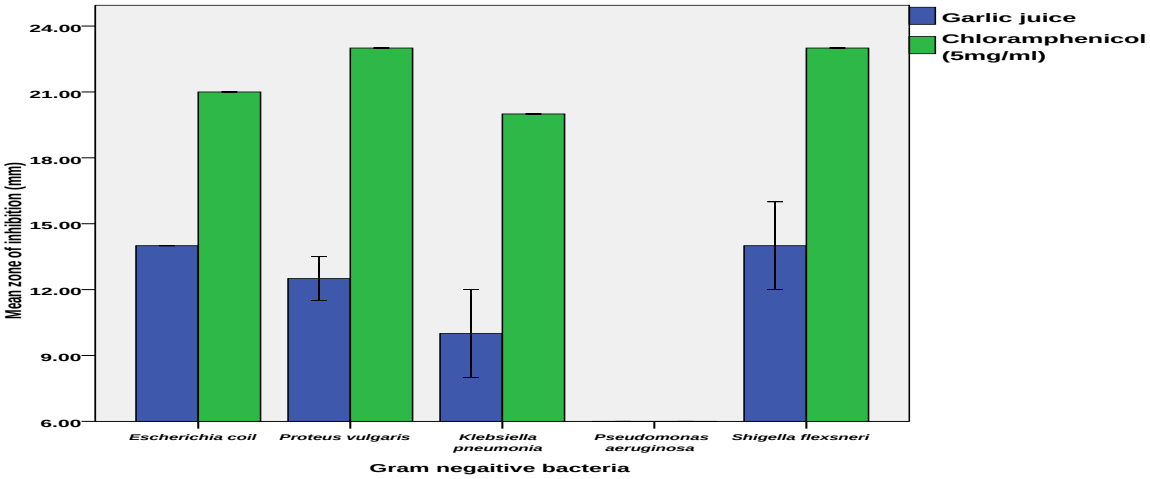


Table-2. Antibacterial activity of garlic juice against gram-negative bacteria

Tested	Zone of Inhibition (mm)				
	Ec	**Pv**	**Kp**	**Pa**	**Sf**
Garlic* (15µl/disc)	14.0±0.0	12.5.0±0.5	10.0±1.0	6.0±0.0	14.0±1.0
Chloramphenicol (15µl/disc)**	21.0	23.0	20.0	6.0	23.0

Ec=*Escherichia coli* ATCC® 25922™, Pv=*Proteus vulgaris* ATCC® 6380™, Kp=*Klebsiella pneumonia* ATCC® 27736™, Pa=*Pseudomonas aeruginosa* ATCC® 9027™ and Sf=*Shigella flexsneri* ATCC® 12022™. . *15 µl form the fresh filtrate (juice) of garlic without additives. ** 15µl from chloramphenicol (5mg/ml). Zone diameter equal 6 mm= no inhibition.

Figure-4. Efficacy of garlic juice on gram-negative isolates compared with the antibiotic (Chloramphenicol)* *The paper disc diameter (6mm) was ommited.



4. Conclusion

The present study offers a scientific basis for the traditional use of fresh garlic against bacterial pathogens. 9 of 10 referenced bacterial strains used in the present investigation were susceptible to the fresh juice of the Sudanese

garlic, particularly the gram-positives. This antibacterial efficacy could be enhanced if the bioactive compounds isolated and purified. The results also provide evidence that garlic might be apotential source of new antibacterial agent and suggested to examine its extracts against the multi-drug resistant bacteria. It is also recommended to consider garlic as important food supplement and introduced in nutrition as a basic condiment, which may help in the control of the microbial pathogens besides its other benefits on health.

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