

Antibacterial activity of allium sativum mixture with nano ZnO on pathogenic bacterial

Huda Rameez Hadi^{1*}, Entissar Razzaq Ebrahim², Muntaha R. Ibraheem³

¹Al Karkh University of Science, Baghdad, Iraq

²University of Raparin, College of Agriculture, Department of Horticulture

³University of Baghdad, AL Khwarizmi College of Engineering, Biomedical Engineering Department

Abstract

Allium Sativum (garlic clove) has essential biological and chemical activities and a wide range uses as antibacterial and antifungal beside that as anticancer. The biological activity of Allium sativum extract was examined in this study. The investigation of Infrared spectroscopy (FTIR) with Fourier transform was done and showed the content analysis of the FTIR test results of garlic extract which focused on oxygen hydrogen bonding, carbon-hydrogen bond, C=O, C=C, and carbon-oxygen functional groups are present., confirming a complex mixture of alcohols, alkanes, carbonyls, alkenes, and polysaccharides in the garlic extract. Also, this research summarizes biological effects were evaluated by testing the antibacterial activity of different concentrations of aqueous Allium sativum extracts against *E. coli* and *Klebsiella pneumoniae* as gram-negative and *Staphylococcus aureus*, *Enterococcus faecalis* as a gram-positive bacterium and try to enhance its antibacterial effect by using ZnO nanoparticle with garlic extract. So, this study concludes that Allium Sativum (garlic clove) extract has beneficial therapeutic effects better when combined with ZnO nanoparticle and t also shows potential as a valuable herbal therapy due to its chemical composition and pharmacological properties.

Keywords: Garlic extract Garlic (Allium Sativum), Nano ZnO, antibacterial, Pathogenic bacteria

Introduction

In Iraq, one of the major public health challenges is the rising rate of infectious diseases caused by various bacteria and the increasing resistance of these bacteria to antibiotics. This poses a serious threat to human health, making it essential to search for alternative antibacterial agents Allium sativum, or garlic, is a popular therapeutic herb. It is used in traditional treatment in medicine for ages to cure a variety of human diseases (1). It's used as an alternative medicine due to its availability, low side effects, and efficacy make it a valuable source of novel, safe, and natural medicinal compounds. Beside that garlic considered as one of the top natural and supplementary medicine sources (2).

Recent studies shows that garlic is one of preserves from inflammation and enhances the immune system, also demonstrate improvement in several health conditions, such as hyperglycemia and cardiovascular disorders, and resists diseases associated with infection. However, many nanoparticles are used for potent antibacterial properties against pathogenic microorganisms, spatially Zinc oxide nanoparticles (ZnO NPs) Which

used as antibacterial against *Escherichia coli* and *Staphylococcus aureus*. The nanoparticles antimicrobial act by many mechanism-like restricted vital enzymes, or by binding to bacterial cell walls, compromising cell structure, and ultimately impeding bacterial proliferation. One of the most common important and effective compounds is the Sulphur, which responsible for its antibacterial properties, since these compounds have been shown to restrict the growth of both gram-positive and gram-negative bacterial strains.

Besides that, the concentration and the potency of these reactive components vary due to garlic's chemical composition. The utilization of nanoparticles to augment the antibacterial efficacy of garlic extract has emerged as a promising technique to enhance its bioactive potential. Numerous studies have investigated the synergistic effects of garlic and nanoparticles; however, the precise interactions and mechanisms remain uncertain. In This study discusses the garlic extract antimicrobial properties of, ZnO nanoparticles, and the mixture effect on four bacterial strains. The results could contribute to the development of alternative antibacterial medicines to replace current medications that have diminished

efficacy owing to bacterial resistance (6,7).

Methodology and Materials

Bacteria Strains and ZNO source

The bacterial strains used in this study (*Enterococcus faecalis*, *Escherichia coli*, and *Staphylococcus aureus*) beside *Klebsiella pneumoniae* were used from previously reported microbiological strains from Baghdad University's biotechnology department, while the Zinc oxide (Sigma-Aldrich, USA) supplied a suspension of nanoparticles (ZnO NP), in many concentrations, with an average particle size of 35 nm.

The liquid extract was made by mixing one liter of filtered water and 95% methanol with powdered *Allium sativum*. After that Vacuum evaporation at 60°C and 40°C used to remove the filtrate under non-liquid conditions.

Culture and inoculum preparation

To initiate bacterial growth, all tested strains were reactivated by inoculating them onto their respective agar media. Cultivation was carried out on brain heart infusion (BHI) agar plates, The samples were incubated at 37°C for 24 hours in an inverted position. Post-incubation, individual colonies were selected, transferred into designated broth media for sub-culturing. The bacterial suspensions were adjusted to a concentration of 1.5×10^8 CFU/mL by using the McFarland standard in sterile 0.85% sodium chloride solution before evaluating the garlic extract as an antimicrobial.

Preparation of garlic extract

The *Allium sativum* bulbs, chopped into small pieces after rinsing with tap water then squashed the chopped garlic and dried at 40°C for seven days in oven, then pulverized into fine powder using an electric blender. A 20 g sample of the garlic powder was boiled in 100 mL of deionized water for 30 minutes. The mixture was subsequently filtered through Whatman No. 1 filter paper and stored at 4°C until further use.

Antibacterial activity

The Antibacterial effect evaluated by using well

diffusion method before that the minimum inhibitory concentration (MIC) of both garlic extract and zinc oxide (ZnO) nanoparticles was determined using a modified microdilution technique in 96-well microplates. Garlic extract concentrations ranged from 120 to 0.12 mg/mL, while ZnO nanoparticle concentrations varied from 80 to 0.78 mg/mL. Plates were incubated at 37°C for 18 to 24 hours to evaluate antibacterial efficacy. The MIC values were determined in triplicate to ensure accuracy and consistency. In agar well diffusion method used to test antibacterial effects, sterile agar plates were spread with 0.1 mL of standardized bacterial suspension using an L-shaped spreader. Then, a sterile 5 mm corn borer was used to make three uniform wells in the agar. Using a micropipette, 100 µL of each tested concentration (15 mg/mL for garlic extract, 2.5 mg/mL for ZnO, and their mixture in equal volume) was added into the wells. Plates were left on a clean bench for 1 hour to allow the extracts to diffuse into the agar. Then, after incubation at 37°C for 24 hours the diameter of the clear zone around each well (inhibition zone) was measured in millimeters to assess antibacterial activity (9). Each antibacterial assay was repeated three times independently, and the mean value of the inhibition zone diameters was determined and recorded as the final inhibition zone for each set.

Result and Dissection

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The antimicrobial effects were seen due to active components in the garlic extract were released into the surrounding media. The extract tested on different bacterial strains showing different result to the extracts, indicating that various chemical components were extracted from garlic. many factors induce antimicrobial activity like pH of the extract, with the aqueous extract having the highest pH. The One of the theories that the chemical compounds in the garlic extract are interacting with and disrupt bacterial cell membranes, leading to bacterial cell death. This theory was confirmed by FTIR analysis (fourier Transform Infrared Spectroscopy) of the garlic extract which showed similar spectral patterns, by presence of specific functional groups such as phenolics, phenolic acids, and alkaloids. These groups can act as capping agents when combined with nano

Zinc Oxide (ZnO), developing the antibacterial effectiveness of the extract. The FTIR spectra displayed multiple peaks, indicating the complex biological nature of the garlic extract. (10,11).

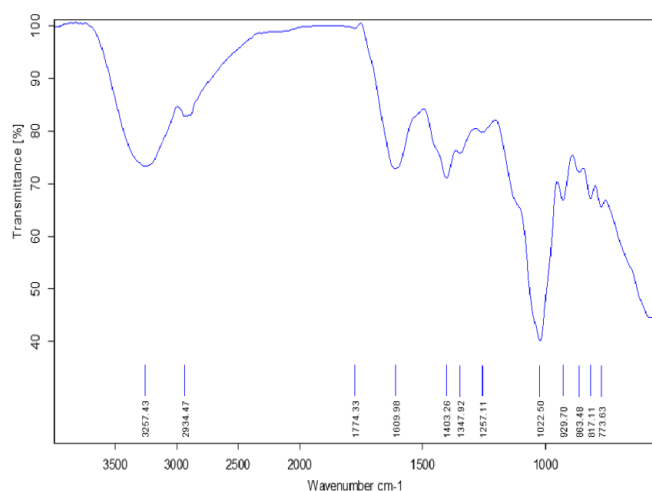
The FTIR analysis, shown in Figure 1, displays various absorption bands at the following wavenumbers: 3257.43 cm^{-1} (broad stretch corresponding to hydroxyl groups, OH, involved in hydrogen bonding), 2934.47 cm^{-1} (OH stretching), 1774.33 cm^{-1} (carboxylic acids), 1609.98 cm^{-1} (primary amides), 1403.26 cm^{-1} (alcohols), 1347.92 cm^{-1} (ethers, C-O stretching), 1257.11 cm^{-1} (esters), and 1022.50 cm^{-1} indicating amide and ether groups. Additional peaks appeared at 929.70, 863.48–817.11, 773.63, and 698.71–610.08 cm^{-1} , which correspond

to halogen functionalities. These multiple bands suggest, the detection of fluorine, along with the foundation of two or more bands indicating stretching vibration of C-C and C-Br (Table 1).

The FTIR spectrum of the current garlic extract is consistent with previous studies that identified similar functional groups in garlic extracts solution. [12,13]. Depending on the FTIR spectrum described above, it can be concluded that phenolic compounds, organosulfur compounds, amino acids, carboxylic groups, and proteins are the main active components responsible for the antimicrobial efficacy of garlic extract. This conclusion is supported by multiple studies that have identified these key phytochemicals in garlic extract. (14,15).

Table 1. FTIR analysis and corresponding functional groups

Number of waves\cm	Structures	Functional Group
900-700	CCl, CC or CH or CO stretch	halogen functionalities, DNA and RNA backbones
1200-1010	CO stretch	Ethers, Amide
1465-1440	CH ₃ , CO	Methyl groups, inorganic carbonate
1475-1400	CH ₂	Methylene groups aliphatic, inorganic carbonate
1670-1615	CC stretch	Alkenes
1800-1680	CO stretch	Carbonyls groups, ester fatty acid
2850-2100	CH and CH ₂	Aliphatic group
3000-2850	CH stretch	Alkyl groups aliphatic group, aromatic group
3550-3000	NH stretch	Primary amines, aminoacidic group
3600-3200	OH stretch	Alcohols, water, polyphenol



FTIR spectrum of prepared *Allium Sativum* (garlic clove)

Fig 1. FITR spectrum of prepared allium sativum

Effects garlic extract on bacterial growth

The antimicrobial effects of the nanoparticles and garlic extract were tested against two types of gram-positive bacteria (*S. aureus* and *Enterococcus faecalis*) and two types of gram-negative bacteria (*E. coli* and *K. pneumoniae*) using the agar well diffusion method. After finding the minimum inhibitory concentration (MIC) for both ZnO nanoparticles and garlic extract, the sizes of the clear zones where bacteria did not grow (inhibition zones) were measured. The results are shown in the figure below and in (Table 2). Garlic extract has also been found effective against many other bacteria, including *Bacillus cereus*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Micrococcus flavus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, and *Staphylococcus aureus*. [16-17].

Table 2: Effects of nanoparticles (supernatant) materials and garlic extract on bacterial growth by agar well diffusion method

Concentration (mg\ml)	Inhibition Zone (mm)			
	<i>S. aureus</i>	<i>Enterococcus faecalis</i>	<i>E. coli</i>	<i>K. pneumonia</i>
Garlic Extract	25	13	17	17
ZnO nanoparticle	15	11	13	15
Mixed from both(v\ v)	27	23	18	23

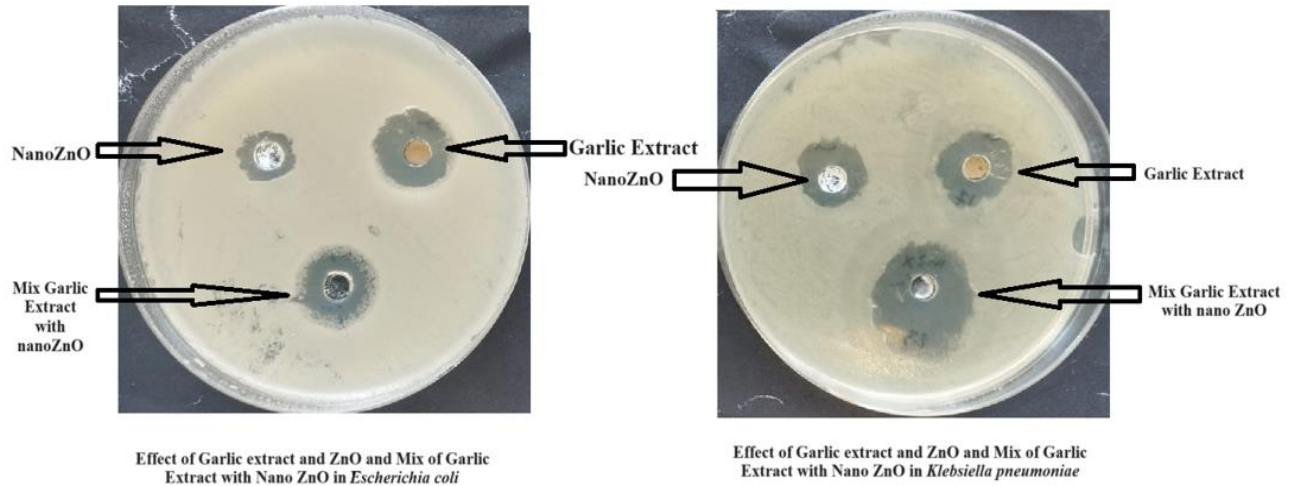


Fig 2. Nano ZNO

The findings revealed that garlic extract exerted a comparatively stronger against the four tested bacterial strains compared to nano ZnO alone, and this effect was further enhanced when both were combined. Overall, all tested bacteria—both gram-positive as well as gram-negative—were sensitive to garlic extract and nanoparticles across the concentrations adopted for this research. However, reduction in growth varied depending on the bacterial species (18)

These results align alongside previous studies on the antibacterial activities of spices from South India, as well as Chinese and Desi varieties, which demonstrated effectiveness against a broad range of bacteria including *Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Micrococcus flavus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and various *Salmonella* species (19, 22).

The enhanced antibacterial activity observed with the combination of garlic extract and nanoparticles,

as shown by larger inhibition zones in the agar well diffusion assay, may result from increased absorption and better penetration of the garlic compounds into bacterial cell walls. This synergistic effect likely improves the ability to kill bacteria and inhibit their growth more effectively than either agent alone (20,21).

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