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Antibacterial and Anticandidal Activity of Ethanolic Extract of Immature Flower Buds of *Syzygium aromaticum*

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ABSTRACT

Microbial resistance to antibiotics has been raised over time, creating a serious burden and an issue that requires a quick response. As a result, interest has shifted to medicinal plants as natural, effective, and cost-efficient alternatives. The goal of this study was to assess the antibacterial and antifungal activities of the dried buds of the clove's flowers, which were extracted in 250 ml of ethanol using the Soxhlet apparatus. Subsequently, DMSO stock solutions were utilized to create concentrations of 100, 50, 25, 12.5, 6.25, and ultimately 3.125 mg/ml. Following that, the agar-well diffusion technique was utilized for the evaluation of the antibacterial activities of the clove extract against gram-positive bacteria (*Staphylococcus aureus* and Group A *Streptococcus* bacteria) and gram-negative bacteria (*Escherichia coli*), as well as *Candida albicans*, isolated and identified through the pediatric hospital's medical laboratory west of Gaza city. Lastly, the results of this study clearly showed that the ethanolic extract of dried immature flower buds of clove has anti-*S. aureus* and anti-group A *Streptococcus* effects, as well as for the *C. albicans* used in the present study, with the exception of *E. coli*, which demonstrated resistance to the plant extract at all concentrations tested. While the lowest concentrations that showed anti-bacterial activity were revealed to be 12.5 and 25 mg/ml for anti-*S. aureus* and anti-group A *Streptococcus*, respectively, with inhibition zone diameters of 2 and 1 mm, on the other hand, 25 mg/ml with a 2 mm inhibition zone was determined to have the lowest concentration that had anti-*C. albicans* activity. This study concluded that cloves can be employed as a plant with anti-bacterial and anti-fungal characteristics, but additional research is required.

Keywords: *Syzygium aromaticum*, *Candida albicans,* Group *a Streptococcus*, and Ethanolic extract.

INTRODUCTION:

Globally, infectious diseases have been recognized as a major cause of morbidity and mortality, particularly in developing countries, accounting for over half of all morbidity documented in tropical countries due to the high incidence of bacterial infection in those countries (Iwu *et al.,* 1999; Lewis & Ausubel, 2006). The detection and development of antibiotics is without a doubt one of the most significant and successful contributions of modern science and technology to the fight against infectious diseases. However, the occurrence of antimicrobial resistance among microbial strains, as well as the increasing occurrence of such resistance, restrict the efficiency of several antibiotics' therapeutic results (Jelager *et al.,* 1998; ATEŞ and TURGAY, 2003). Nonetheless, the continuous development of

microbial resistance to currently available antibiotics is producing further issues by increasing the number of incidents of infection, lengthening recovery periods, and resulting in avoidable deaths. Because of these circumstances, there is a significant demand for innovative and effective antimicrobial agents (Bokhari, 2009; Aly & Bafeel, 2010). Besides that, the World Health Organization has stated that antimicrobial resistance (AMR) is a complicated global public health concern, and that no single or simple strategy will be effective in preventing the establishment and spread of disease-causing organisms resistant to current antimicrobial drugs. Antimicrobial resistance is a natural process in microbes that has been exacerbated by the indiscriminate and unwise use of these antimicrobial drugs, as well as their random abuse of people and animals. The current shortage of novel antimicrobials to replace those that have become ineffective adds to the necessity to keep existing treatments effective (W. H. O, 2014). Based on the aforementioned and the developing antibiotic resistance, alternative, therapeutic, and preventative measures that are safe, cost-effective, and beneficial to humanity are required. Numerous different plants, herbs, and spices have been utilized for this purpose for a long time (Lee, 2013). As a result, since ancient times and throughout the ages, attention has been drawn to handling plants as a treatment for many diseases in all prior cultures and civilizations for long periods of time, in addition to as a crucial part of traditional medicine. Furthermore, recent studies have proven that plants are a potential source of several antimicrobial agents because they produce a variety of active metabolites that serve as natural protection against microbial infections (Mahmoud *et al.,* 2004; Amer *et al.,* 2006).

Since ancient times, plants have been a significant source of phytochemicals that have been utilized to maintain human health. As stated by the World Health Organization, more than half of all existing therapeutic drugs are derived from natural products. 80% of the world's population utilizes plant extracts or their active ingredients as folk medicine in traditional therapies. Nevertheless, traditionally, useful therapeutic compounds have been found in medicinal plants. By providing pharmacopoeia that may be used to create new pharmaceuticals with innovative mechanisms of action, green pharmacy has the potential to become the basis for drug development (Nassar *et al.,* 2007; Kirbağ *et al.,* 2009; Karuppiah and Rajaram, 2012). Clove (*Syzygium aromaticum* (L.) or *Eugenia aromaticum*) is one of the oldest and most significant spices in the East. It is a member of the *Myrtaceae* family. Commercial cloves are made from dried, unopened flower buds. Furthermore, both whole and powdered cloves are used to enhance the flavor of meat and rice meals, as well as curry and masala powders. It is highly valued in medicine for its carminative, stimulant, and natural anthelmintic properties. It is also commonly used in Europe and Asia, where it is smoked as a type of cigarette known locally as kretek in Indonesia and in a few coffeehouses in the West. In recent years, it has been recognized as a useful fish analgesic for a number of invasive and non-invasive fisheries management and research strategies (Prince and Powell, 2000; Srivastava *et al.,* 2003). *Syzygium* species have been demonstrated to have antibacterial and anti-inflammatory activities. *Syzygium aromaticum* buds have traditionally been utilized as a diuretic, stomachic, cardio tonic, aromatic spice and condiment with carminative and tonic properties. Likewise, clove essential oil has been shown to have antibacterial activity against a variety of multi-resistant Staphylococcus epidermidis and oral pathogens (Kirbağ *et al.,* 2009; Karuppiah & Rajaram, 2012; Shahen *et al*., 2019).

Otherwise, cloves are the aromatic, dried buds of a tree (*Syzygium aromaticum*) that are used as a spice in almost all dishes across the world. Dried clove bud contains carbohydrates, fixed oil, volatile steam oil, resins, tannins, proteins, cellulose, pentosene, and mineral components. Besides, cloves have also produced a number of non-volatile chemicals such as tannins, sterols, triterpenes, and flavonoids (Ayoola *et al.,* 2008). The purpose of this study is to assess the antifungal and antibacterial activities of an ethanolic extract of dried clove buds against pathogenic strains identified and gathered from one of the most significant pediatric hospitals in Gaza, Palestine.

MATERIALS AND METHODS:

Plant material collection and processing

The plant material (dried immature clove flower buds) was purchased from a spice shop in Gaza city, Pales-

tine. Subsequently, the plant material was thoroughly washed with water and dried in the shade, away from sunlight. After drying, it was meticulously ground using a mill to get a fine powder. This powder was reserved dry and away from moisture until it was used in the extraction procedure to produce the crude extract required for the current investigation.

Preparation of plant extract

The crude plant extract was prepared using the soxhlet apparatus and a temperature adjustment of 70 ° C for two days intermittently for an average of 8 hours each day by mixing 200 ml of 70% ethyl alcohol with 20 g of the dry plant powder acquired in the previous step. The extract was recovered after two days by evaporation to remove the alcohol residues used in the process, which was carried out in a 45 °C oven. The residual evaporation extract was collected in sterile containers and kept in the freezer until use (Mishra & Kalyani, 201 4).

Preparation of plant extracts concentrations

A modified approach of Almola's, (2010) was performed, in which 100 mg of the ethanolic extract was dissolved in 1 ml of DiMethyl Sulphoxide (DMSO). As a result, an ethanolic extract standard concentration of 100 mg/ml of stock was achieved. After that, 0.22 µm membrane filters were utilized to sterilize the obtained solution (Almola, 2010). The ethanolic extract was then serially diluted to achieve the concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/ml.

Preparation of inocula

The microbiological stock cultures utilized in this study were maintained at 4 °C on nutritional agar plates. Along with *Candida albicans*, the Central Microbiology Laboratory at Al-Nasr Governmental Hospital for Children in Gaza clinically isolated and identified three bacterial strains, including *E. coli* (gram -), *S. aureus* (gram +), and Group A *Streptococcus* (gram +). After that, a loop of the culture was transferred to 5 ml of Brain Heart Infusion broth and incubated for 24 hours at 37 °C to generate active cultures for the current investigation (Jouda *et al.,* 2015).

Determination of Antibacterial Activity

Each tested bacteria was cultivated in nutrient broth medium, and 0.1 ml of pure cultures (4x106 CFU/ml) was used to inoculate each chilled Muller Hinton agar plate. A suspension of the tested bacteria was also placed on MHA medium. Consequently, filter paper discs (5 mm in diameter) were inoculated with the tested bacteria before being impregnated with 20 µl of plant extract (at concentrations of 100, 50, 25, 12. 5, 6.25, and 3.125 mg/ml). The plates were then incubated for 24 hours at 37 °C. Given that each experiment was repeated three times for greater precision, the average diameter of the inhibitory zone was measured in millimeters after incubation to assess the antibacterial activities of the dry extract of immature clove flower buds. The positive control was ciprofloxacin, whereas the negative control was DMSO (Perez, 1990; Al Masoudi *et al.,* 2013).

Determination of Anticandidal Activity

To evaluate the antifungal activity of an ethanolic extract of dried immature clove flower buds against *Candida albicans*, a loop of the test strain was inoculated in 25 ml of dextrose potato broth and shaken on a rotary shaker for 24 hours to activate the given tested *candida* strain. As suggested previously, PDA plates were created for the agar diffusion technique to assess antifungal activity *in vitro* (Hammer *et al.,* 1999). It should be mentioned that nystatin 50 µg was used as a positive control. On the other hand, DMSO served as a negative control. Filter paper discs (5 mm in diameter) were inoculated with the tested *Candida albicans* and then impregnated with 20 µg of plant extract (at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml). To test the antifungal activities of dried immature clove flower buds, each experiment was conducted three times, and the average diameter of the inhibitory zone was measured in mm.

RESULTS:

With the exception of *E. coli*, which demonstrated resistance to the plant extract at all concentrations tested, the results of this study clearly demonstrated that the ethanolic extract of dried immature flower buds of clove has an anti-bacterial effect, as well as for the *C. albicans* used in the present study.

Antibacterial activity

The findings of this investigation, as previously stated, demonstrated that the ethanolic extract of dried cloves had a clear and significant impact against *S. aureus* at various concentrations. At the concentrations utilized

in this investigation, the inhibitory zones were measured to be 5, 4, 3, and 2 mm in diameter at 100, 50, 25, and 12.5 mg / ml, respectively. The other concentrations in the current investigation, 6.25 mg/ml and 3.125 mg/ml, exhibited no discernible anti-*S. aureus* activity. Based on these findings, the lowest dose employed in this investigation that indicated anti-*S. aureus* activity was 12.25 mg/ml, with a 2 mm inhibition zone. On the other hand, three of the five concentrations proved positive for the antibacterial effect of the plant extract utilized in this investigation against Group *A Streptococcus*. The inhibitory zone diameters were determined to be 4, 2, and 1 mm for each of the following concentrations: 100, 50, and 25 mg/ml. While the other three concentrations of 12.5, 6.25, and 3.125 mg/ml had no anti-*Streptococcus* impact, the lowest concentration observed in this investigation for the ethanolic extract of cloves that had an anti-*Streptococcus* effect was 25 mg/ml with an inhibition zone 1 mm in diameter. The plant extract used did not exhibit any anti-bacterial activity against gram-negative bacteria *E. coli* at any of the five concentrations utilized in this investigation, in contrast to the anti-bacterial effect shown by the plant ethanolic extract used against gram-positive bacteria *S. aureus* and *Streptococcus*. **Table 1**, below provides more information on the ethanolic extract of dried, immature clove flower buds' anti-bacterial and anti *C. albicans* activities.

Table 1: Inhibition zones diameter (mm) (average), of the antibacterial and anti *C. albicans* activities of the ethanolic extract of dried immature flower buds of clove at different concentrations.

Organism	Inhibition zones diameter (mm) 100mg/ml	Inhibition zones diameter (mm) 50 mg/ml	Inhibition zones diameter (mm) 25 mg/ml	Inhibition zones diameter (mm)12.5 mg/ml
S. aureus	5	4	3	2
Streptococcus	4	2	1	0
E. coli	0	0	0	0
C. albicans	5	3	2	0

While **Fig. 1** below illustrates the antibacterial activity of the ethanolic extract of dried immature clove flower buds against some of gram-positive and gram-negative bacteria.

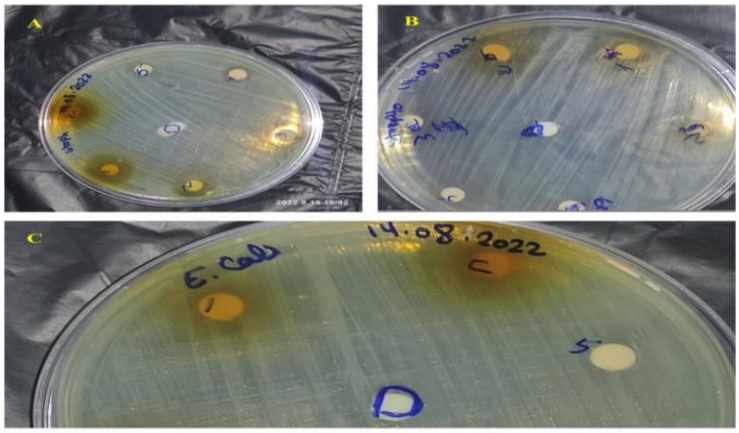

Fig. 1: The antibacterial activity of the ethanolic extract of dried immature clove flower buds against; *S. aureus* (A), *Streptococcus* (B) and *E. coli* (C).

Anticandidal activity

The results of this investigation proved definitely that the ethanolic extract of immature dried flower buds of clove has anti *C. albicans* activity at numerous concentrations. Furthermore, these findings did not differ significantly from prior findings testing the antibacterial activity of the same extract. While three of the five concentrations used for the ethanolic extract of the used plant demonstrated clear activity against *C. albicans*, the results of this study confirmed the measurement of the following inhibition zones with diameters of 5, 3, and 2 mm for each of the following concentrations of 100, 50, and 25 mg / ml, respectively. Based on these findings, the lowest concen-

tration that had an anti *C. albicans* activity was found to be 25 mg/ml, with an inhibition zone diameter of 2 mm. The results are shown in further details in table No. (1), above. While **Fig. 2** below illustrates the anti *C. albicans* activity of the ethanolic extract of dried immature clove flower buds.

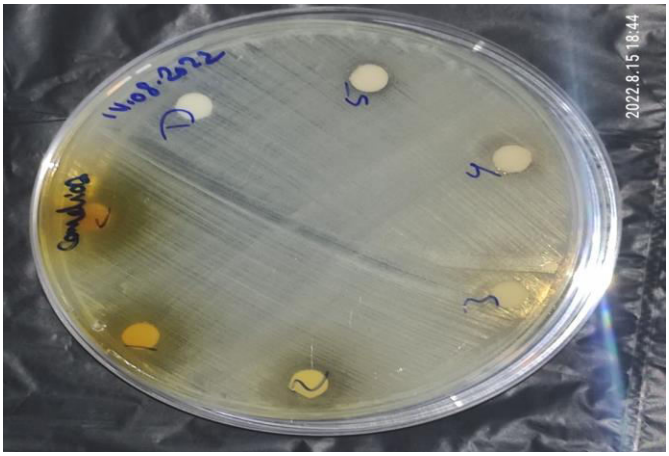

Fig. 2: The anti *C. albicans* activity of the ethanolic extract of dried immature clove flower buds.

DISCUSSION:

Although antibiotics have revolutionized medicine and the treatment of infectious diseases, there is a real problem on the horizon: the rise in microbial resistance to antibiotics, which has created an urgent need for humanity to find safe, cost-effective, and beneficial treatment and preventive alternatives. For a long time, attention has been drawn to the use of herbs, spices, and medicinal plants, as it was discovered that they have beneficial qualities in the treatment and prevention of a wide range of diseases caused by microbes (Ferrazzano *et al.,* 2009; Lee, 2013; Mathur & Dhillon, 2018). One of the most significant of these widely utilized medicinal plants is the clove (*Syzygium aromaticum*), a plant that is grown in numerous Southeast Asian countries, China, and other regions. It is worth remembering that it, like thyme, is a common ingredient in cooking spices around the world. The ability of its extracts to kill a variety of microorganisms, including bacteria and certain fungus, served as further proof of its antibacterial properties. The antibacterial properties of clove essential oils are assumed to be caused by the presence of eugenol, oleic acids, and lipids (Hammer *et al.,* 1999; Nzeako *et al.,* 2006). Overall, the results of this investigation showed that dried clove flower buds' ethanolic extract has antibacterial and anti-*Candida* properties. More particular, all concentrations tested in this study - aside from 6.25 and 3.125 mg/ml - found anti *S. aureus* activity. The inhibition zone had a diameter of 2 mm, and the lowest concentration utilized in this research that demonstrated anti *S. aureus* activity was determined to be 12.5 mg/ml. The results of this investigation into these bacteria are in line with those of Mishra and Kalyani, who revealed that clove extract, had an anti *S. aureus* impact. Furthermore, their research discovered that using the Soxhlet apparatus generated better results than other extraction methods. The study's findings were attributed to the clove's high concentration of antimicrobial active chemicals, the most significant of which are tannins, followed by saponins, terepenoids, sugars, steroids, and glucosides (Mishra & Kalyani, 2014). The current study found no significant antibacterial effect of the ethanolic extract of clove flower buds against *E. coli*, which demonstrated resistance to this extract at all concentrations tested, in contrast to the findings of Hoque *et al*, they discovered that clove has the ability to degrade the bacterial cell membrane and thus prevent the production of β-lactamase in the *E. coli* cell. While the difference between the two investigations is that, the current study employed ethanolic extract, whereas Hoque *et al*. used essential clove oil. Furthermore, the researchers attributed their findings to the presence of a high concentration of eugenol in clove essential oil, which has a high power to kill bacterial cells via the aforementioned method (Hoque 2008). Surprisingly, the findings of this study contradict those of Gittings *et al.* While this investigation found no anti *E. coli* activity, Gittings *et al,* found that cloves and cinnamon were extremely effective against

E. coli and *K. pneumoniae*. This disparity in results can be explained by their use of clove essential oil rather than the ethanolic extract utilized in this investigation, given that 72-90% of the essential oil produced from cloves included eugenol. It is a chemical that is thought to have a direct influence on microorganisms in general, and its concentration is probably definitely greater than that of the ethanolic extract (Alexa, *et al.,* 2020; Ginting *et al.,* 2021). The results of this study for Group A *Streptococcus* showed that the ethanolic extract of clove flower buds had a clear impact, as the lowest concentration that had an antibacterial effect on these bacteria was 25 mg / ml and the diameter of the zone of inhibition was only 1 mm. This study's findings were comparable to those of MP and Rajeshkumar's in that clove extract had an anti-*Streptococcus* impact on both (MP & Rajeshkumar, 2022). Furthermore, the outcomes of this study strongly indicated that the anti *C. albicans* ethanolic extract is effective and powerful, since the lowest concentration that produced an anti-*C. albicans* effect was 25 mg/ml and has an inhibition zone diameter of just 2 mm. The conclusions of this study agree with those of MP and Rajeshkumar's investigations, as well as Musa and Chehri's. The clove has an anti-*Candida albicans* impact in general (Musa & Chehri, 2021; MP & Rajeshkumar, 2022). Various researches have revealed that the physiologically active chemicals contained in cloves include kaempferol and vanillic acid. Cloves' main bioactive components are secondary metabolites such as tannins, alkaloids, and phenols, which are responsible for their antibacterial and antifungal activities. These chemicals are thought to be responsible for clove's ability to resist bacteria, fungus, and insects (Liu *et al.,* 1997; Kumar *et al.,* 2012; Alexa *et al.,* 2022). In addition to the aforementioned characteristics, clove essential oil contains a higher concentration of eugenol than extracts, which is responsible for its strong biological and antibacterial activities. Both eugenol and the essential phenolic compounds in the essential oil have been proven to denature proteins and interact with phospholipids in the cell membrane, causing it to become more permeable. Thus, it may inhibit a wide range of Gram-negative and Gram-positive bacteria, as well as many types of fungi, by this mechanism, which explains another part of the clove's potential to have antibacterial characteristics in general (Walsh *et al.,* 2003; Chaieb *et al.,* 2007). Notwithstanding, the results of this study clearly showed that the antibacterial effect of Gram-positive bacteria was greater than that of Gram negative bacteria, as the ethanolic extract of clove flower buds had an antibacterial effect on Gram-positive bacteria Group A *streptococcus* and *S. aureus*, but not on Gram-negative bacteria. However, at the concentrations tested, it had no discernible impact *on E. coli*, a gram-negative. These findings are consistent with the findings of research conducted by Behbahani *et al.* Researchers discovered that clove inhibited Gram-positive bacteria more than Gram-negative bacteria (Behbahani *et al.,* 2019). The difference in the effect of plant extracts on Gram-positive bacteria and Gram-negative bacteria is attributed to Gram-negative bacteria's resistance to antibiotics and other drugs, as well as possibly plant extracts, while this resistance can be explained by the structure and composition of the Gram-negative bacteria cell wall. The cell wall of these bacteria is composed of three distinct layers that serve as an envelope and barrier to certain physiologically active components. An outside polysaccharide membrane, a peptidoglycan layer, and an inside or cytoplasmic membrane make up these layers. Gram-positive bacteria, on the other hand, lack this outer membrane in comparison to Gram-negative bacteria because it serves as a barrier with limited permeability, preventing some molecules from entering the bacterial cell, such as medications and antibiotics (Yu *et al.,* 2022). Despite the fact that most studies and research indicate that cloves, whether ethanolic extract or essential oil, have a demonstrated impact on most bacteria in general. Some differences in results between these studies can be explained by a variety of factors, including possible differences in the strains of microbes used in various studies, differences in habits between geographical regions in terms of the indiscriminate use of antibiotics, which affects the long-term resistance of these microbes to these antibiotics, as well as differences in techniques used in studies and research, as well as differences in extraction method. These variations are also likely to be due to differences in the genotype of the plant used, and we cannot rule out the potential of geographical and climatic factors altering the chemical composition of the plant used, as well as the plant's preservation procedures. Eventually, the

microbiological samples utilized in this study were clinical samples isolated from children attending Al-Nasr Hospital for Children in Gaza City, Palestine, where these microbial samples were isolated and identified through the pediatric hospital's medical laboratory. Furthermore, these are clinical samples of pathogenic bacteria isolated from patients, the majority of which are resistant to a variety of antibiotics, which may explain one of the study's findings and significance.

CONCLUSION:

The ethanolic extract of dried immature clove flower was shown to exhibit anti-bacterial and anti-fungal characteristics, including anti *S. aureus*, anti-group A *Streptococcus*, and anti-*Candida albicans* activities, although there was no activity against *E. coli*. As a consequence, the analysis revealed that the impact on gram-positive and gram-negative bacteria varies, with the activity against gram-positive bacteria being higher and more effective. This study also revealed that anti *S. aureus* was the most effective and had the greatest impact of all the activities carried out. Eventually, this study revealed that cloves might be used as a plant with anti-bacterial and anti-fungal properties, but more research is needed.

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CONFLICTS OF INTEREST:

The authors declare that they have no conflicts of interest with relation to the publication of this research.

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