Medical Science Journal for Advance Research

Vol. 5, No.4, December 2024
e-ISSN: 2774-4892

A Comparison Study of Garlic and Propolis Extract as Biological Activity on *Staphylococcus aureus* Isolated from Diabetic Foot Patients

Sabreen G. Ghazal [1], Zeena Kareem Gihad [2], and Hanan Saad[3]

[1]*Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Iraq.*

2 Department of Community Health Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Iraq.

[3] *Department of Vision Screening Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Iraq.*

***E-Mail**: sabreen.almummar.chm@atu.edu.iq , zina.jehad@atu.edu.iq , hanan.hashem@atu.edu.iq

ABSTRACT

Background: *Plant extracts play a crucial role in biological activity in recent years the extraction of some active material from some plants indicates good activity challenging the excess types of bacteria without side effects.* ***Methods:*** *Simple extraction is used to extract garlic using water as a solvent and ethanol for propolis. the Garlic and Propolis extract used on the Staphylococcus aureus which was isolated and identified from diabetic foot patients after culturing the bacteria on Mullar Hinton media the biological activity was measured for the Garlic and Propolis extract.* ***Results:*** *The elevated concentration of the garlic extracts (C) produced elevated inhibition by S. aureus (32 mm); the smallest inhibition zone was detected by Staphylococcus aureus (19 mm) in the minimal concentration of the garlic extracts. While the elevated concentration of the propolis extracts produced the elevated inhibition zone by S. aureus (20mm), the smallest inhibition zone (14mm) was detected by Staphylococcus aureus in the low concentration of propolis extract. The inhibition zone of the garlic extracts (at 5% concentration) was the larger than the propolis extract at 5g\ml against S. aureus (32 vs 20 mm). The MIC method by S. aureus of the garlic extract resulted 12.5 mg/ml, while, the propolis extract resulted 3.2 mg/ml. The MBC recognized by the lack the growth of the tested bacteria that inoculated from the inhibition well initial from the maximum concentration (100 mg/ml) reached to the concentration equivalent the minimal MIC. The garlic extract exhibited an absence growth of bacteria for total inoculation which confirmations potentially bactericidal action to S. aureus have an MBC at 12.5 mg/ml.* ***Conclusion:*** *The elevated concentration of the garlic extracts produced the elevated inhibition towards S. aureus as well as the least inhibition zone detected in the minimal concentration of the garlic extracts towards Staphylococcus aureus. Whatever, the concentration of the garlic extracts that used in the present study, the inhibition zone was larger as compared with propolis. The results indicate that the garlic extract was more active than the propolis extract. The results prove the efficiency of garlic extract, which makes them suitable for medical treatment used as an ointment form or tablet to treat ulcers suffered by patients with chronic diabetes.*

Keywords: Diabetic patient, Garlic extract, Propolis extract, Staphylococcus aureus.

Article Information
Received: September 26, 2024; Revised: October 30, 2024; Online: December, 2024

INTRODUCTION

Diabetic Foot Ulcers (DFUs) are a serious complication of diabetes that can lead to severe consequences, including the need for amputation, hospitalization, and even death. The impact of DFUs is particularly significant in developing countries where healthcare facilities may be lacking and awareness of this condition is lower(1). The burden of diabetes-related foot complications is expected to increase in the future, highlighting the importance of improving resources and access

https://doi.org/10.46966/msjar.v5i4.230

Published by: READ INSTITUTE

to specialists for the treatment of DFUs[2]. Therefore, it has become necessary to find easily available herbal extracts to treat it[3]. The constant emergence of the bacterial resistance to several antibiotics may bring about lethal infections due to the production of both Gram-positive and Gram-negative bacteria[4]. High doses of these antibiotics also can affect balance in the intestinal microflora and, possibly, intensify the spreading of methicillin resistant Staphylococcus aureus[5].

Traditionally plant and their extracts have been used in treatment of infections due to pathogens. It was also ascertained that many plants exhibit anti-microbial activity[6], such as the Allium sativum L., and propolis. Garlic (Allium sativum Linn); belonging to Liliaceae family related to onion is known to possess medicinal values. That is why it is relevance to modern medicine: The juice contains more than 200 different chemicals. Allicin the main constituent in garlic known as allyl 2-propenethiosulfinate or diallyl thiosulfinate possesses high antimicrobial action. Allicin is produced as a result of garlic which is chopped or crushed[8]. Another plant extract is Propolis which is a resin collected by honey bees from plants having antioxidant, antibiotic, antiviral, local anaesthetic and anti-inflammatory benefits[9].(All these are due to its constituent which includes resins, aromatic oils, flavonoids, vanillin, isovanillon, caffeic acid, benzoic acid, ascorbic acid, benzyl alcohol, cinnamic acid, etc. A lot of studies have been conducted to determine the effect of propolis on antioxidant and antimicrobial activities[10] .The recent increase in antibiotic resistant strains has led to revival of interest in the antibacterial properties of the herbal plants and propolis. Some seasoning plants have been reported to have varying degree of antibacterial properties; however, garlic possesses outstanding antibacterial characteristics and has the history of uses in traditional medicine for the treatment of infectious diseases, especially against the Gram-positive bacterium S. aureus [11].

PATIENT AND METHOD

Preparation of Garlic Extract including Collection

Garlic used in this study was fresh garlic of Allium sativum species bought from the local market and was prepared as follows. The first procedure included washing the garlic with running water, washing it again with ethanol then with distilled water. The cleaned garlic was then dried using an incubator Most of the steps were realized aseptically**.** Secondly, The garlic bulbs were then peeled, weighed, and sliced into small slices with a sterilized knife. This used an electric grinder whereby these pieces were grinded into a puree**.** To eliminate any solid matter, the puree was poured to a sterile cheesecloth through which it was filtered**.** The obtained crude extract was kept in the sterile and capped bottle at the temperature 4ºC – 8ºC for the subsequent experiments according to the protocol described in the work of [12].(The garlic extracts were further diluted with distilled water in such a manner that three analyte concentrations were prepared. Concentration A was prepared by mixing 2. with 7 Minute Simple ingredients needed; 5 ml Garlic Extract. 5 ml of sterile distilled water Only. Concentration B was prepared from the garlic extract by mixing 5 ml of it with 5 ml of sterile distilled water. Finally, Concentration C was prepared by mixing 7. garlic solution for 5 ml with 2. The addition of the distilled water is done by using 5 milliliters of it.

Collection and preparation of Propolis

Five grams of propolis powder dissolved in (100) ml of ethyl alcohol at a concentration of (70%). After that, it was transferred to a shaking device for an hour and then left. The material was placed in a dark bottle and kept in the laboratory for ten days. After that, the material was filtered using filter papers, then the filtrate was evaporated using a German bath device (water path) until a thick mass of sticky resinous material was obtained. Propolis extracts were diluted with distill For preparation of the 1st concentration (A), 2. This was done by addition

of 5 ml propolis extract to 7. 5 ml of sterile distill water. For the second concentration (B), dilution of propolis extract involved mixing propolis extract with 5 ml of sterile distilled water. The third concentration (C) was made by blending 7. When examining the efficacy of propolis used for respiratory diseases, trachea infections in particular, the following dosages were used: 5 ml of propolis extract with 2. 5 ml of Distilled Water which should be sterile.

Microorganisms

24 isolates of staph aureus isolated from diabetic foot patients were obtained from Al-Amin Center for Scientific Research in Najaf.

Disc Diffusion Method Procedure

Therefore, to assess antimicrobial activity the presence of extracts was tested. A bacterial suspension was adjusted to a 0. 5 McFarland standard and spread on to Mueller–Hinton agar plates with the help of cotton swab. An appropriate stereility filter paper disks of 6mm diameter was later used and each disk was spotted with 10µl of the desired concentration of the plant extract. For each extract, three concentrations were prepared: The second one is the following: 5, 5, and 7. 5 µl to all garlic and propolis extracts. The agar plates were then placed in incubator at 37°C for the next 24 hours. Incubation was then done and the antimicrobial effect was tested by a measurement of the inhibition zone diameter[13].(The outcomes thereof are the inhibition zones of garlic and propolis extracts in Table 1 and Figure 1.

Determination of Minimum inhibitory concentration (MIC)(garlic extract, propolis extract)

Antibacterial potentials of garlic and propolis extract on standard bacterial strains was further investigated by determining their minimum inhibitory concentration micron. Minimum inhibitory concentration (MIC) value of garlic and propolis extracts were also evaluated through Broth microdilution method following the protocols of clinical and laboratories standards institute. Dilutions of the extracts twofold were done and the concentration tested for were 100µg/ml, 50µg/ml, 25µg/ml and 12. 5, 6. 25, 3. 12, 1. 56, 0. 78, 0. 39, and 0. 195 mg/ml, and were re-spread into two 96-well microplates. Alkaline bacterial suspensions grown to the logarithmic phase were diluted to a turbidity of 0. 5 McFarland standard, which corresponds to 1 × 10^8 cfu/ml [15.[Afterwards bacteria were inoculated in to the wells of each plate and the plate were incubated at 37°C for 24 hours. Next, resazurin was added into each well followed by incubation for further 30 min to determine any possible variations of the observed color in comparison with the control. Wells containing only bacteria suspension and nutrient broth added, represented positive growth controls while nutrient broth added with bacteria suspension and garlic or propolis extracts represented negative growth control.

RESULTS

Disc diffusion method

After 24 hours have passed from the incubation process of selecting 24 *Staphylococcus aureus* isolates, the damping diameters formed around the pits filled with 1 ml of different concentrations for each extract are measured in millimeters, measured by a ruler. The results of the inhibition zone diameters for the samples treated by garlic extract are shown in (Tab. 1). The *S. aureus* cultured on agar plate with garlic extract zone of inhibition is presented in (Fig. 1) the antibacterial activity of garlic extracts and propolis extract separately, which are shown in (Fig.s 2 and 4). The *S. aureus* cultured on agar plate with propolis extract zone of inhibition is presented in (Fig. 3). The results of the inhibition zone diameters for the samples treated by propolis extract are shown in (Tab. 2). The whole different garlic extract concentrations and propolis extract concentrations exhibited diverse inhibition

zones, indicating to an antibacterial effect of Garlic and Propolis Extract on the *Staphylococcus aureus* shown in (Fig. .5). The elevated concentration of the garlic extracts (C) produced the elevated inhibition by *S. aureus* (32 mm); the smallest inhibition zone was detected by *Staphylococcus aureus* (19 mm) in the minimal concentration of the garlic extracts. While the elevated concentration of the propolis extracts produced the elevated inhibition zone by *S. aureus* (20mm), the smallest inhibition zone (14mm) was detected by *Staphylococcus aureus* in the low concentration of propolis extract. Whatever the garlic extracts concentration used, the inhibition zone was larger as compared with inhibition zone created by propolis at the same concentration. The inhibition zone of the garlic extracts (at 5% concentration) was the larger than the propolis extract at 5g\ml against *S. aureus* (32 vs 20 mm).

The MIC method

The result of MIC method by the garlic extract of *S. aureus* resulted 12.5 mg/ml, while, the propolis extract resulted 3.2 mg/ml. The MBC recognized by the lack the growth of the tested bacteria that inoculated from the inhibition well initial from the maximum concentration (100 mg/ml) reached to the concentration equivalent the minimal MIC. The effect of garlic extraction and propolis on the bacteria *Staphylococcus aureus* by the MIC method shown in Fig. (6,7). The garlic extract exhibited an absence growth of bacteria for total inoculation which confirmations potentially bactericidal action to *S. aureus* have an MBC at 12.5 mg/ml.

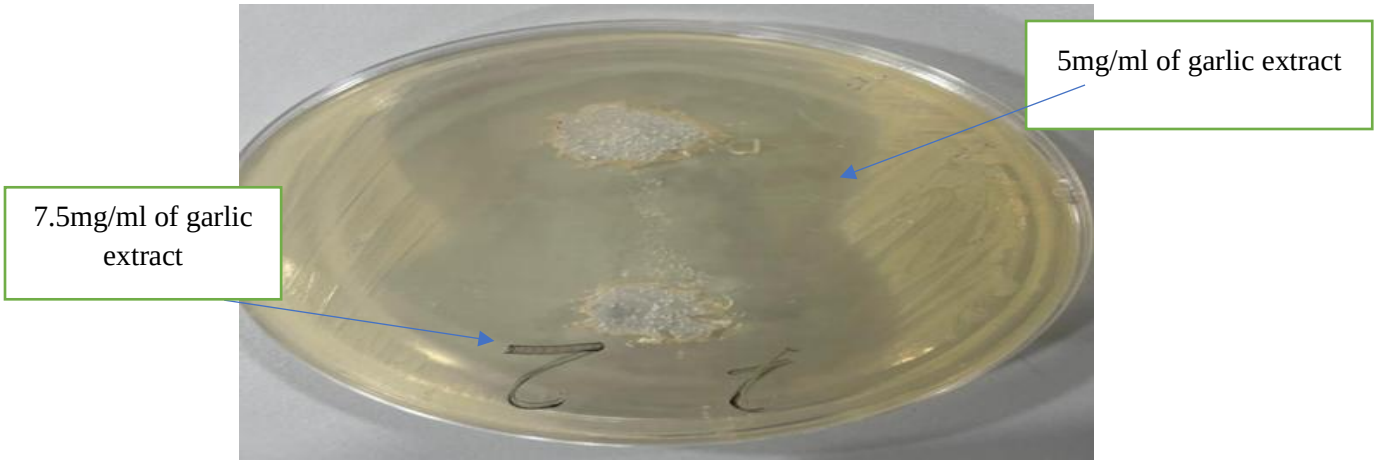

Figure 1. *S. aureus* cultured on agar plate with garlic extract zone of inhibition.

Table (1): The inhibitory diameters of the by garlic extract

Inhibition zone (mm)	Concentration of garlic extract
19	2.5
26	5
32	7.5

Table (2): The inhibitory diameters of the by propolis extract

Inhibition zone (mm)	Concentration of propolis extract
14	2.5
18	5
20	7.5

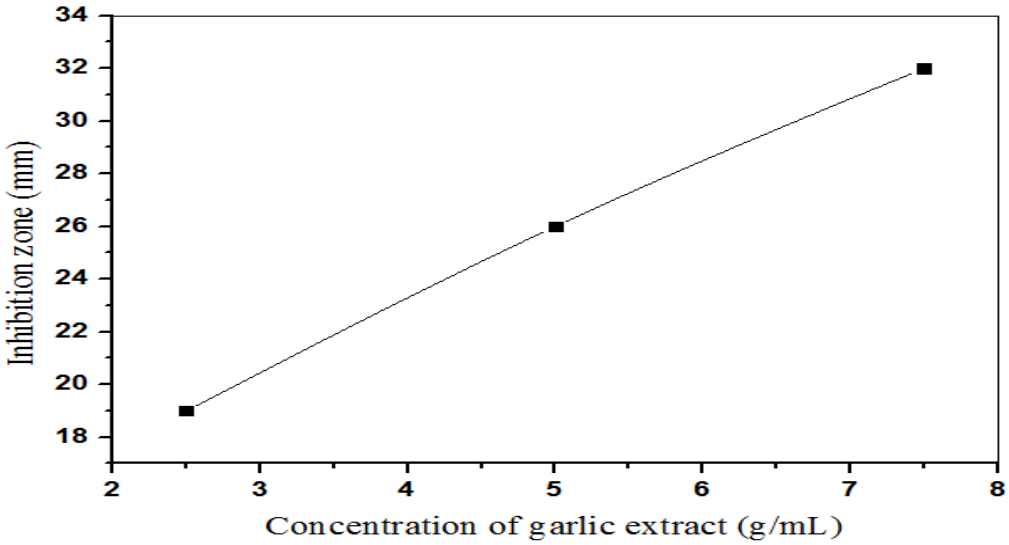

Figure (2) Antibacterial activity by garlic extracts.

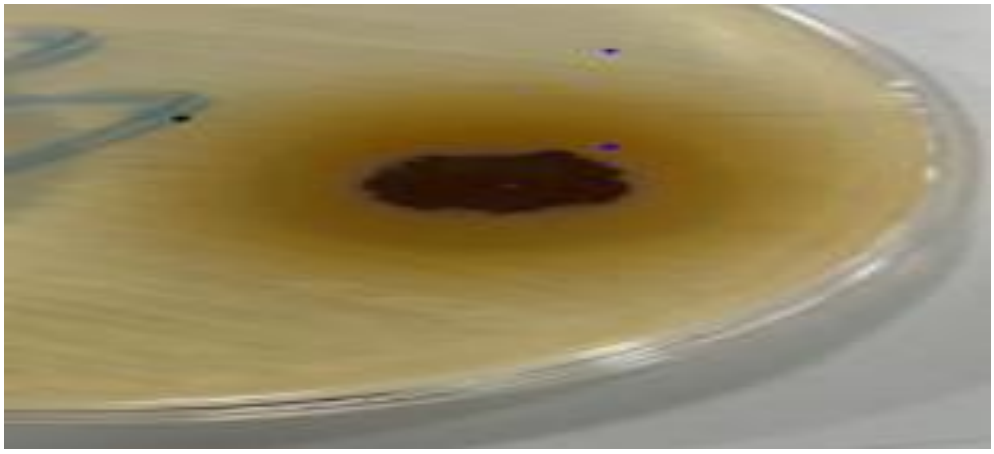

Figure (3) *S. aureus* cultured on agar plate with propolis extract zone of inhibition

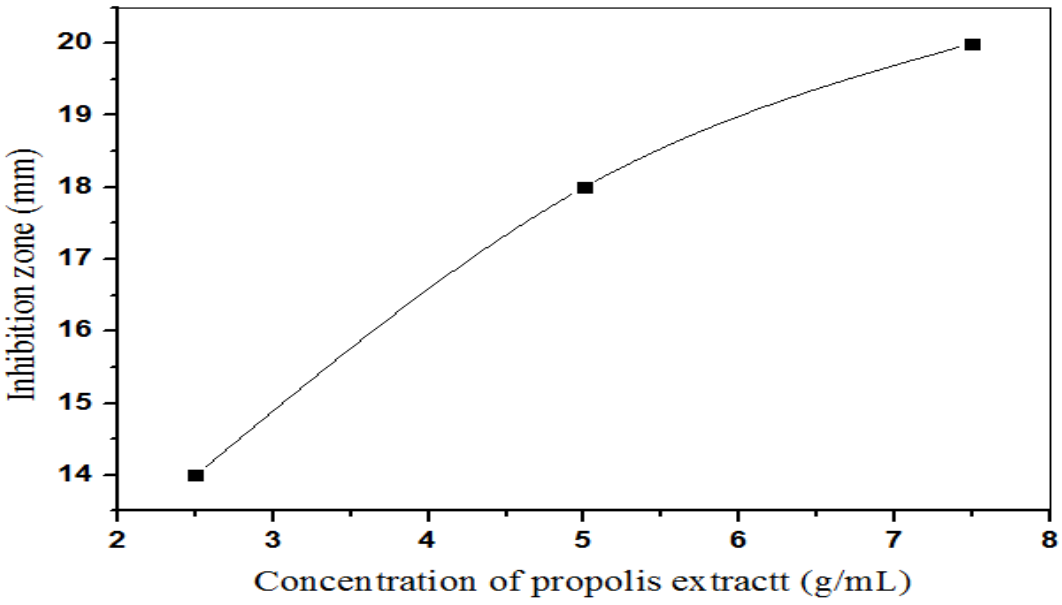

Figure (4) Antibacterial activity by propolis.

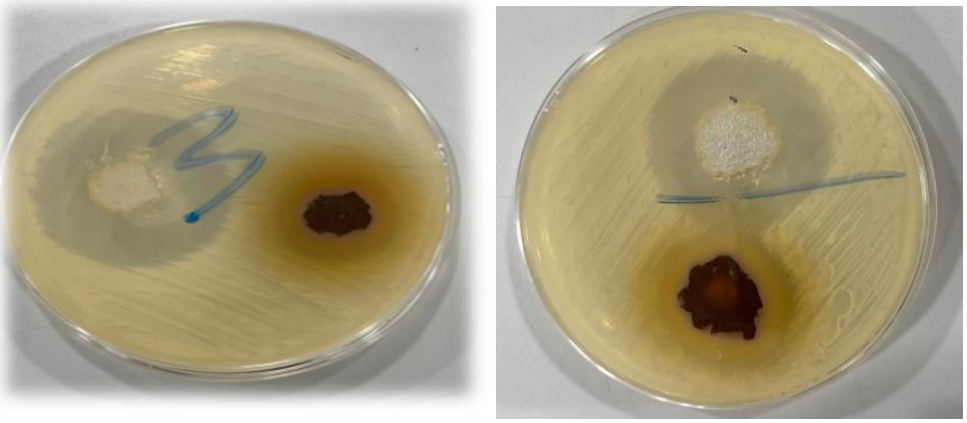

Figure 5 Effect of Garlic and Propolis Extract on the *Staphylococcus aureus*

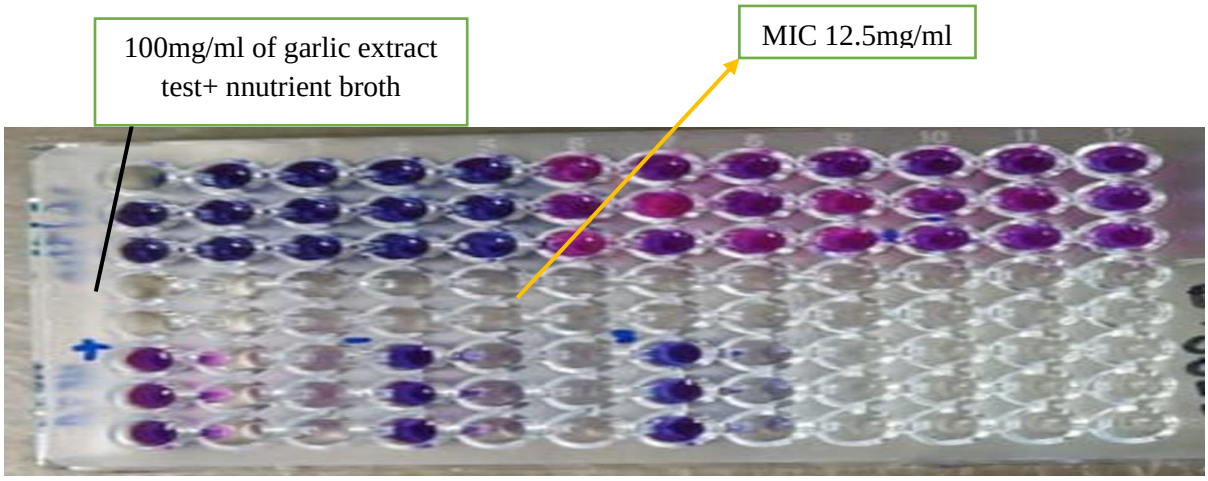

Figure (6) show effect of garlic extraction on bacteria *Staphylococcus aureus* by the MIC method

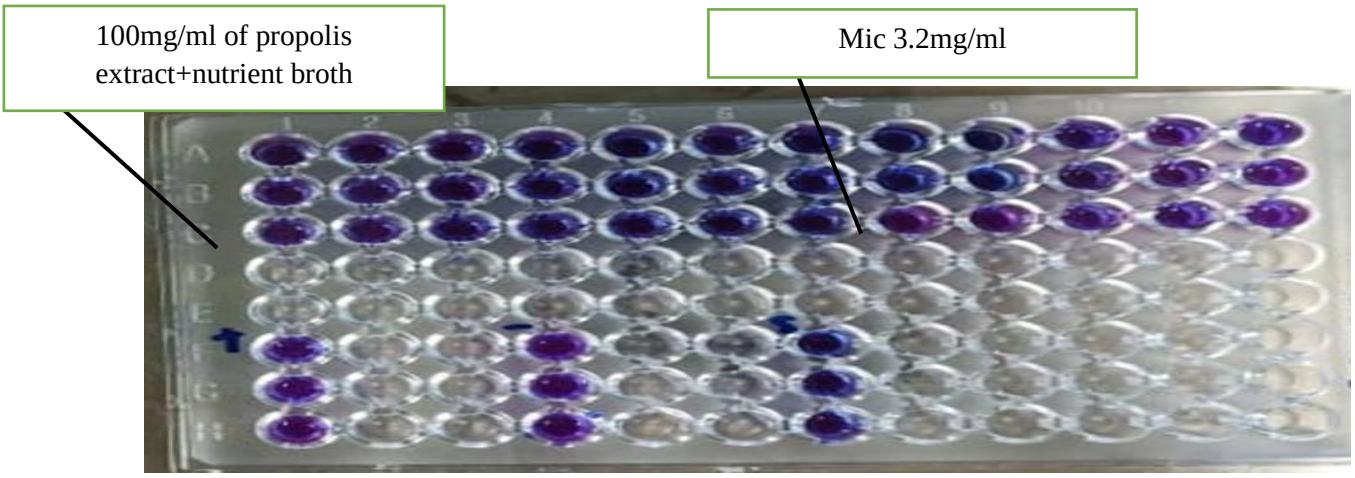

Figure (7) Show effect of propolis extract on bacteria *Staphylococcus aureus* by the MIC method.

Medical Science Journal for Advance Research

Vol. 5, No.4, December 2024
e-ISSN: 2774-4892

DISCUSSION

Disc diffusion method

In recent years, the resistance of bacteria to antibiotics used in treatments has increased. This has led to an increase in the interest of scientists in the antibacterial activity of herbal plants as well as the study of the antibacterial activity of propolis. Garlic has been used as an effective source to eliminate bacteria and a traditional medicine against infectious diseases. Apparent antibacterial activity against *S. aureus.* this result agree with the study of Guzmán [16] that evaluate of propolis and garlic extracts to inhibit *Staphylococcus aureus* growth, also show that combinations garlic and propolis only small concentrations inhibit growth of *Staphylococcus aureus*, garlic and propolis have an antagonistic effect at high concentration.

Garlic extract proved to have a high inhibitory effect against *Staphylococcus aureus* compared with Propoli extract, and this was noticed when using concentrated solutions of it. When using highest concentration of garlic and propolis extract (7.5mg /ml), larger inhibiting zones, this study was agree with the study of Mohammed [17] that show the highest concentration D of garlic extract towards *S. aureus* with maximum inhibiting zones 33mm. Also agree with the study of Borozan [18].

The MIC method

The garlic extract MIC on *S. aureus* arisen with 12.5 mg/ml concentration. These results achieved that garlic is a good antibacterial against other Gram-positive, that must be investigated by further studies. The result of present study slightly diverse with Nejad [19] results that presented MIC of the garlic extract was 7.5 mg/ml to inhibit growth of *S. aureus*. While, the study of Mohammed [17] that show MIC of the garlic extract was 1.56 mg/ml. The garlic extract exhibited an absence growth of bacteria for total inoculation which confirmations potentially bactericidal action to *S. aureus* have an MBC at 12.5 mg/ml bacteriocidal. While, the study of Mohammed [17] that show MBC of garlic extract against *S. aureus* bactericidal at 100 mg/ml, so bacterial growth with (MIC)lowest concentration 1.56 mg/ml reached the concentration (50 mg/ml) signifying that the effect consider only bacteriostatic on these concentration. Propolis is extra operating alongside Gram-positive bacteria observed by [20,21,22]. They presented that the propolis has the antibacterial effect anti Gram-positive bacteria with (MIC between 0.08 mg/ml to 5 mg/ml).

CONCLUSIONS

Garlic and Propolis extract synthesis by a very simple, safe, economical, and fast method. It was found in this study that the efficiency of Garlic extract in inhibiting *Staphlococcus aureus* isolated from diabetic foot patients compared to propolis extract. The diameter of the inhibition reflects the size of the susceptibility of microbes. The results prove the efficiency of garlic extract, which makes it suitable for medical treatment used as an ointment form or tablet to treat ulcers suffered by patients with chronic diabetes. In brief, the results we got in this study show that fresh garlic extract and propolis extract have antibacterial properties whose biological effectiveness has been estimated.

https://doi.org/10.46966/msjar.v5i4.230

Published by: READ INSTITUTE

REFERENCES

1. Jalilian, M., Ahmadi Sarbarzeh, P., & Oubari, S. (2020). Factors related to severity of diabetic foot ulcer: a systematic review. *Diabetes, Metabolic Syndrome and Obesity*, 1835-1842.
2. Peter-Riesch, B. (2016). The diabetic foot: the never-ending challenge. *Novelties in diabetes*, *31*, 108-134.
3. Cheesman, M. J., Ilanko, A., Blonk, B., & Cock, I. E. (2017). Developing new antimicrobial therapies:are synergistic combinations of plant extracts/compoundswithconventiona l antibiotics the solution?. *Pharmacognosy reviews*, *11*(22), 57.
4. Medina, E., & Pieper, D. H. (2016). Tackling threats and future problems of multidrug-resistant bacteria. *How to overcome the antibiotic crisis: facts, challenges, technologies and future perspectives*, 3-33.
5. Fymat, A. L. (2017). Antibiotics and antibiotic resistance. *Biomed J Sci & Tech Res*, *1*(1), 1-16.
6. Hassan, A. and Himayat Ullah. Antibacterial and Antifungal Activities of the Medicinal Plant Veronica biloba", Journal of Chemistry. 2019; 2, 1-7.
7. Mazumdar, T. C. (2019). Isolation and characterization of marker compounds from the leaves of allium cepa linn. and study of their biological activities.
8. Morales-González, J. A., Madrigal-Bujaidar, E., Sánchez-Gutiérrez, M., Izquierdo-Vega, J. A., Valadez-Vega, M. D. C., Álvarez-González, I., ... & Madrigal-Santillán, E. (2019). Garlic (Allium sativum L.): A brief review of its antigenotoxic effects. *Foods*, *8*(8), 343
9. Cauich-Kumul, R., & Campos, M. R. S. (2019). Bee propolis: Properties, chemical composition, applications, and potential health effects. In *Bioactive compounds* (pp. 227-243). Woodhead Publishing.
10. Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., ... & Dash, C. K. (2019). Composition and functional properties of propolis (bee glue): A review. *Saudi journal of biological sciences*, *26*(7), 1695-1703.
11. Upadhyay, S., Rautela, I., Kumar, S., Rawat, B. S., Kumar, V., & Gupta, S. M. (2023). Garlic and Leek Sprouts. In Advances in Plant Sprouts: Phytochemistry and Biofunctionalities (pp. 427-438). Cham: Springer International Publishing.
12. Fagbemi, Josephine Ferdinand, Ugoji, Esther, Adenipekun, Tayo & Adelowotan, Omotoyin. Evaluation of the antimicrobial properties of unripe banana (Musa sapientum L.), lemon grass (Cymbopogon citratus S.) and turmeric (Curcuma longa L.) on pathogens. African Journal of Biotechnology. 2009; 8(7), 1176-1182.
13. Ulusoylu, M., Öndersev, D.V. Soyogul, Ü. Gürkan, E. & Tuzlaci, E. The cytotoxic and the biological (antibacterial and antifungal) activities of Centaurea iberica & Ferulago confusa. Journal of Faculty of Pharmacy of Gazi University. 2001; 18, 75-80.
14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI

supplement M100 (ISBN 978-1-68440-170-3 (Print); ISBN 978-1-68440-171-0 (Electronic)). Clinical and Laboratory Standards Institute, USA, 2023.
15. Yilmaz, M. T. Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains. Turkish Journal of Medical Sciences. 2012; 42, 1423–1429.
16. Guzmán, E. L., & Cruz, F. J. M. (2017). Combinations of Extracts of Propolis and Other Compounds Against Methicillin-Resistant Staphylococcus aureus. InTech. doi: 10.5772/66219
17. Mohammed M. F., Raman N., Alhoot M. A. 2020. Antibacterial Activities of Allium Sativum (Garlic) Extracts Against Staphylococcus aureus and Escherichia Coli. European Journal of Molecular & Clinical Medicine. Volume 7, Issue 11, 2020 ISSN 2515-8260.
18. Borozan A. B., Popescu S., Madosa E., Ciulca A., Moldovan C., Gergen I. Comparative Study on the Antimicrobial Activity of Propolis, Catechin, Quercetin and Gallic Acid. (2023). Not Bot Horti Agrobo 51(2):12826.
19. Nejad A. S. M, Shabani, S., Bayat, M., & Hosseini, S.E. Antibacterial Effect of Garlic Aqueous Extract on Staphylococcus aureus in Hamburger. Jundishapur journal of microbiology. 2014; 7(11), e13134.
20. Mohdaly AA, Mahmoud AA, Roby MH, Smetanska I, Ramadan MF (2015). Phenolic extract from propolis and bee pollen: composition, antioxidant and antibacterial activities. Journal of Food Biochemistry 39(5):538-547.
21. Ghasemi FS, Eshraghi SS, Andalibi F, Hooshyar H, Kalantar-Neyestanaki D, Samadi A, Fatahi-Bafghi M (2017). Antibacterial effect of propolis extract in oil against different bacteria. Zahedan Journal of Research in Medical Sciences. 19(3):e7225.
22. AL-Ani I, Zimmermann S, Reichling J, Wink M (2018). Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. Medicines 5(1):2.

