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A Comparative Study on The Effect of Four Oil Medicinal Plant Extracts on Four Multi-Antibiotic-Resistant Bacterial Groups Isolated from Diabetic Foot Patients

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ABSTRACT

One dangerous side effect of diabetes is diabetic foot ulcers. Patients with diabetic foot ulcers frequently develop multidrug-resistant organisms (MDROs) as a result of the ulcer's long healing process, improper antibiotic use, repeated hospital stays, neuropathy, nephropathy, and peripheral vascular disease. **Objective:** The aim of this research is to examine the potential antibacterial qualities of oil medicinal plants extracts and their prospective as a source of additional anti-infection compounds. **Results**: forty-nine isolates from each bacterial group (*Escherichia coli*, *pseudomonus spp.*, *Proteus mirablis*, *Staphylococcus aureus*) isolated from diabetic foot ulcer and recorded resistance to different antibiotic categories. These isolates treated with four kind of essential oil plant extracts which were (*Syzygium Aromaticum* (Clove), *Camellia sinensis* (green tea), *Nigella sativa* (Black caraway) and *Allium sativum* (garlic)). the result of Scanning electron microscopic (SEM) images showed a normal shape of cells not treated with any type of ethanolic plant extracts where it appeared without any change in morphological appearance While the results of the microscopic examination showed that the presence of media containing microorganism treated with ethanolic plant extracts caused cell deformations, wrinkles, loss of cell shapes and malformations (increases in length and decrease in width) preposition *E. coli* while SEM showed cell depressions, loss of regular cell shapes. Erosion on the outer surface of cells indicates the death of cells and loss of cellular contents in case of *Pseudomonas aeruginosa* As for *staph. aureus* SEM showed cell depressions and dimples on outer surface of the cells. on the other hand, irregularities, diminished cells number and perforation on the outer surface of cells were detected also showed formation of depressions and dimples on the outer surface of the cells. However, abnormalities, a decrease in the number of cells, and perforation on the outer surface of the cells were observed in *proteus mirabilis*.

Keywords: Multidrug-resistant organisms, diabetic foot patients, essential plant oils, Scanning electron microscope

INTRODUCTION

One of the most common effects of diabetes is diabetic foot ulcers (DFUs), which are partly linked to peripheral neuropathy and peripheral vascular dysfunction. According to estimates, 19–34% of diabetic individuals will experience DFUs at some point in their lives. A non-traumatic lower limb amputation is performed on about 85% of these patients (Stramann *et al*., 2020) Aromatic herbs and their derivatives have long been recognized for their therapeutic benefits by the culinary, cosmetic, and medical industries (Lahlou., 2004) . Moreover, it has been demonstrated that several of these plants generate potent secondary metabolites, such as phenolic compounds, which are found in essential oils and have potent antibacterial and insecticidal effects. These results have led to the usage of these chemicals in natural therapies, complementary and alternative medicine, and a range of pharmaceutical products (Rios and Recio ., 2005). Particularly in underdeveloped nations, infectious infections are a leading source of morbidity and mortality. Furthermore, antibiotic resistance is a significant worldwide health issue that compromises patient prognoses, increases treatment costs and demands on the healthcare system due to longer treatment durations and the need of second or third lines of antibiotics (Ventola ., 2015)

The rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immunocompromised individuals, and financial considerations in developing countries all contribute to the global need for alternative prevention and treatment options and products for foot diabietic diseases that are safe, effective, and cost-effective (Lahlou., 2004).

Natural products created from medicinal plants have shown to be a rich supply of biologically active molecules, many of which have been used to develop novel main chemicals for drugs. In the case of microorganism-caused diseases, the increasing resistance of many common pathogens to commonly employed therapeutic medicines, such as antibiotics and antiviral drugs, has resulted in a new interest in the identification of novel antiinfective chemicals (Rios and Recio ., 2005).

Through mutations or DNA exchange (horizontal gene transfer), bacteria will unavoidably continue to evolve strategies to modify pertinent characteristics in order to withstand exposure to novel antibiotics. This will lead to the emergence of what are known as "superbugs." This typically indicates when an organism is resistant to two or more distinct kinds of antibiotics (Gufe *et al*., 2022)**.** *Staphylococcus aureus* is the most often isolated bacteria in cases of osteomyelitis, despite the fact that polymicrobial infections are more common (Lipsky and Van Asten ., 2023). The objective of this study is undertaken to examine the potential antibacterial qualities of some medicinal plants (alternative safe natural antibiotics) and their prospective as a source of additional anti-infection compounds like diabetic foot infection.

MATERIALS AND METHODS

1. Collection of samples

200 diabetic foot ulcers (DFUs) had swabs taken from males and females admitted in Mansoura University Hospitals from July 2021 to July 2022. Samples were collected under aseptic conditions. The laboratory procedures were carried out at: (i) Microbiology Diagnostic and Infection Control Unit (MDICU) of the Medical Microbiology and Immunology department at the Faculty of Medicine, Mansoura University. (ii) Medical Microbiology and Immunology

department at the Faculty of Medicine, Microbiology department at the faculty of science, and Electron Microscopy Unit at Mansoura University.

2. Microbiological analysis

Media used for isolation, purification, identification, maintenance and sensitivity of the bacterial isolate were: MacConkey agar medium, Blood agar medium, Chocolate Agar medium, Nutrient agar and Mueller Hinton agar medium for sensitivity test, and then the biochemical tests performed for cultures to identify the bacteria. After that direct microscopic examination (gram stain film) performed Culture: samples will be inoculated on blood agar, MacConkey agar and Chocolate agar plates and incubated aerobic and anaerobic at 37° C for 24-28hrs. Identification: positive growth will be identified by Gram stained film, colony morphology and biochemical reactions namely, catalase, oxidase, urease, Simmons citrate utilization and methyl red as per the standard method (El Ghallab *et al*., 2020) and (Rebolledo *et al*., 2021) . An antimicrobial sensitivity testing was performed on all identified isolates caused diabetic foot infection by the disc-diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Faujdar and Sharma 2020) to detect the resistant isolates to different antibiotics used during study for further studies.

3. Antimicrobial activity of oil medicinal plant extracts against all infected diabetic foot isolates

The susceptibility of four different medicinal plants oil extracts (Table 1) against four bacterial groups (Escherichia coli, *Pseudomonus* spp., *Proteus mirablis* and Staphylococcus aureus) which were isolated from Diabetic foot ulcer (DFU) patients were detected. Plants was collected from various herbalists and markets in Mansoura and Zagazig cities then confirmed in the Department of Botany, Faculty of Science, Zagazig University.

Table 1: Family, scientific, English name and parts used from each plant in preparing extracts.

Family	Scientific name	English name	Used part
Myrtaceae	*Syzygium Aromaticum*	Clove	Flowers
Theaceae	*Camellia sinensis*	green tea	Leaf
Ranunculaceae	*Nigella sativa*	Black caraway	Seed
Amaryllidaceae	*Allium sativum*	garlic	Root

4. Preparation of herbal plant extract

50 g of dried leaves for each medicinal plants were extracted with 100 ml ethanol by maceration, then filtered through a Buchner funnel with Whatman filter paper number 3, then evaporated under reduced pressure to dryness at 45ºC. The dry extract was suspended in 1% DMSO and sterilized via filtration through a 0.45 µm membrane filter before using in bioassay.

5. **Antibacterial activity of some oil plant extracts against MDR isolates (Well diffusion method)**

MDR isolates used during this study treated with medicinal plant extracts by the well diffusion method. The antibacterial activity against the growth of the pathogenic bacteria recorded as the diameter (cm) of the clear inhibition zone surrounding the discs. Controls had solvent (DMSO) without test plants. The minimum inhibitory concentration (MIC) for the each plant extract of the test plants was determined as the lowest conc. of the extract inhibiting the visible growth of the bacteria on the agar plate (Gupta *et al*., 2018)**.** Inhibition zone was measured in millimeters. The antibacterial activity was done in triplicates to confirm the antibacterial activity

6. **Studying antibacterial activity of most active oil extracts on MDR isolates by detecting the morphological changes by Scanning Electron Microscopy (SEM) examination.**

Steps were done in Electron Microscope Unit in Faculty of Agriculture, Mansoura University. All types of isolates are prepared to be subjected to the plant extracts. Administer the most potent oil extracts to MDR isolates at concentrations equivalent to their Minimum Inhibitory Concentration (MIC) values. Incorporate appropriate measures (unprocessed microorganisms, positive controls) to ensure correct regulation. Treated and control isolates were reared overnight at 37ºC and then placed into the primary fixative and microwaved (MW) under vacuum conditions in a Pelco Biowave (Ted Pella, Inc.). Progressively desiccate the immobilized bacterial samples by subjecting them to a sequence of ethanol concentrations. Substituting the dehydrating agent with a transitional fluid and carrying out critical point drying is done to avoid the structural collapse while capturing images (Nongkhlaw and Joshi 2017). Position the prepared samples onto SEM stubs and insert them into the SEM chamber. Utilize SEM to examine the outer layer of the bacterial cells. SEM has the capability to generate high-resolution, three-dimensional pictures of the surface of the sample, enabling precise examination of morphological alterations.

7. **Statistical analysis**

The data collected were analyzed using SPSS version 20 software, and descriptive statistics were produced in percentage form for the qualitative variables. The chi-square test was used to compare and correlate categorical variables. For correlation results, any p-value less than 0.05 are considered statistically significant.

8. **Ethical considerations**

Our study is validated by a provincial health ethics commission and authorized by the hospital director. The confidentiality of collected information and the anonymity of the participants were guaranteed. The survey team was trained to respect ethical procedures such as professional secrecy.

RESULTS

Antimicrobial activity of medicinal plant oil extracts against MDR clinical *Staphylococcus aureus*, *E. coli*, *Proteus mirabilis* and *Pseudomonas* sp. isolated from diabetic foot patients.

In the current study, four ethanolic crude extracts derived from different parts of four herbal plants species traditionally used in Egypt and belonging to different families were screened for their antibacterial activity against clinical against MDR clinical *Staphylococcus aureus*, *E. coli*, *Proteus mirabilis* and *Pseudomonas* sp. isolated from diabetic foot patients

The diameter of the inhibition zones of ethanolic crude extracts of Dimas (Dimeyhylsulfoxide), Green tea, Clove, *Allium sativum* and *Nigella sativa* against *Staphylococcus aureus*, *E. Coli*, *Proteus mirabilis* and *Pseudomonas* sp were tabulated in Tables (2, 3, 4 and 5) and shown in Figures (1, 2 3 and 4). The current study showed, as recorded in table below that there was statistically significant difference between strain resistance to Dimas, Green tea, clove, *Allium sativum* and *Nigella sativa* while all types of bacteria were resistant to Dimas (Table 2). Regarding to Green tea, *Pseudomonas* sp. had the highest sensitivity with mean inhibition zones of 7.76 (Table 3).

Table 2: Antimicrobial activity of DMSO (Dimethylsulfoxide) against some MDR clinical bacterial isolates isolated from diabetic foot patients

MDE resistant clinical bacterial isolates	Diameter of inhibition zone (mm) Samples (n =49)				F (p value)
	Min	Max	Mean	SD	
S. aureus	**0.0**	**0.0**	**0.0**	**0.0**	
E. coli	**0.0**	**0.0**	**0.0**	**0.0**	
P. mirabilis	**0.0**	**0.0**	**0.0**	**0.0**	
***Pseud.* sp**	**0.0**	**0.0**	**0.0**	**0.0**	

Table 3: Antimicrobial activity of green tea against some MDR clinical bacterial isolates isolated from diabetic foot patients

MDE resistant clinical bacterial isolates	Diameter of inhibition zone (mm) Samples (n =49)				F (p value) = 3.10 (0.02*)
	Min	Max	Mean	SD	
S. aureus	0.0	18.0	4.25	5.86	
E. coli	0.0	22.0	4.63	5.67	
P. mirabilis	0.0	20.0	4.93	6.60	
***Pseud.* sp**	0.0	18.0	7.76	6.72	

As regard to clove all stains reported high sensitivity with high mean inhibition zones (Table 4, and Plate 1).

Table 4: Antimicrobial activity of clove oil extract against some MDR clinical bacterial isolates isolated from diabetic foot patients

MDE resistant clinical bacterial isolates	Diameter of inhibition zone (mm) Samples (n =49)				F (p value) 19.87 (0.001*)
	Min	Max	Mean	SD	
S. aureus	**0.0**	**30.0**	**20.2**	**4.81**	
E. coli	**11.0**	**20.0**	**15.95**	**2.78**	

P. mirabilis	**11.0**	**35.0**	**20.72**	**3.42**	
***Pseud.* sp**	**10.0**	**20.0**	**17.63**	**2.35**	

E coli was sensitive to *Allium sativum* with the highest mean inhibition zones of 20.29 ranging from 0 – 30.0 (Table 5). *Staphylococcus aureus* was slightly sensitive to *Nigella sativa* with inhibition zones ranged from 0 – 15 mm mean value of 1.34 (Table 6).

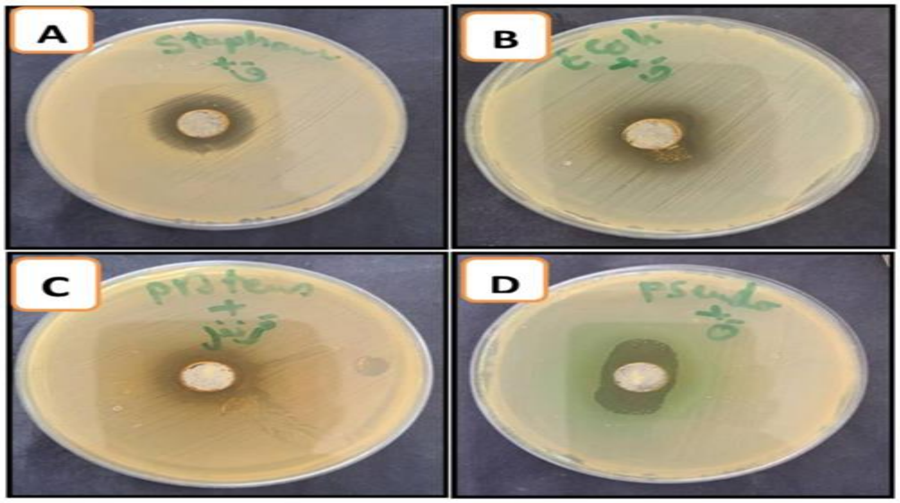

Plate 1: Inhibition zone of ethanolic oil extract of clove (*Syzygium aromaticum*) against some MDR clinical bacterial isolates (A): *Staphylococcus aureus* (B): *E. coli* (C): *Proteus mirabilis* (D): *Pseudomonus* sp

Table 5: Antimicrobial activity of *Allium sativum* against some MDR clinical bacterial isolates isolated from diabetic foot patients

MDE resistant clinical bacterial isolates	Diameter of inhibition zone (mm) Samples (n =49)				F (p value) 3.10 (0.02*)
	Min	Max	Mean	SD	
S. aureus	**0.0**	**10.0**	**0.596**	**2.31**	
E. coli	**0.0**	**30.0**	**20.29**	**4.81**	
P. mirabilis	**0.0**	**0.0**	**.000**	**0.0**	
***Pseud.* sp**	**0.0**	**0.0**	**.000**	**0.0**	

Of all extracts, the ethanolic extract of clove was the most active one with inhibition zone diameters ranging between 20mm-35mm followed by green tea with inhibition zone diameters ranging between 18mm-22mm.

Table 6: Antimicrobial activity of *Nigella sativa* against some MDR clinical bacterial isolates isolated from diabetic foot patients

MDE resistant clinical bacterial isolates	Diameter of inhibition zone (mm) Samples (n =49)				F (p value) 4.17 (0.007*)
	Min	Max	Mean	SD	
S. aureus	**0.0**	**15.0**	**1.34**	**3.64**	

E. coli	**0.0**	**0.0**	**0.0**	**0.0**	
P. mirabilis	**0.0**	**10.0**	**0.72**	**2.42**	
***Pseud.* sp**	**0.0**	**0.0**	**0.0**	**0.0**	

Electron microscopic examination

The most effective medicinal plant extract against MDR clinical *Staphylococcus aureus, E. coli, Proteus mirabilis and Pseudomonas* sp. isolated from diabetic foot patients was clove. These isolates were examined under electron microscope before and after treatment with this oil plant extract.

Scanning Electron microscopic examination (SEM) of some MDR clinical bacterial isolates under the effect of clove oil extracts

There are various stages involved in preparing bacterial samples for Scanning Electron Microscopy (SEM) inspection in order to guarantee appropriate coating, dehydration, and preservation. The structural integrity of bacterial cells must be preserved through stabilization, which is accomplished with the application of fixatives like formaldehyde or glutaraldehyde. The samples are cleaned by completely washing them in buffer solutions. Dehydration is achieved by immersing them in different concentrations of ethanol solutions in order to avoid collapse during SEM imaging. A transitional fluid can be employed in place of ethanol while maintaining the sample's structural integrity through the application of critical point drying. Desiccated samples are securely attached to carbon tape or conductive glue on SEM stubs. To enhance image quality, a protective layer is placed to the samples' surface. The coating is applied using a sputter coater. After that, the samples are carefully moved into the SEM chamber. By modifying variables like voltage, working distance, and aperture size, the imaging conditions are tuned. After that, pictures are examined to evaluate the samples' structural qualities at various magnifications.

Escherichia coli

E. coli is a rod-like form and belongs to the Enterobacteriaceae family. It is classified as a Gram-negative bacterium. The cells have a cylindrical shape with rounded extremities, like little rods (Figure 1). The typical size of an *E. coli* cell is around 2 micrometers in length and 0.5 micrometers in diameter. *E. coli* cells are typically seen alone or in pairs. The outer membrane of the bacteria includes lipopolysaccharides, which play a role in the bacterium's ability to cause disease. Under microscopic examination, *E. coli* cells exhibit a pink or red coloration when subjected to Gram staining, which is caused by the application of a counterstain The rod-shaped cells may be observed using light microscopy. SEM examination shows that it is normal shape of cells without any change in morphological appearance (**Figure 1A**). SEM images showed that the presence of media containing E. coli treated with the used clove oil extract which caused cell deformations, wrinkles, loss of cell shapes (**Figure 1B**) and malformations (increases in length and decrease in width)..

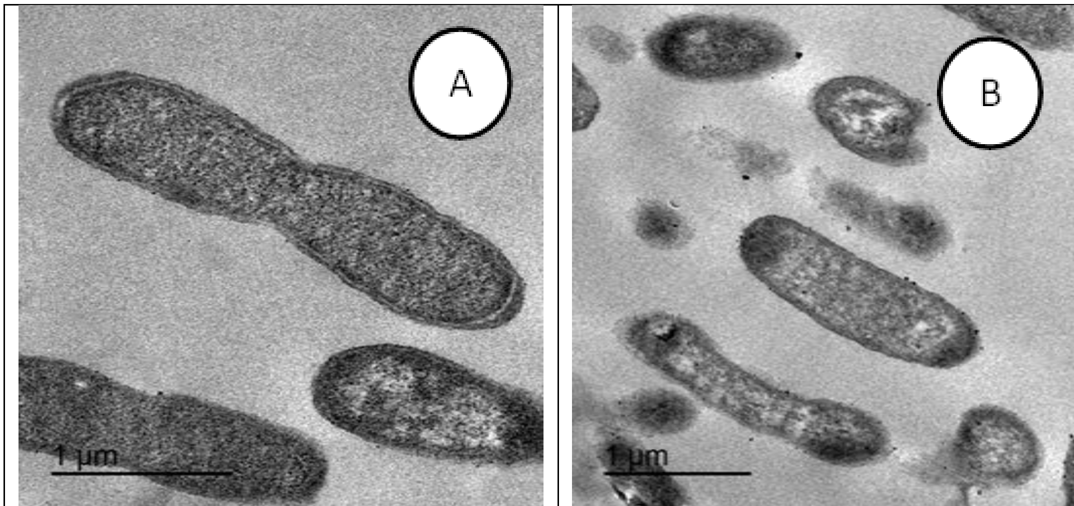

Fig.1. **Scanning electron microscopic examination of *E. coli* before (A) and after (B) treated with clove oil extract.**

Pseudomonas aeruginosa

Pseudomonas aeruginosa bacteria often exhibit a morphology characterized by a rod-shaped or bacillary structure. They have an extended shape and may vary in length, but often have a straight or slightly curved look. The cell surface of *Pseudomonas* has a rather uniform texture when seen under scanning electron microscopy (SEM). It is noted normal shape of cells without any change in morphological appearance **(Figure 2 A)**. SEM images showed that the presence of media containing *Pseudomonas aeruginosa* treated with clove oil plant extracts which caused cell depressions, loss of regular cell shapes. Erosion on the outer surface of cells indicates the death of cells and loss of cellular contents **(Figure 2B)**.

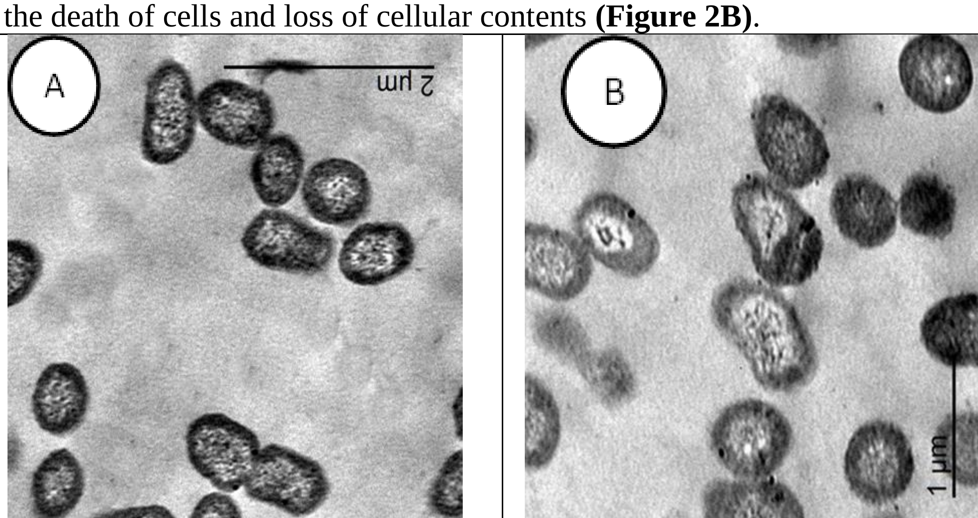

Fig. 2. Scanning electron microscopic examination of *Pseudomonas aeruginosa* before (A) and after (B) treated with clove oil extract.

Staphylococcus aureus

Staphylococcus aureus bacteria are spherical, cocci-shaped, round cells with distinctive clusters resembling grapes. They divide in multiple planes, creating irregular clusters, known as "irregular cell division," which contributes to their distinctive appearance under SEM. It is noted normal shape of cells without any change in morphological appearance **(Figure 3A)**. SEM images showed that *Staphylococcus aureus* treated with plant extracts which caused cell depressions and dimples on outer surface of the cells .on the other hand irregularities ,diminished cells number and perforation on the outer surface of cells **(Figure 3B).**

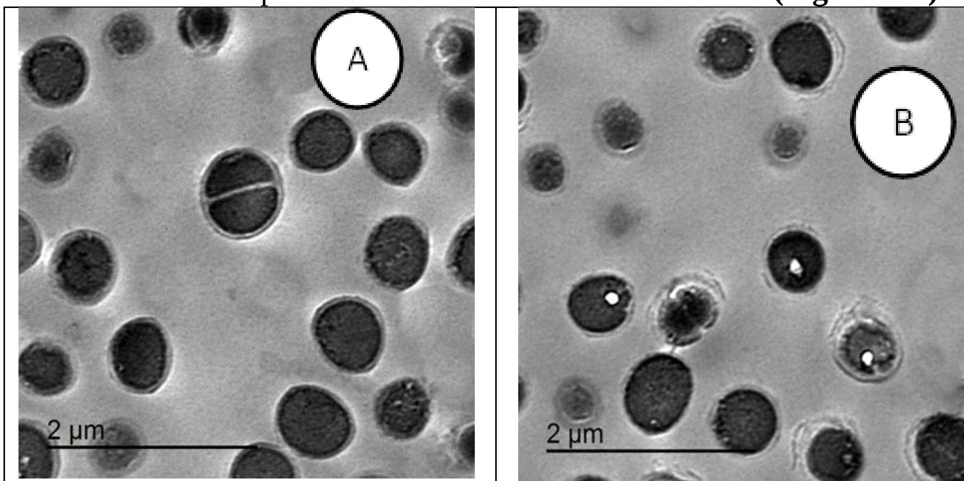

Fig. 3. Scanning electron microscopic examination of *Staphylococcus aureus* before (A) and after (B) treated with clove oil extract.

Proteus mirbilis

Regarding to *Proteus mirabilis*, it is noted normal shape of cells without any change in morphological appearance **(Figure 4A)**, while SEM images revealed the cells were subjected to treatment with clove oil plant extracts, resulting in the formation of depressions and dimples on the outer surface of the cells. However, abnormalities, a decrease in the number of cells, and perforation on the outer surface of the cells were observed **(Figure 4B)**

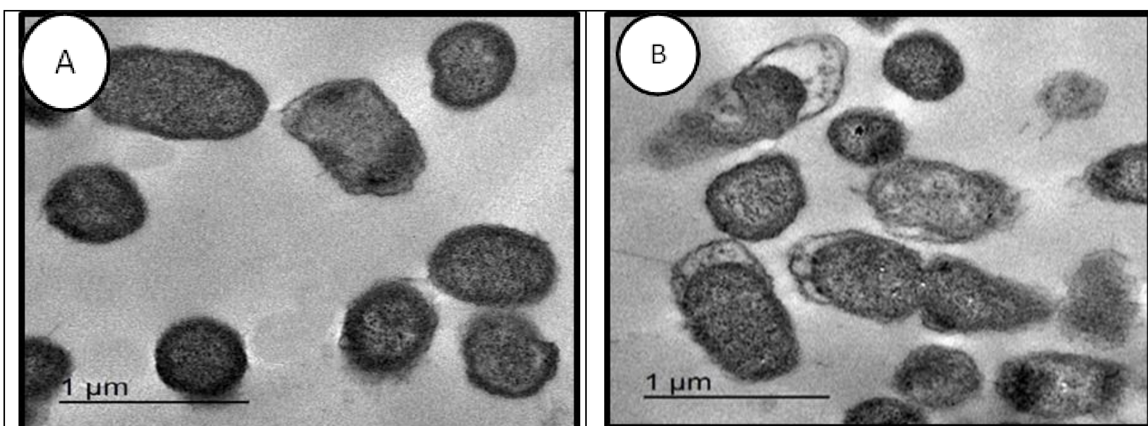

Fig. 4. Scanning electron microscopic examination of Staphylococcus aureus before (A) and after (B) treated with clove oil extract.

DISCUSSION

Important study is being done to determine how plant components affect the growth and survival of bacteria that often grow on diabetic feet. Specifically, the effect of plant extracts on bacteria isolated from the feet of diabetic foot patients is being studied. The purpose of this study is to demonstrate how different plant extracts affect the growth of bacteria that has been isolated from diabetic foot ulcer patients' feet. This method is significant since it might assist in selecting the best course of action for diabetics' foot infections.

An antibiotic can be used as a successful treatment if the bacteria are susceptible to it. On the other hand, a different course of action would be required if the bacteria are resistant to the antibiotic (Liu *et al*., 2019). Patients with diabetes may develop sores on their feet due to the harmful microorganism Staphylococcus aureus, commonly known as staph bacterium (Anafo *et al*., 2021). Since it decreases the effectiveness of antibiotic therapy, the rise of antibiotic resistance in Staphylococcus aureus is a serious concern in the medical community (Mukherjee *et al*., 2021).

It is concerning that *Staphylococcus aureus* is becoming more resistant to antibiotics because these infections are associated with a higher risk of disease and mortality (Ghurch and Mckillipe 2021). When treating *Staphylococcus aureus* infections, the existence of antibiotic resistance suggests the need for other therapeutic alternatives (Anafo *et al*., 2021).

Gram-negative bacteria with a gram-negative cell wall are called Proteus mirabilis, and they are members of the Enterobacteriaceae family. It is the cause of many illnesses, including up to 44% of urinary tract infections linked to catheter use and more than 3% of infections obtained in healthcare settings (Maneno *et al*., 2023). The significant levels of antibiotic resistance found in *Pseudomonas*, particularly to many commonly used antibiotics, pose a serious threat to the effective treatment of bacterial illnesses (Bhardwaj *et al*., 2023).

In our findings, there was statistically significant difference between strain resistance to Dimas, Green tea, Clove, *Allium sativum* and *Nigella sativa* while all types of bacteria were resistant to Dimas. Regarding to Green tea, *Pseudomonas* had the highest sensitivity with mean inhibition zones of 7.76. As regard to Clove all stains reported high sensitivity with high mean inhibition zones. *E coli* was sensitive to *Allium sativum* with the highest mean inhibition zones of 20.29 ranging from 0 – 30.0. Staphylococcus aureus was slightly sensitive to Nigella sativa with inhibition zones ranged from 0 – 15 and mean value of 1.34

Many bioactive ingredients included in green tea have been well studied for their potential antibacterial properties (Reygaert , 2018). One class of chemicals that contributes to these effects are called catechins, a kind of flavonoid [(19)]. The most common and most studied catechin in green tea is called epigallocatechin gallate (EGCG) (Almatroodi *et al*., 2020). *Pseudomonas aeruginosa* is among the many bacterial species against which EGCG has demonstrated antibacterial effectiveness (Buchmann *et al*., 2022). It can hinder Pseudomonas development and virulence by breaking down bacterial cell membranes and interfering with essential cellular functions.

Pseudomonas aeruginosa is known for its capacity to form biofilms, which are bacterial aggregations encased in a matrix (Jacobs *et al*., 2022). EGCG may be a viable option for the treatment or prevention of biofilm-related diseases since it has shown the capacity to prevent Pseudomonas from producing biofilms [(23)]. To regulate the activation of virulence proteins, *Pseudomonas aeruginosa* uses quorum sensing, a communication mechanism among bacterial cells (Asfahl *et al*., 2022). The ability of EGCG to interfere with quorum sensing mechanisms has been investigated; this could reduce Pseudomonas pathogenicity (Hengge , 2019).

Antioxidant properties are possessed by EGCG (Messire and Berteina-Raboin.,2023) Antioxidants can foster an environment that helps the immune system combat bacterial infections, even though they might not be able to actively eradicate germs (Pandey *et al*., 2021). Because of its antibacterial properties—more especially, how it affects various bacterial strains—clove, which is produced from the Syzygium aromaticum plant, has been the subject of scientific inquiry (Haro *et al*., 2021). According to Ishaq *et al*. (2019), clove has a broad spectrum of antibacterial activity against several types of bacteria (Rosarior *et al.*, 2021). Both Gram-positive and Gram-negative bacteria are included in this. Clove oil contains a phenolic compound called eugenol, which is commonly identified as the active ingredient that has an antibacterial action (Nada *et al*., 2022) .

Eugenol, has the ability to disturb the cell membranes of bacteria, resulting in the demise of the cells (Ulanowska and Olas, 2021). Thus, clove oil possesses the potential to serve as a natural substitute for controlling the growth of germs (Haro-Gonzalez *et al*., 2021). The effects of garlic, or *Allium sativum* as it is known scientifically, on a variety of bacteria, including *E. coli*, have been studied in great detail (Altuntas and Korukluoglu, 2019). Allicin, a substance found in garlic, is well known for having antibacterial properties (El-Saber Batiha *et al*., (2020). *E. coli* and other bacterial species can't grow as easily when exposed to allicin (Bhatwalkar *et al*., 2021). It disrupts a number of biological functions in bacteria, which stops them from reproducing and causes their cells to die.

According to Liu *et al*. (2021), garlic extracts, including garlic oil and aqueous extracts, have shown antibacterial capabilities against *E. coli*. It is possible for these extracts to successfully prevent *E. coli* from growing in a lab setting that is under control. It has been found that garlic extracts prevent the growth of bacterial biofilms (Bhatwalkar *et al*., 2021). The bacterium *Escherichia coli* have the ability to form biofilms, which are collections of bacteria encased in a protective matrix (Singh *et al*., 2021). Disrupting the formation of biofilms is necessary to stop bacterial infections from becoming chronic (Hemmati et al., 2021).

Conclusions:

According to the present study, it can be concluded that the essential oils derived from several plant sources exhibit significant antibacterial activity, indicating their potential as strong antibacterial agents. These organic compounds have demonstrated effectiveness against bacteria that are resistant to many drugs, which are often difficult to treat with traditional antibiotics. The study highlights how different essential oils differ in their ability to fight microorganisms. Certain oils exhibited substantial antibacterial activity against the investigated microorganisms, whereas other oils showed varying degrees of efficacy. These results suggest that the choice of essential oil can significantly affect how well complementary therapy modalities work. Contribution Potential in Treating Diabetic Foot Infections: Diabetic foot infections pose a serious risk to health, especially when they are caused by bacteria resistant to many medications. The research suggests that essential oils may be utilized in addition to conventional treatments to improve the prognosis of patients for specific infections. Research Directions for the Future: The encouraging results of this study encourage further research in this area. In order to determine the safety and efficacy of essential oils in treating diabetic foot ulcers, future research should give priority to optimizing essential oil formulations, investigating their mechanisms of action, and conducting clinical investigations. The study shows the growing importance of natural and holistic approaches in medicine. In keeping with the global trend toward complementary and sustainable medicine, essential oils offer a viable and eco-friendly replacement for synthetic antibiotics. In light of the growing issue of antibiotic resistance, research into natural remedies, such as essential oils, has significant promise for treating bacterial illnesses. This work encourages a multidisciplinary approach to addressing the numerous problems associated with diabetic foot infections and contributes to the growing body of knowledge in the field of alternative medicine. As we proceed, it is imperative to maintain the highest standards of evidence-based healthcare while making use of the potential of natural medicines.

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Conflicts of interest

The authors declare that there is no conflict if interests.

Ethical approval

All ethical procedures have been respected and our study have been validated by provincial ethics commission

REFERENCES

Almatroodi, S.A.; Almatroudi, A.; Khan, A.A.; Alhumaydhi, F.A., Alsahli, M.A. and Rahmani, A. H. (2020). Potential therapeutic targets of epigallocatechin gallate (EGCG), the most abundant catechin in green tea, and its role in the therapy of various types of cancer. Molecules, 25: 3146-3154.

Altuntas, S. and Korukluoglu, M. (2019). Growth and effect of garlic (Allium sativum) on selected beneficial bacteria. Food Science and Technology, 39: 897-904.

Anafo, R.B.; Atiase, Y.; Dayie, N.T.; Kotey, F.C.; Tetteh-Quarcoo, P.B.; Duodu, S. and Donkor, E.S. (2021). Methicillin-resistant *Staphylococcus aureus* (MRSA) infection of diabetic foot ulcers at a tertiary care hospital in Accra, Ghana. Pathogens, 10: 937-944.

Asfahl, K.L.; Smalley, N.E.; Chang, A.P. and Dandekar, A.A. (2022). Genetic and transcriptomic characteristics of RhlR-dependent quorum sensing in cystic fibrosis isolates of *Pseudomonas aeruginosa*. Msystems, 7: e00113-22.

Bhardwaj, S.; Bhatia, S.; Singh, S. and Franco Jr, F. (2021). Growing emergence of drug-resistant *Pseudomonas aeruginosa* and attenuation of its virulence using quorum sensing inhibitors: A critical review. Iranian Journal of Basic Medical Sciences, 24: 699.

Bhatwalkar, S.B.; Mondal, R.; Krishna, S.B.N.; Adam, J.K.; Govender, P. and Anupam, R. (2021). Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). Frontiers in Microbiology, 12: 1869.

Buchmann, D.; Schultze, N.; Borchardt, J.; Böttcher, I.; Schaufler, K. and Guenther, S. (2022). Synergistic antimicrobial activities of epigallocatechin gallate, myricetin, daidzein, gallic acid, epicatechin, 3-hydroxy-6-methoxyflavone and genistein combined with antibiotics against ESKAPE pathogens. Journal of Applied Microbiology, 132: 949-963.

Church, N.A. and McKillip, J.L. (2021). Antibiotic resistance crisis: challenges and imperatives. Biologia, 76: 1535-1550.

El Ghallab, Y.; Al Jahid, A.; Jamal Eddine, J.; Ait Haj Said, A.; Zarayby, L. and Derfoufi, S. (2020). *Syzygium aromaticum* L.: Phytochemical investigation and comparison of the scavenging activity of essential oil, extracts and eugenol. Advances in Traditional Medicine, 20: 153-158.

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

Fathima, A. and Rao, J.R. (2016). Selective toxicity of Catechin—A natural flavonoid towards bacteria. Applied microbiology and biotechnology, 100: 6395-6402.

Faujdar, S.S.; Bisht, D. and Sharma, A. (2020). Antibacterial activity of *Syzygium aromaticum* (clove) against uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent?. Journal of family medicine and primary care, 9: 180.

Gufe, C.; Karembera, B.; Marumure, J. and Makuvara, Z. (2022). Lead, silver nitrate and antibiotic resistance in bacteria isolated from Nile tilapia (*Oreochromis niloticus*) in anthropogenically polluted Lake Chivero, Zimbabwe. Cogent Food & Agriculture, 8: 2082.

Gupta, D.I..; Kumar, M.U. and Gupta, V.I.. (2018). An in vitro investigation of antimicrobial efficacy of *Euphorbia hirta* and *Murraya koenigii* against selected pathogenic microorganisms. Asian J Pharm Clin Res, 11: 359-363.

Haro-González, J.N.; Castillo-Herrera, G.A.; Martínez-Velázquez, M. and Espinosa-Andrews, H. (2021). Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules, 26: 6387.

Hemmati, F.; Rezaee, M.A.; Ebrahimzadeh, S.; Yousefi, L.; Nouri, R.; Kafil, H.S. and Gholizadeh, P. (2021). Novel strategies to combat bacterial biofilms. Molecular biotechnology, 63: 569-586.

Hengge, R. (2019). Targeting bacterial biofilms by the green tea polyphenol EGCG. Molecules, 24: 2403.

Ishaq, A.; Syed, Q.A.; Khan, M.I. and Zia, M.A. (2019). Characterising and optimising antioxidant and antimicrobial properties of clove extracts against food-borne pathogenic bacteria. International Food Research Journal, 26: 1165-1172.

Jacobs, H.M.; O'Neal, L.; Lopatto, E.; Wozniak, D.J.; Bjarnsholt, T. and Parsek, M. R. (2022). Mucoid *Pseudomonas aeruginosa* can produce calcium-gelled biofilms independent of the matrix components Psl and CdrA. Journal of bacteriology, 204: e00568-21.

Lahlou, M. (2004). Methods to study the phytochemistry and bioactivity of essential oils. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 18: 435-448.

Lipsky, B.A. and van Asten, S. A. (2023). An Evidence-Based Approach to Treating Osteomyelitis. In *Functional Limb Salvage: The Multidisciplinary Team Approach* (pp. 175-186). Cham: Springer International Publishing.

Liu, Y.; Li, R.; Xiao, X. and Wang, Z. (2019). Antibiotic adjuvants: an alternative approach to overcome multi-drug resistant Gram-negative bacteria. Critical reviews in microbiology, 45: 301-314.

Lu, L.; Hu, W.; Tian, Z.; Yuan, D.; Yi, G.; Zhou, Y. and Li, M. (2019). Developing natural products as potential anti-biofilm agents. Chinese medicine, 14: 1-17.

Maneno, E.; Baravuga., A.; Mzula., AA. and Katakweba. (2023). Antimicrobial susceptibility patterns and molecular phylogenetics of *Proteus mirabilis* isolated from domestic rats: An environmental driver to antimicrobial resistance in public health in Arusha Tanzania. German journal of microbiology, doi: 10.51585/gjm.2023.1.0022.

Messire, G.; Serreau, R. and Berteina-Raboin, S. (2023). Antioxidant Effects of Catechins (EGCG), Andrographolide, and Curcuminoids Compounds for Skin Protection, Cosmetics, and Dermatological Uses: An Update. Antioxidants, 12: 1317.

Mukherjee, R.; Priyadarshini, A.; Pandey, R.P. and Raj, V.S. (2021). Antimicrobial resistance in *Staphylococcus aureus*. Insights into drug resistance in *Staphylococcus aureus*, 85: 11-20.

Nada, H.G.; Mohsen, R.; Zaki, M.E. and Aly, A.A. (2022). Evaluation of chemical composition, antioxidant, antibiofilm and antibacterial potency of essential oil extracted from gamma irradiated clove (*Eugenia caryophyllata*) buds. Journal of Food Measurement and Characterization, 3: 1-14.

Nongkhlaw, F.M.W.and Joshi,S.R. (2017). Microscopic study on colonization and antimicrobial property of endophytic bacteria associated with ethnomedicinal plants of Meghalaya. Journal of microscopy and ultrastructure, 5: 132-139.

Pandey, R.P.; Mukherjee, R.; Priyadarshini, A.; Gupta, A.; Vibhuti, A.; Leal, E and Lyu, X. (2021). Potential of nanoparticles encapsulated drugs for possible inhibition of the antimicrobial resistance development. Biomedicine & Pharmacotherapy, 141: 111943.

Rebolledo, V.; Otero, M.C.; Delgado, J.M.; Torres, F.; Herrera, M.; Ríos, M. and Rodríguez-Díaz, M. (2021). Phytochemical profile and antioxidant activity of extracts of the peruvian peppertree Schinus areira L. from Chile. Saudi Journal of Biological Sciences, *28*: 1052-1062.

Reygaert, W. C. (2018). Green tea catechins: Their use in treating and preventing infectious diseases. BioMed research international, 20: 18-23.

Ríos JL and Recio MC (2005). Medicinal plants and anti-microbial activity. J Ethnopharmacol 100: 80–84.

Rosarior, V.L.; Lim, P.S., Wong, W.K.; Yue, C.S.; Yam, H.C. and Tan, S.A. (2021). Antioxidant-rich clove extract, a strong antimicrobial agent against urinary tract infections-causing bacteria in vitro. Tropical Life Sciences Research, 32: 45.

Singh, S.; Datta, S.; Narayanan, K.B. and Rajnish, K.N. (2021). Bacterial exo-polysaccharides in biofilms: Role in antimicrobial resistance and treatments. Journal of Genetic Engineering and Biotechnology, 19: 1-19.

Stratmann, B.; Costea, T.C.; Nolte, C.; Hiller, J.; Schmidt, J.; Reindel, J., and Tschoepe, D. (2020). Effect of cold atmospheric plasma therapy vs standard therapy placebo on wound healing in patients with diabetic foot ulcers: a randomized clinical trial. JAMA network open, 3: e2010411-e2010411.

Ulanowska, M. and Olas, B. (2021). Biological Properties and prospects for the application of eugenol—A review. International Journal of Molecular Sciences, 22: 3671.

Ventola, C.L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics, 40: 277.