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Comparison of the Antibacterial Activity of the Extracts of Three Cultivars of Garlic (*Allium sativum* L.)

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KEYWORDS

Allium sativum L., Allicin, inhibition zone, immune responses, local, Chinese, Egyptian

ABSTRACT:

This study was conducted on three varieties of garlic plant *Allium sativum* L. (local - Chinese - Egyptian) belonging to the Alliaceae family. Samples were collected from the local market because it is a crop of great economic and nutritional value and has medicinal properties such as contributing to blood thinning, enhancing resistance against intestinal pathogens, enhancing immune responses, reducing allergies, and being a source of vitamins, antioxidants, fiber, and minerals. The study aimed to test the antibacterial activity of garlic plant extracts against Gram-negative and Gram-positive bacteria (*Staphylococcus, Streptococcus, Salmonella, and Escherichia coli*), in addition to testing their sensitivity to some antibiotics. The results showed a clear inhibition zone and sensitivity in the local variety for all four species, while *(Strepto.)* was sensitive to the extracts of the three varieties. The (Chinese and Egyptian) extracts, however, were resistant to *(E. coli, Salmo, and Staph)*. This is attributed to the different concentrations of active ingredients among the varieties and their concentration in the local variety. These differences may also be influenced by soil variability, the agrochemical composition of the soil, and climatic conditions. The bacterial species showed clear resistance to some antibiotics, with the exception of (CTX 30), which had an inhibitory effect on *(Strepto.* and *Staph.)*, and (NV/NET 30), which had an effect on *(E. coli.)*

1. Introduction

Plants have continued as a source of medicinal compounds to play a vital role in maintaining human health since ancient times. Recently, medicinal plants have received special attention, as they are an important health and economic source in developed and developing countries, where the global role of plants in treating diseases has become clear. They have provided the basis for global medical systems in the history of humanity is that more than 60% of the world's population and 80% of developing countries depend directly on it to obtain their medicines (El-Mokasabi, 2014). There has been a continuous increase in the search for alternative and effective food preservation compounds aiming to partially or completely replace antimicrobial chemical additives (Jitu, *et al.,* 2011). Extract from many plants used as flavoring agents and spices in foods and beverages has been used therapeutically for centuries (Kumar, *et al.,* 2010). However, there are few data on the antimicrobial activities of most medicinal plants (Priscila, *et al.,* 2007).

Garlic (*Allium sativum*) Family: Amaryllidaceous is an aromatic herbal spice and one of the oldest and most important herbs that has been used since ancient times in traditional medicine. It is the second type of Allium widely used along with onions (*Allium cepa* L). Studies show that it may be it is native to Asia, but has long been naturalized in Europe, North Africa, Mexico and around the world. Garlic is used as a treatment against many common diseases such as colds, influenza, snakebites, and high blood pressure (Barnes, *et al.,* 2002). It also reduces cholesterol in the blood, the severity of atherosclerosis, and cardiovascular diseases, strengthens immunity, and has antimicrobial and anti-aging properties, as well as it has anti-inflammatory and protective effects against stroke, coronary thrombosis, atherosclerosis, platelet aggregation and heart disorders (Sainani, *et al.,* 1979). Garlic contains about 2,000 biologically active components, and scientists have obtained a variety of compositions and physiological activities of garlic, depending on processing and extraction methods (Bazaraliyeva, *et al.,* 2022). This activity is mainly due to the presence of sulfur compounds in their formulations (Batiha, *et al.,* 2020). Where contains a variety of biologically active compounds, including organosulfur compounds, saponins, phenolic compounds, and polysaccharides

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(Koca, & Tasci, 2016), water (65%), fiber, and adenosine. pectin, fructan, carbohydrates, fatty acids, essential amino acids, nicotinic acid, phospholipids, prostaglandins, lectins, enzymes, sulfur-containing compounds in addition to other biologically active compounds. Such polyphenols are mainly flavonoids, minerals (Ca, Fe, I, K, Mg, Na, Zn) and vitamins (A, B1, B2, B6, C). The main sulfur compound in garlic is Alliin, which is converted to Allicin by the enzyme

Garlic (*Allium sativum* L) has shown broad antibiotic activity against Gram-negative and Gram-positive bacteria including Escherichia, Salmonella, Staphylococcus, Streptococcus, Rlebsiella, Proteus, Bacillus, Clostridium, Helicobacter, Pylori (Srinivasan, *et al.,* 2009) and on diarrheal organisms, where Allicin, the active component of garlic, acts by partially inhibiting DNA and protein synthesis as well as completely inhibiting DNA synthesis as its primary target (Alli, *et al.,* 2011), the susceptibility of bacteria to garlic may also depend on the structural differences of the bacterial strains.

Recently, garlic has been shown to be effective against a large number of Gram-positive and Gram-negative bacteria and acid-fast bacillus (AFB). These studies included species: Salmonella, Escherichia coli (Adler & Beuchat, 2002), Pseudomonas, Proteus, Staphylococcus aureus (Cavallito & Bailey, 1944), Escherichia coli, Salmonella (Johnson & Vaughn, 1969), Klebsiella (Jezowa & Rafinski, 1966), Micrococcus, Bacillus subtulis (Sharma *et al.,* 1977), Clostridium (De Witt *et al.,* 1979), Mycobacterium (Delaha & Garagusi, 1985) and Helicobacter (O'Gara *et al.,* 2000). It has also been shown that garlic extract, especially Allicin, has bactericidal effects on some vancomycin-resistant Enterococcus (VRE) bacteria (Jonkers *et al.,* 1999), garlic extracts have also been reported to be effective in reducing insulin resistance (Padiya & Banerjee, 2013). Due to the multiple uses of antimicrobial agents in medicine, the effectiveness of existing antimicrobial agents has decreased due to the resistance of organisms to those agents. In addition to the magnitude of the problem of drug resistance, some researchers have chosen to develop alternative strategies. Garlic has been shown to have synergistic effectiveness with antibiotics in resisting microbes. This is evident. Especially in Staphylococcus aureus and enter pathogenic bacteria, such as Shigella and Salmonella species (Roberta & Fabiana, 2010).

2. Materials and Methods

i. Sample processing: this study was conducted on three varieties of garlic plants (local - Chinese - Egyptian). Samples were collected during the month of March 2023 from the local market, and dry and wet samples were prepared and prepared.

ii. Preparing extracts: Dry samples: We weigh 30 grams of the plant, cut the samples into small slices, and place them in a preheated oven at 180°. Then we turn off the oven, take out the samples, and then weigh them after drying. Wet samples: We weigh 30 grams of the plant, add 100 ml of distilled water, shake the bottle well, and leave for 12 hours. Then perform the extraction process and keep the extract until used.

iii. Types of bacteria under study:

- Staphylococcus aureus is a Gram-positive bacterium (Woodford & Livermore, 2009).
- Streptococcus, which is a gram-positive, non-motile bacteria and is a diverse group of cocci usually arranged in pairs or chains (as opposed to the clusters formed by staphylococci) (Patrick, *et al.,* 2012).
- Salmonella, which is a gram-negative bacteria (Ryan, *et al.,* 2017).
- Escherichia coli, which is a motile Gram-negative bacillus bacterium, ranging in length from 1-3 mm (Mellies *et al,* 2007).

iv. Primary phytochemical tests: alkaloids, sugars, resins, tannins, saponins, flavonoids, starch, phenols and glycosides were detected based on (Adedayo, *et al.,* 2001)

v. Biological tests: Antimicrobial sensitivity was tested in order to determine the antimicrobial activity of the previously obtained plant extract on the aforementioned microbial species based on the method (Chakraborty M. & Mitra A, 2008). In addition to a sensitivity test against some antibiotics as a comparison.

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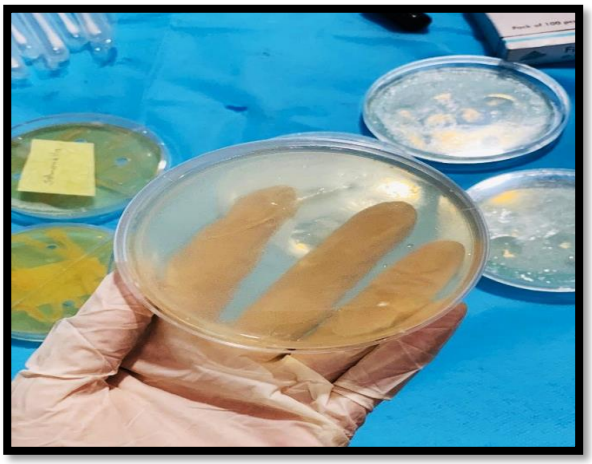

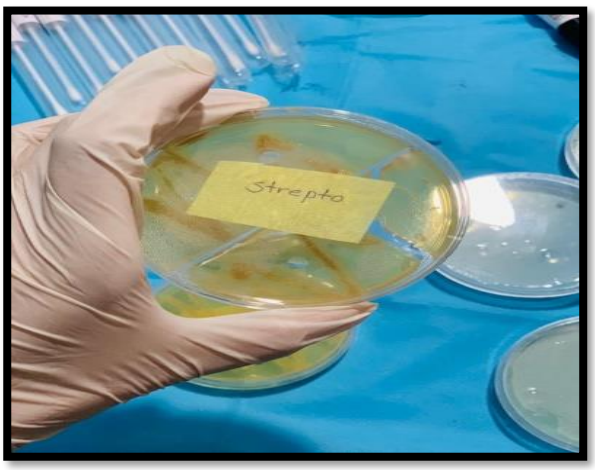

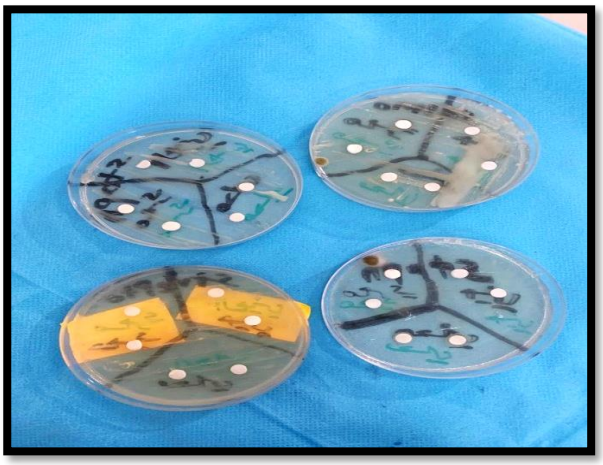

3. Results and Discussion

- **Phytochemical Tests for *Allium sativum* L**

The results of the initial detection test conducted on the extracts showed color notes and colored sediments. Through their interpretation, we concluded that there are several chemical families as shown in the following Table 1.

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Table 1

Chemical families	Color/evidence of presence	Local	Chinese	Egyptian
Alkaloids	Orange color	+	+	+
Sugars	Red precipitate	+	++	+
Resins	-	+	+	+
Tannins	Green color	++	+	+
Saponins	Foam appears	+	+	+
Flavonoids	Yellow color	++	+	+
Starch	-	-	-	-
Phenols	Black color	+	+	+
Glycosides	Reddish brown	+	+	+

(+) Positive, (++) High concentration, (-) Negative

As in Table (1), the tests showed the presence of the aforementioned active substances in the three varieties at different concentrations, where tannins were the highest in concentration in the sweetener, while flavonoids were in high concentration in the sweetener, while the absence of starch in the three varieties was evident.

- **Biological Tests for *Allium sativum* L**

The results are read by calculating the area of the inhibition zone (mm) that appears around the disk saturated with the extract. In table (2) shows the sensitivity of Strepto bacteria. Extracts of the three types are present and are more sensitive in the sweetener, while bacteria (*E.coli*, *Salmo*. & *Staph*.), have shown resistance to two types (Chinese and Egyptian).

Table 2

Type of Bacteria / Type of Garlic	***Staphylococcus* (*Staph*.)**	***Streptococcus* (*Strepto.*)**	***Salmonella* (*Salmo.*)**	***Escherichia coli* (*E.coli*)**
Local	++	++	+	++
Chinese	-	+	-	-
Egyptian	-	+	-	-

(++) More sensitive to extract, (+) Less sensitive to extract, (-) Resistance of bacteria to extract

As for Table (3), the results show resistance to most antibiotics, except for the antibiotic (CTX 30) for Bacterial (*Strepto*. and *Staph*.), the zone of sensitivity and inhibition was clear, while the (*E. coli*.) bacteria were very sensitive to two types of antibiotics (NV/NET 30) while showing resistance to the rest of the antibiotics. Whereas, (*Salmon*.) bacteria are absent from this.

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Table 3

Type of Bacteria / Type of Antibiotic	*Staphylococcus* (*Staph.*)	*Streptococcus* (*Strepto.*)	*Salmonella* (*Salmo.*)	*Escherichia coli* (*E.coli*)
AZM 15	-	-	*	-
CRO 30	-	-	*	-
CTX 30	+	+	*	-
NV 30	-	-	*	+
NET 30	-	-	*	+
TE 10	-	-	*	-

(+) Bacterial sensitivity to the antibiotic, (-) Bacterial resistance to the antibiotic, (*) Not testing

AZM 15: Azithromycin, CRO 30: Ceftriaxone, CTX 30: Cefotaxime, NT/NET 30: Netilmicin, TE 10: Tetracycline

Microbial resistance to antibiotics has increased, prompting scientists to focus on discovering antimicrobial activity and searching for new, natural, plant-based alternative antibiotics that are cost-effective and an important contribution to global health. One of these plants is garlic (*Allium sativum*). Garlic has been used for a long time by societies. As a traditional medicine against infectious diseases, it is available in the form of capsules and powder supplements (Mustafa, *et al.,* 2020). Through chemical detection of the extracts, it was clear the presence of active substances in the garlic plant, due to the many previous studies on it and its nutritional and medicinal importance (Reiter *et al.* 2020). The results showed areas of growth inhibition that were sensitive to Strepto bacteria. Extracts of the three types are present and more sensitive in (local), while bacteria (*E.coli, Salmo. & Staph*.) Showed resistance to two types (Chinese and Egyptian). By comparing them with some types of antibiotics, it was found that the effectiveness of garlic extracts is more sensitive than the above-mentioned antibiotics. This effect has been attributed to the presence of sulfur compounds, especially the compound Allicin, where the more concentrated it is, the stronger the effect on bacteria (Trivedi & Bhatt, 2015). This brings us to the conclusion that the concentration of active substances in the local variety is high, so it had a clear effect through the results. . The study presented by El-Mahmood, 2009 concluded; (Jafari. & Shadi., 2019) Garlic has the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria (*Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Pseudomonas aeruginosa*), that it has a broad spectrum of activity and can be used in the formulation of new broad-spectrum antibacterial substances. Several studies have confirmed and are consistent with our results that garlic has an activity that opposes microbial activity, which supports the theory of using garlic as an alternative to antibiotics or alongside traditional antibiotics.

4. Conclusion

Allium sativum (Garlic) is among the oldest cultivated plants. As it has been used medically for thousands of years, it is widely recommended to treat diseases such as asthma, bronchitis, influenza, some other diseases, as antibiotics and most medications on the market have shown unwanted symptoms, the emergence of disease-resistant microbes, and toxic effects associated with these medications.

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