Original Article

Effects of Prepared Garlic (Allium Sativum) Extracts on C. Albicans and s. Aureus. Isolated from Oral Cavity

Noralhuda Ahmed Hamzah[1*], Faehaa Azher Al- Mashhadane[2], Suhad M. Hamdoon[3]

[1*,2,3]Department of Dental Basic Sciences, University of Mosul, Mosul, Iraq.

E-mail: [1*]nooralhuda.20dep4@student.uomosul.edu.iq, [2]faehaaazher@uomosul.edu.iq, [3]suhadhamdoon@uomosul.edu.iq

[1*]ORCID: 0000-0003-0118-7578, [2]ORCD: 0000-0001-5122-2170, [3]ORCD: 0000-0001-6154-6961

Abstract

Background: *Candida albicans (C. albicans)* and *Staphylococcus aureus (S. aureus)* are among the most prevalent hospital-acquired and healthcare-associated pathogens. It causes severe morbidity and mortality and remains a serious clinical threat even with appropriate treatment. Therefore, more attention should be taken to the herbals and plants to extract new pharmacologically active molecules to fight against these pathogens. Garlic has been known as the most effective plant species in the treatment of bacterial infections. **Aim:** This study aimed to prepare different types of Garlic (*Allium sativum*) extracts and evaluate the antimicrobial effect against S. *aureus* and *C. albicans*, two important and common pathogens isolated from the oral cavity. **Methods:** The antimicrobial activity against both *C. albicans* and *S. aureus* of garlic extracts was evaluated by the macro-dilution method to measure the minimum inhibitory concentration (MIC) of different garlic extracts (Ethanolic Garlic extract (EGE), Aqueous Garlic extract (AGE) and Fresh Garlic extract (FGE)). Kirby-Bauer method (well diffusion method) that measures the zone of inhibition of these extracts was also used. Both methods were compared to the mouthwash containing 0.12% chlorhexidine as a control positive. **Results and Discussion**: MICs for the three types of garlic extract (EGE, AGE, and FGE) were 200mg/ml against both *C.albicans* and *S.aureus*, showing no growth compared with 0.12% chlorhexidine. The means zones of inhibition (ZOI) measured by well diffusion method for 200mg/ml of 20µl of each garlic extract (EGE, AGE, FGE) for *C.albicans* were 29 mm, 31.5mm, 32.25mm, and 12mm for EGE, AGE, FGE, and control, respectively. On the Other hand, the means of ZOI measured by well diffusion method for 200mg/ml of 20µl of each garlic extract (EGE, AGE, FGE) for *S.aureus* were 30mm, 31.5mm, 29mm, and 11.5mm for EGE, AGE, FGE, and control positive, respectively. **Conclusions:** garlic extracts (EGE, AGE and FGE) produced marked antimicrobial properties against C.*albicans* and *S.aureus* and may be a promising source for the development of effective and safe alternative against these pathogens.

Keywords: Allium Sativum, S. Aureus, C. Albicans, Zones of Inhibition, Minimum Inhibitory Concentrate.

DOI: 10.47750/pnr.2022.13.S03.122

INTRODUCTION

The incidence and prevalence of invasive infections caused by fungi have increased in recent decades, and the number of antifungal drugs available is still quite limited. Also, there is a problem associated with increased resistance of pathogenic fungi to the agents used in the therapeutic regimen (Silva *et al*., 2019).

Candida albicans and *Staphylococcus aureus* are exceptional human hospital-acquired and healthcare-associated pathogens, causing severe morbidity and mortality. They are the most common fungal and bacterial agents isolated from bloodstream infections worldwide. *Candida albicans* is a commensal fungus and is the most common fungal species in the human microbiota. It colonizes healthy individuals' oral cavity, skin, and gastrointestinal and reproductive tract (Hernandez-Cuellar *et al*., 2022).

It is also an opportunistic pathogen, able to cause both superficial and systemic infections, mainly in immunocompromised patients. On the other hand, *Staphylococcus aureus* is a commensal bacterium found on healthy individuals' skin and mucous membranes. *S. aureus* also colonizes the nasal cavity of around 30% of the human population (Hernandez-Cuellar *et al*., 2002; Carolus, Dyck, and Dijck, 2019; Hu *et al*., 2021; Nobile and Johnson, 2015).

Recently, antibiotics exhibited resistance to pathogenic microorganisms, adverse effects related to these drugs, and toxicity restricting their use. Therefore, more attention should be taken to the herbals and plants to extract new pharmacologically active molecules (El-Saber Batiha *et al*., 2020).

Allium sativum (Garlic) has been grown on all continents for thousands of years. It is known as one effective plant specie in the treatment of bacterial infections. It has been widely investigated due to the recent emergence of bacterial strains resistant to traditional antibiotics. *Allium sativum* extract inhibited the growth of a broad range of bacteria, including multidrug-resistant strains with bactericidal or bacteriostatic effects (Magryś *et al*., 2021; Bhatwalkar *et al*., 2021).

Garlic (*Allium sativum L*.) is a type of bulbous flowering plant in the genus Allium.it is an aromatic herbaceous plant widely used worldwide as food and a traditional remedy for many medical conditions. It is present as fresh or dried bulbs of *Allium Sativum* possessing composite nature. Garlic has a potent antimicrobial activity attributed to its contents of multiple organosulfur compounds (Nakamoto et al., 2020). It also has protein, sugar, vitamins, and mineral such as selenium (Kıraç et al., 2022). Garlic (*Allium sativum L*.) has been used for culinary and medical purposes in many cultures and old-world civilizations, such as Sumerians, Egyptians, Ancient Chinese, and Indians (Abidullah *et al*., 2021). Nowadays, this plant is used as a spice in cooking and is usually used as a remedy for bacterial, fungal, and viral infections (Anggraeni, Kamaluddin, and Theodorus, 2020). Over time, there have been numerous studies and researches on the effect of garlic, and its benefits in the prevention and treatment of many diseases and health problems (Farid, Yousry, and Safwat, 2022). Also, garlic preparations and prescriptions are increased because it has multiple antibacterial effects even against multidrug-resistant bacteria and is reported antiviral, antifungal, and anti-parasite (Abdel Hamed and Eman Alaa, 2021). Garlic is available in different prepared forms such as fresh or crude garlic extracts, garlic powder and other solid dosage forms, but it is usually prepared as macerated in oil, aqueous or alcoholic solvents, the difference in preparation methods affects its chemical composition and biological activity (Bhatwalkar *et al*., 2021).

S. aureus is gram-positive cocci that cause several dangerous infections resistant to treatment due to biofilm formation and can acclimate to different environmental conditions (Idrees *et al*., 2021). In addition, it is considered one of the most multiple drug-resistant bacteria in humans (Abidullah *et al*., 2021). *C. albicans* is the most prevalent and aggressive fungal pathogen in the oral mucosa (Sasi *et al*., 2021). The treatment of fungi faces high resistance in many cases. This leads to increase doses and the emergence of side effects.

Thus, this study aimed to prepare different Garlic (Allium sativum) extracts and evaluate their antimicrobial effect against two essential and common pathogens isolated from the oral cavity, which are *S. aureus and C. albicans*.

MATERIALS AND METHODS

2.1. Garlic (Allium sativum) extracts preparations

Three types of garlic extracts (Fresh garlic extract (FGE), Macerated Aqueous garlic extract (AGE) and Ethanolic garlic extract (EGE)) have been prepared from dried garlic that was purchased from local markets in Mosul city /Iraq (Imported from China).

2.1.1. Fresh Garlic extract (FGE)

The Fresh Garlic extract (FGE) was prepared on the same day as microbiological tests according to the Abdelraheem (2019) technique.

The garlic was peeled, and the outer peel was removed to obtain garlic cloves. Then, the peeled cloves were weighed to be 50g. The cloves were washed with sterile water. All tools have been sterilized. The garlic was crushed using a special garlic press and then placed in an electric mixer after adding 100 mL of distilled water to obtain a homogenized mixture. The mixture was filtered, squeezed, and then centrifuged for 15 min. Next, the extract was sterilized by filtration using 0.2 µm pore filter. Aliquots were used immediately for microbiological testing. The final extract concentration was calculated to be 23.7% (w/v) (237mg/mL) by subtracting the weight of the precipitate from the weight of the original Garlic bulbs (Figure 1).

Figure 1. FGE (yellow color) after filtration

2.1.2. Macerated Aqueous garlic extract (AGE)

The Macerated Aqueous garlic extract (AGE) was prepared by maceration method according to the Bienvenue *et al*., (2021) technique.

The dry garlic cloves were peeled. A total of 50g of peeled garlic cloves was taken. The cloves were washed with sterile water, and sterilized tools were used for crushing garlic using a special garlic press and grinder. Crushed garlic paste was mixed with 100mL of distilled water in a bottle and shaking it occasionally for five days at 3-5ºC to obtain a fine mixture which was then filtered and centrifuged for 15 min. Next, the extract was sterilized by filtration using 0.20 µm pore filter before using the extract against isolated microorganisms. The final concentration of extract was calculated by subtracting the weight of the precipitate from the weight of the original Garlic bulbs. Finally, aliquots were stored in at -20°C until required (Figure 2).

Figure 2. AGE (orange)

2.1.3. Ethanolic garlic extract (EGE)

The Ethanolic garlic extract (EGE) was prepared by maceration method according to the Sasi *et al*., (2021) technique.

The same steps were made in the peeling and cleaning dry garlic cloves, and 50g of garlic cloves undergo crushing and grinding. Then, the crushed garlic paste was mixed with 100mL of Ethyl alcohol (99%) in a bottle and shaken occasionally for five days at 2-5°C. The extract was filtered and centrifuged for 15 min. Finally, it was put in a rotary evaporator (Figure 3) for evaporation of all ethanol. The remaining extract was collected and weighted (Figure 4). A dilution with distilled water was performed to produce the concentration of 200 mg/mL before its use in the experiment.

Figure 3. rotary evaporator to evaporate ethanol.

Figure 4. EGE (green) and AGE (orange).

2.2. Antimicrobial analysis

2.2.1. Microbial samples and cultures Preparation

Two important pathogens *S.aureus* and *Candida albicans* were isolated from unstimulated human saliva, cultivated on thier selective media (Manitol salt agar for *S.aureus*) and (HiCrome Candida Diffrential Agar for Candida spp.), examined by a Gram staining technique for each one: gram-positive *S.aureus* and gram-positive fungi *Candida albicans.*, identified by vitek 2 system, To prepare the microbial culture, each microorganism was cultivated in a tube containing sterile nutrient broth for 18h. Then, it was adjusted to equal the turbidity of tube 0.5 McFarland standards.

2.2.2. McFarland standard preparation method

The McFarland standard preparation method was done according to the Bhadra *et al*., (2022) technique.

The purpose of this standard preparation is to adjust bacterial growth density depending on broth culture's turbidity. For antimicrobial sensitivity tests, the bacterial suspension of the organism should be equivalent to the 0.5 McFarland standard. Original McFarland standards were prepared by adding 0.5 mL of 1% W/V barium chloride to 99.5 mL of 1% V/V sulfuric acid with vigorous mixing to maintain a suspension. To achieve the standard density of the suspension, it the turbidity was adjusted by measuring the absorbance by a spectrophotometer at 625nm which must range between (0.08 to 0.13) which matued with 0.5 % McFarland standard represents ($1.5*10^8$) cells/ml in the microbial broth culture. So, a comparison can be made manually by holding both the standard and the inoculums broth culture tube side by side on the Wickerham card and comparing the vision of the black lines through both suspensions. If the two tubes are not matched, broth can be adjusted by adding normal saline to dilute it or by adding broth to increase the microbial density.

2.2.3. Preparation of culture media

2.2.3.1 Müller-Hinton agar (Oxoid Company, England)

This medium is a standard medium for antimicrobial sensitivity tests, prepared by adding 38g to 1L of distilled water, mixing the constituents by heating gently and stirring using a magnetic stirrer, pH adjusted to 7, and sterilized by autoclaving.

2.2.3.2 Nutrient broth

It was prepared by adding 25g of powdered medium in 1L of distilled water mixing the constituents by heating gently and stirring using a magnetic stirrer, then dispensing into tubes, sterilized by autoclaving.

2.2.3.3 Mannitol Salt Agar

It was prepared by adding 108g of powdered medium to 1L of distilled water, mixing the constituents by heating gently and stirring using a magnetic stirrer, then sterilizing by autoclaving.

2.2.3.4 HiCromeTM Candida Diffrential Agar (HIMEDIA)

It was prepared by adding 42.72 g to 1L of purified distilled water, mixing the constituents by stirring using a magnetic stirrer,hat to boiling with out autoclaving,pour into sterial petri plates cultivated for 48 h *Candida albicans* formed green colonies.

2.3. Antimicrobial sensitivity tests

2.3.1. Broth dilution method to determine the minimum inhibitory concentration (MIC)

These tests were carried out by determining the minimum inhibitory concentration (MIC) of the FGE, AGE, and EGE using the broth dilution method according to the Kowalska-Krochmal and Dudek-Wicher (2021).

2.3.1.1 Preparation of Garlic stock solutions (EGE, AGE, FGE)

The garlic extracts stock solutions were sterilized by filtration and dispensed a small volume of extract stock solution into sterile test tubes tightly sealed and stored at -20°C to be used as needed. For the preparation of inoculums, two different identified microorganisms were used: Gram-positive *S. aureus. and C. albicans.* Each organism's overnight broth culture (18h) was adjusted to equal the turbidity of tube 0.5 MCfaland slandered ($1.5*10^8$) cells/ml. The preparation of the dilutions series was done by using four test tubs. The first one was prepared by adding 9mL of sterile nutrient broth mixed with 1mL of (EGE) stock solution. In the same way, the second tube was prepared by mixing 9mL of sterile nutrient broth with 1mL of (AGE) stock solution. The third tube was prepared by adding 9mL of sterile nutrient broth mixed with 1mL of (FGE) stock solution; the fourth tube was prepared by adding 9mL of sterile nutrient broth mixed with 1mL of chlorohexidine.

2.3.1.2 Inoculation

A total of 100µl of the adjusted inoculum was added to each tube in the dilution series. One tube containing broth with microorganisms without garlic extract was prepared and considered a control positive; another tube containing broth was only considered a control negative. After inoculation, the tubes in the series dilution were incubated for 24hr at 37°C. The interpretation was carried out by measuring and comparing the absorbance, reflecting the microbial growth in the tubes. The growth analysis was carried out by spectrophotometer at 625 nm.

2.3.2 Well diffusion method

The well diffusion method (modified Kirby- Bauer method) recommended by world health organization was carried out according to (Abidullah *et al*., 2021). Muller-Hinton agar plates were prepared. Five wells (5mm diameter) were punched aseptically. In each plate with a copper ring sterilized by alcohol flaming, each organism's overnight broth culture (18h) was prepared and adjusted to equal the turbidity of tube 0.5 McFarland standard. The plate was inoculated by transferring 100µl of each adjusted microbial culture, evenly distributed by L form glass sterilized by flaming with alcohol. Each punch was filled with 20µl of 200 mg/mL of each prepared garlic extracts (AGE) and (EGE). To the last two wells, one was filled with sterilized distal water as control negative, and the other with 20µl 0.12% chlorhexidine as control positive. After 24h of incubation, the diameter of the clear zones were measured.

Results and Discussion

3.1. Results

3.1.1 Determination of the minimum inhibitory concentration (MIC) for garlic extracts against different oral microorganisms

Table 1 shows the minimum inhibitory concentration of different garlic extracts and chlorohexidine against *Candida albicans.* Table 2 shows the minimum inhibitory concentration of different garlic extracts and chlorohexidine against *S. aureus*. Finally, table 3 shows the minimum inhibitory concentration (MIC) of different Garlic extracts.

3.1.2 The antimicrobial effect of different garlic extracts on different oral microorganisms by well diffusion method (modified Kirby-Bauer method)

The antimicrobial effect of garlic extracts *on C. albicans.* Figure 5 shows the antimicrobial activity of garlic extracts on *Candida albicans.* After using the same concentration of each garlic extract, 20µl of 200mg/ml mouth wash containing 0.12% chlorhexidine was used as a control positive. The means of inhibition zones measured were 29 mm, 31.5mm, 32.25 mm, and 12mm for EGE, AGE, FGE, and control, respectively (Table 4).

3.1.3 The antimicrobial effect of garlic extracts on S. aureus.

The well diffusion method for evaluating the antibacterial activity (Figure 6) of garlic extract at 20µl of 200mg/mL concentration against S. aureus. showed mean inhibition zones of 30mm, 31.5 mm, 29mm, and 11.5mm for EGE, AGE, FGE, and control positive, respectively (Table 5).

3.2. Discussions

In recent years, extensive abuse and empirical treatment of antibiotics led to increasing drug resistance. This threatened the entire world, and the need to develop new antibacterial agents used alone as alternative medicine or in combination with other antibiotics as a synergistic effect has become an urgent necessity.

Infectious diseases due to the emergence of multidrug-resistant microorganisms became the main cause of mortality worldwide (Ramzan *et al*., 2022). Also**,** *C. albicans* is an opportunistic fungal pathogen evolving widespread in normal people and immunocompromised patients (Ashrit *et al*., 2022). It is a commensal of the mucosal surfaces of the oral cavity and gastrointestinal tract responsible for infections linked to high mortality and high drug resistance worldwide, so we need to search for newer antifungal natural agents (Rai *et al*., 2022).

The World Health Organization (WHO) claims that 80% of the world's population still uses traditional medicine, including herb-based treatments (Ramzan *et al*., 2022), because herbal plants such as garlic are good sources of bioactive compounds for drugs development (Dahiya *et al*., 2022).

This research focused on the antimicrobial effect of Garlic *(Allium sativum)* against multidrug-resistant human microbe such *C. albicans* and *S. aureus* with their respective minimum inhibitory concentrations and inhibition zone.

It used chlorhexidine mouth wash (0.12%) as control positive owing to its potent chemotherapeutic efficacy and golden role in reducing oral biofilm. In addition, it demonstrated swift antibacterial, antifungal, and antivirus action and maintained its effectiveness even at low concentrations level (Deus and Ouanounou, 2022). Three methods were used to prepare the extracts of garlic because many studies confirmed that the methods of preparations and the type of solvent used in extraction affect the effectiveness and constituencies of extracts (Bhatwalkar *et al*., 2021; Melguizo-Rodríguez *et al*., 2022).

Garlic has a variety of bioactive compounds, including organosulfur compounds, saponins, phenolic compounds, and polysaccharides. The most significant benefits of garlic are due to the presence of organosulfur compounds, which differ significantly in their effectiveness and chemical properties between intact garlic cloves from crushed or macerated garlic cloves (Nadeem *et al*., 2021). The entire garlic mainly contains Allium. Precursor compounds are converted to Allicin and an array of thoisulfinates due to an enzyme called alliinase released after garlic cutting and crushing (Melguizo-Rodríguez *et al*., 2022; Nadeem *et al*., 2021). Allicin is the most potent organosulfur in garlic. An oily, slightly yellow liquid gives garlic its unique odor, representing approximately 70% of the thiosulfinates in garlic problems (Farid, Yousry, and Safwat, 2022).

Bhatwalkar *et al*. (2021) reported that concentrated ethanolic or watery extracts of garlic yield products rich in allicin. Also, Ashrit *et al*., (2022) reported that allicin is unstable and easily breaks down but is more stable in the aqueous-based extract. Although Allicin is highly unstable and rapidly oxidized; volatile, it quickly changes into a series of other sulfur-containing compounds. The most important one is Ajoene and vinyl dithiins (Zhu and Zeng, 2020). Ajoene is most stable and abundant in macerate of garlic, is formed from a chemical reaction involving two allicin molecules (Cho, Ryu, and Surh, 2019). It has two isomers *Z* and *E*, and has attracted attention for its pharmacological activity underscores in the development of new therapeutic drugs (Yoneda *et al*., 2022). Ajoene has no distinctive smell. Still, content in low concentration than allicin (Zhu and Zeng, 2020) also possesses inhibitory effects on bacteria as allicin (Nakamoto *et al*., 2020).

In the current study, the inhibitory effects of garlic extracts on *C. albicans and S.aurues were* evaluated by MIC & agar well diffusion methods. The MIC result of three extracts (EGE, AGE & FGE) against *C.albicans was* 200 mg/mL (Table 3). EGE at a 50 mg/mL concentration shows lower effect when compared with AGE and FGE, as shown in (Table 1).

Garlic extracts show a significant inhibitory effect against *C. albicans,* as shown in Figure 5. Compared with the chlorohexidine effect, EGE's zone of inhibition increased by 2.4-fold more than the inhibition zone of chlorhexidine while the zone of inhibition of AGE and FGE increased by 2.6 and 2.7-fold respectively the inhibition zone of chlorohexidine. FGE showed the most significant inhibition zone, followed by AGE and then EGE, as shown in Table 4. This study's results were consistent with that mentioned by Bhatwalkar *et al*., 2021, who reported analysis by using HPLC of ethanolic garlic extract (EGE) and showed it contains many thiosulfates - the major was allicin. Although (EGE) had allicin, AGE is more effective because of other antibacterial chemical elements, resulting in synergistic or additive effects. The results of this study are also compatible with Ashrit *et al., (*2022) who found that AGE is more potent than EGE and related that when ethanol was evaporated, some of the volatile constituents of garlic were also evaporated, possibly leading to this difference.

Regarding the antimicrobial effect of garlic extracts *on S. aureus,* the MIC of three extracts against *S.aureus* were equal at 200mg/mL while approximately similar inhibition effect with a slight difference at a concentration of 100 mg/mL as shown in Table 2. The inhibitory effect of the three types was strong and more potent than the chlorohexidine effect shown in Figure 6.

The measurement of the inhibition zone of (EGE) is increased by about 2.6-fold compared to chlorohexidine's inhibition zone. In contrast (AGE) and (FGE) increased by 2.7 and 2.5-fold respectively than the inhibition zone of chlorohexidine. AGE revealed the most significant inhibition zone, followed by EGE and FGE, as shown in Table 5. These results are compatible with a previous literature review that demonstrated that FGE exhibited a significant inhibitory effect on methicillin-resistant *S. aureus.* and *C. albicans* (Zhu and Zeng, 2020).

The possible mechanism of action that explains antimicrobial effect of garlic is they have a broad spectrum antibacterial effect in a dose-dependent manner (Zhu and Zeng, 2020). It has various properties, such as bactericidal, antitoxin, and antibiofilm formation, which is the leading cause of bacterial resistance to the antibiotic. It regulates quorum sensing activity in an extreme range of bacteria, among them multidrug-resistant strains (MDRS) (Nakamoto *et al*., 2020). Allicin can penetrate cell membranes and bind covalently with sulfhydryl groups of proteins or enzymes in bacteria leading to microbial apoptosis (Bhatwalkar *et al*., 2021). The antifungal mechanism of garlic extracts mainly includes the ability to penetrate the cell membrane and destroy its integrity, leading to cellular collapse and altering gene expression. (Zhu and Zeng, 2020). Garlic can induce Proteomic change, anti-biofilm, and antifungal susceptibility of *C. albicans.* (Ashraf, El-Barrawy, and Omran, 2022).

CONCLUSIONS

The increased prevalence of infections caused by resistant microbial strains dictates the need to develop a new antimicrobial agent. This study revealed that garlic extracts (EGE AGE FGE) produced marked antimicrobial properties against *C. albicans* and *S. aureus* that could be a source of new drugs shortly. First, however, a Garlic extract exhibits antimicrobial activity is of interest. Still, the comparison between results is often incurable and needs standardized preparation techniques, identical growth and incubation conditions, and accuracy in measurement. Therefore, additional studies should be conducted focusing on approaches to produce more effective garlic extracts and incorporating them in a new pharmaceutical form to overcome the most critical obstacles, including the instability of the Garlic active substances.

DECLARATIONS

5.1. Study Limitations

This study is limited to the technique used regarding sample extraction, the conditions, parameters selected, types of equipment, incubation conditions, and measurement accuracy. Other studies should be performed to endorse these results.

5.2. Acknowledgements

Authors are greatly appreciative to the University of Mosul / College of Dentistry for their kind assessment to reach best quality of this research.

5.3. Funding source: self-funding

5.4. Competing Interests: non

5.5. Ethical Approval

This study conducted in College of Dentistry, University of Mosul and the scientific laboratories in Mosul city/Iraq. The study was done under the approval number UoM.Dent/A.L.17/22. by both the scientific and Ethical academic committees of basic science department of college of dentistry/ university of Mosul.

Table 1. MIC of Garlic extracts and Chlorohexidine on C. albicans

Type of Garlic extract	**Concentration 1 (200mg/ml)**	**Concentration 2 (100mg/ml)**	**Concentration 3 (50mg/ml)**	**Chlorohexidine Control +0.12%**	**+ve *C. albicans* growth**
EGE	0.000	0.006	0.116	0.186	0.531
AGE	0.000	0.001	0.005		
FGE	0.000	0.005	0.006		

Table 2. MIC of Garlic extracts and Chlorohexidine on S. aureus.

Type of Garlic extract	**Concentration 1 (200mg/ml)**	**Concentration 2 (100mg/ml)**	**Concentration 3 (50mg/ml)**	**Chlorohexidine Control +0.12%**	**+ve *S.aureus* growth**
EGE	0.000	0.001	0.045	0.170	0.485
AGE	0.000	0.006	0.340		
FGE	0.000	0.079	0.205		

Table 3. Minimum inhibitory concentration (MIC) of different Garlic extracts.

Organism	**MIC (mg/ml) of EGE**	**MIC (mg/ml) of AGE**	**MIC (mg/ml) of FGE**
C. albicans	200	200	200
S. aureus.	200	200	200

Table 4. The antibacterial activity of garlic extracts and chlorohexidine mouth wash on C. albicans

Type of Garlic extract (20µl)	**Means of ZOI (mm)**
EGE	29
AGE	31.5
FGE	32.25
Control (+)	12
Control (-)	0

Notes: EGE; Ethanolic Garlic extract, AGE; Aqueous Garlic extract, FGE; Fresh Garlic extract, Control (+) = Chlorohexidine; Control (-) = Water; ZOI = Zone of inhibition

Table 5. The antibacterial activity of garlic extracts and chlorohexidine mouth wash on S.aureus.

Type of Garlic extract (20µl)	Means of ZOI (mm)
EGE	30
AGE	31.5
FGE	29
Control (+)	11.5
Control (-)	0

Notes: EGE; Ethanolic Garlic extract, AGE; Aqueous Garlic extract, FGE; Fresh Garlic extract, Control (+) = Chlorohexidine; Control (-) = Water; ZOI = Zone of inhibition

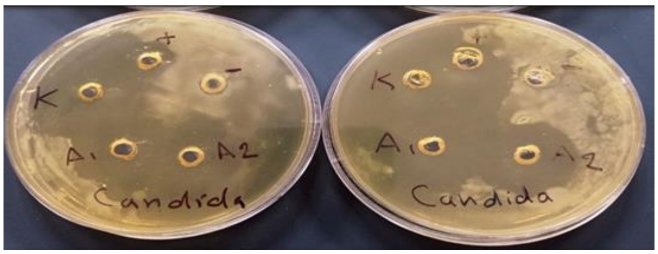
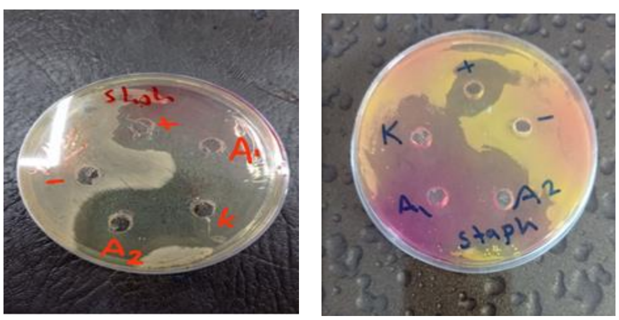


Figure 5: The antibacterial activity of garlic extracts and chlorohexidine mouth wash on C. albicans by well diffusion method

Notes: K (Ethanolic Garlic extract), A1(Aqueous Garlic extract), A2 (Fresh Garlic extract),Control +(Chlorohexidine) Control – (Water).



A B

Figurer 6: The antibacterial activity of garlic extracts and chlorohexidine mouth wash on S.aureus.

Notes: K (Ethanolic Garlic extract), A1(Aqueous Garlic extract), A2 (Fresh Garlic extract), Control +(Chlorohexidine), Control – (Water).

References

Silva, P. D. C., Santos, B. L. C. dos., Soares, G. L., Oliveira, W. A. de., (2019). Anti-candida albicans activity of the association of citronelal with anfotericin b or with cetoconazole. Periódico Tchê Química, Vol. 16 N. 31, pp. 250-257. DOI: 10.52571/PTQ.v16.n31.2019.256_Periodico31_pgs_250_257.pdf.

Hernandez-Cuellar, E., Alma Lilián, G.-B., Avelar-Gonzalez, F. J., Díaz, J. M., Santigado, A. S. de., Chávez-Reyes, J., Poblano-Sánchez, E. (2022). Characterization of Candida albicans and Staphylococcus aureus polymicrobial biofilm on different surfacesCaracterización de la biopelícula polimicrobiana de Candida albicans y Staphylococcus aureus sobre diferentes superficies. Revista Iberoamericana de Micología, Vol. 39, N. 2, pp.36-43.

Carolus, H., Dyck, K. V., Dijck, P. V. (2019). Candida albicans and Staphylococcus Species: A Threatening Twosome. Frontiers in Microbiology. Mini Review. Vol. 10., Article 2162. doi: 10.3389/fmicb.2019.02162

Hu, Y.; Niu, Y.; Ye, X.; Zhu, C.; Tong, T.; Zhou, Y.; Zhou, X.; Cheng, L.; Ren, B. (2021). Staphylococcus aureus Synergized with Candida albicans to Increase the Pathogenesis and Drug Resistance in Cutaneous Abscess and Peritonitis Murine Models. Pathogens, Vol. 10, 1036. https://doi.org/10.3390/pathogens10081036

Nobile, C. J., and Johnson, A. D. (2015). Candida albicans biofilms and human disease. Annu. Rev. Microbiol. 69, 71–92. doi: 10.1146/annurev-micro-091014-104330

Magryś, A., Olender, A., Tchórzewska, D. (2021). Antibacterial properties of Allium sativum L. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Arch Microbiol, Vol. 203. N. 5, pp. 2257–2268, doi: 10.1007/s00203-021-02248-z

Bhatwalkar, S. B., Mondal, R., Krishna, S. B. N., Adam, J. K., Govender, P., Anupam, R. (2021). Antibacterial Properties of Organosulfur Compounds of Garlic (Allium sativum). Front. Microbiol. 12:613077. doi: 10.3389/fmicb.2021.613077

El-Saber Batiha, G., Magdy Beshbishy, A., G Wasef, L., Elewa, Y. H., A Al-Sagan, A., El-Hack, A., Prasad Devkota, H. (2020). Chemical constituents and pharmacological activities of Garlic (Allium sativum L.): A review. Nutrients, 12(3), 872.

Nakamoto, M., Kunimura, K., Suzuki, J. I., & Kodera, Y. (2020). Antimicrobial properties of hydrophobic compounds in Garlic: Allicin, vinyldithiin, ajoene and diallyl polysulfides. Experimental and therapeutic medicine, 19(2), 1550-1553.

Kıraç, H., Dalda Şekerci, A., Coşkun, Ö. F., & Gülşen, O. (2022). Morphological and molecular characterization of Garlic (Allium sativum L.) genotypes sampled from Turkey. Genetic Resources and Crop Evolution, 69(5), 1833-1841.

Abidullah, M., Jadhav, P., Sujan, S.S., Shrimanikandan, A.G., Reddy, C.R., Wasan, R.K. (2021). Potential Antibacterial Efficacy of Garlic Extract on Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae: An In vitro Study. J Pharm Bioallied Sci.;13 (Suppl 1): S590-S594. doi: 10.4103/jpbs.JPBS_681_20.

Anggraeni, D., Kamaluddin, K., Theodorus, T. (2020). Effectiveness of Garlic Water Extract Gel (Allium sativum. L) Against Necrotic Factor Alfa Tumors and Mouth Ulcer Diameter in Wistar White Male Rats. Biomedical Journal of Indonesia 6(1): 27-34. DOI:10.32539/bji. v6i1.7970.

Farid, A., Yousry, M., & Safwat, G. (2022). Garlic (Allium sativum Linnaeus) improved inflammation and reduced cryptosporidiosis burden in immunocompromised mice. Journal of Ethnopharmacology, 292, 115174.

Abdel Hamed B.M. and Eman Alaa. (2021). clinical and Microbiological Evaluation of the Effect of Heat Killed Lactobacillus Acidophilus and Garlic Extract on Candida Albicans in a Group of Elderly Denture Wearers. Egyptian Dental Journal. Article 14, Volume 67, Issue 2 - Serial Number 4, pp. 1475-1486. DOI: 10.21608/edj.2021.52408.1414

Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). Staphylococcus aureus biofilm: Morphology, genetics, pathogenesis and treatment strategies. International Journal of Environmental Research and Public Health, 18(14), 7602.

Sasi, M., Kumar, S., Kumar, M., Thapa, S., Prajapati, U., Tak, Y.,and Mekhemar, M. (2021). Garlic (Allium sativum L.) Bioactives and Its Role in Alleviating Oral Pathologies. Antioxidants, 10(11), 1847.

Abdelraheem, W. M. (2019). Fresh garlic extract has a synergistic effect with antibiotics on ESBLs producing E. coli urinary isolates. J. Adv. Microbiol, 14, 1-9.

Bienvenue, D.N., Bong, D.A., Brian, N.Z., Staphane, J., Hortense, G., Charles, M.B. (2021). In Vitro Evaluation of the Efficacy of an Aqueous Extract of Allium Sativum as an Antibacterial Agent on Three Major Periodontal Pathogen. J Oral Dent Health Res., Vol. 3., N. 1,

Bhadra, S., Paik, I., Torres, J. A., Fadanka, S., Gandini, C., Akligoh, H., and Ellington, A. D. (2022). Preparation and Use of Cellular Reagents: A Low-resource Molecular Biology Reagent Platform. Current Protocols, 2(3), e387.

Kowalska-Krochmal, B., and Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens, 10(2), 165.

Ramzan, M., Karobari, M. I., Heboyan, A., Mohamed, R. N., Mustafa, M., Basheer, S. N., Zeshan, B. (2022). Synthesis of silver nanoparticles from extracts of wild ginger (Zingiber zerumbet) with antibacterial activity against selective multidrug resistant oral bacteria. Molecules, 27(6), 2007.

Ashrit, P., Sadanandan, B., Shetty, K., & Vinayamparambath, V. (2022). Polymicrobial Biofilm Dynamics of Multidrug-Resistant Candida albicans and Ampicillin-Resistant Escherichia coli and Antimicrobial Inhibition by Aqueous Garlic Extract. Antibiotics, 11(5), 573.

Rai, L. S., van Wijlick, L., Chauvel, M., d'Enfert, C., Legrand, M., Bachellier-Bassi, S. (2022). Overexpression approaches to advance understanding of Candida albicans. Molecular Microbiology, 117(3), 589-599

Dahiya, A., Prakash, A., Agrawala, P. K., Dutta, A. (2022). Investigation on Oral Toxicity of Diallyl Sulfide: A Principle Organosulfur Compound Derived from Allium Sativum (Garlic) in mice. Defence Life Science Journal, Vol. 7, No. 1, pp. 3-10, DOI: 10.14429/dlsj.7.16972

Deus, F. P., Ouanounou, A. (2022). Chlorhexidine in Dentistry: Pharmacology, Uses, and Adverse Effects. International dental journal. International Dental Journal, Vol. 72, N. 3, pp. 269-277, https://doi.org/10.1016/j.identj.2022.01.005

Melguizo-Rodríguez, L., García-Recio, E., Ruiz, C., De Luna-Bertos, E., Illescas-Montes, R., Costela-Ruiz, V. J. (2022). Biological properties and therapeutic applications of garlic and its components. Food & Function, 13(5), 2415-2426.

Nadeem, M. S., Kazmi, I., Ullah, I., Muhammad, K., Anwar, F. (2021). Allicin, an Antioxidant and Neuroprotective Agent, Ameliorates Cognitive Impairment. Antioxidants (Basel), Vol. 11, N. 1, doi: 10.3390/antiox11010087.

Zhu, X. Y., Zeng, Y. R. (2020). Garlic extract in prosthesis-related infections: a literature review. J Int Med Res., Vol. 48, N. 4, doi: 10.1177/0300060520913778.

Cho, S. J., Ryu, J. H., Surh, Y. J. (2019). Ajoene, a major organosulfide found in crushed garlic, induces NAD (P) H: quinone oxidoreductase expression through nuclear factor E2-related factor-2 activation in human breast epithelial cells. Journal of cancer prevention, 24(2), 112.

Yoneda, T., Kojima, N., Matsumoto, T., Imahori, D., Ohta, T., Yoshida, T., Nakamura, S. (2022). Construction of sulfur-containing compounds with anti-cancer stem cell activity using thioacrolein derived from garlic based on nature-inspired scaffolds. Organic & Biomolecular Chemistry, 20(1), 196-207.

Ashraf, H. M. M., El-Barrawy, M. A., Omran, E. A. (2022). Garlic-induced Proteomic Change, Anti-biofilm and Antifungal Susceptibility of Candida albicans. Egyptian Academic Journal of Biological Sciences, G. Microbiology, 14(1), 11-22.