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

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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.

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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 Batilha et al., 2020).

Allium sativum (Garlic) has been grown on all continents for thousands of years. It is known as one effective plant species 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](image)

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 3-5°C 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).
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.

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 Differential 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 matured with 0.5 % McFarland standard represents (1.5*10⁸) 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 Mülller-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 Differential 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 sterilised 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 McFarland standard. The preparation of the dilutions series was done by using four test tubes. 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 chlorhexidine.

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 thiosulfates 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 thiosulfates 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. aureus* 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 chlorhexidine. 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 chlorhexidine. 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.

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<th>Concentration 2 (100mg/ml)</th>
<th>Concentration 3 (50mg/ml)</th>
<th>Chlorohexidine Control +0.12%</th>
<th>+ve C. albicans growth</th>
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<td>S. aureus</td>
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Table 4. The antibacterial activity of garlic extracts and chlorohexidine mouth wash on C. albicans

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<th>Means of ZOI (mm)</th>
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<td>FGE</td>
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<td>Control (-)</td>
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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.

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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 (Ethanollic Garlic extract), A1(Aqueous Garlic extract), A2 (Fresh Garlic extract), Control +(Chlorohexidine) Control - (Water).

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

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

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