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Original article

Comparative study of alcohol clove extract, TiO2 nanoparticles, and clove extract-TiO2 complex in controlling bacterial growth in burn infections

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ABSTRACT

Background: Individuals with burn injuries (BI) are particularly vulnerable to infections. Microbial infection led to morbidity and mortality. This study explores the frequencies of bacteria associated with BI-infection and detects which one is the highly prevalent species. Also, it detects the microbial activity of three kinds of antimicrobial agents involved: alcohol clove extract. TiO2 nanoparticles; and clove extract mediated by TiO2NP. This study enrolled seventy swabs collected from BI-patients. **Material and methods**: We used routine methods and the VITECK assay to detect bacteria. The dilution microplate assay was used to detect the minimum inhibitory concentration (MIC) for all agents. **Results** of study revealed that VMRSA had a high frequency score of 42.9%, followed by MARSA and pseudomonas aeruginosa at 14.3%. According to microbial activity, clove extract was revealed to have the lowest concentration inhibition growth bacterium at 0.75 mg/ml; additionally, concentration 1.55 mg/ml for TiO2NPs had been virtue inhibition growth bacteria; and also, it had the lowest dilution used as MIC. Furthermore, at a concentration of 0.**75** mg/ml, clove extract mediated by TiO2NPs has a significant microbial activity to inhibit the growth of VMRSA more than a single agent microbial. **Conclusion**: Our finding found that VMRSA is a high-frequency bacterium and also a common bacteria associated with infection-burn injuries in patients. Finally, the clove extract mediated by TiO2NPs at a concentration of 0.75 mg/ml can be considered the MIC for VMRSA. Also, it can be suggested as a potential application in medical felids due to their forceful antimicrobial activity.

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Introduction

Burn wounds are recognized as a major global health issue [1]. In this study, *Staphylococcus aureus* was the most commonly isolated bacterium, consistent with other findings [2]. Conversely, some studies have reported *Pseudomonas aeruginosa* as the predominant organism, which could be attributed to differences in geographical locations and hygienic practices. In this study, the overall prevalence of S. aureus infections was higher compared to other bacterial isolates, aligning with reports by Bhat and Vasaikar [3]. This may be due to cross-contamination via the hands of medical personnel, airborne transmission, and contact with contaminated materials; however, no significant association with age or sex was observed. [4]. *Staphylococcus aureus* is a commensal bacterium of the human microbiota that can become

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opportunistic, often causing skin infections and upper respiratory infections, such as sinusitis and abscesses [5]. A common pathogen isolated from wound-burn patients is *Staphylococcus aureus*. It might form biofilms on injuries of burn and embedded vein which can lead to rebellious infections or even biofilm-associated sepsis [6,7]. It is a catalase-positive reduction of nitrate and a facultative anaerobe [8]. These bacteria can grow without the required oxygen. Many strains of bacteria that are resistant to a wide range of drugs may develop through mutations that accumulate over years of antibiotic use, making them difficult to treat [9].

Recently, several studies have been interested in the extract of plants to treat wound microbial infection [10]. The activity of antimicrobial extracts of a plant or a substance has the ability to kill and inhibit the growth and other activities of microorganisms, finally leading to death. *Syzygium aromaticum* is an aromatic extract from the buds of trees [11]. It has multiple contents, such as oil, resins, proteins, and others. Several studies were focused on their oil. It is widely medicated for special conditions such as aroma and decline pain in dental patients [12]. Moreover, experimental testing of the extract demonstrated a 99.9% reduction in colonization of Escherichia coli and S. aureus within eight hours. Additional research has shown that eugenol, a key constituent of the oil, possesses significant inhibitory effects against various pathogens [13].

Titanium dioxide (TiO2) is a naturally occurring oxide of titanium, known for its low toxicity and minimal biological impact. Due to its classification as a bio-inert material, larger particles of titanium dioxide (>100 nm) have been widely utilized in food products, as well as in various pharmaceuticals and cosmetics, including sunscreens and toothpaste [14]. The nanoparticles have the strong activity amicrobial and antioxidant. this activity increased associated with extract plant [15] Overall, Ag and TiO2 nanoparticles show notable potential in developing antibacterial materials efficient against both Gram-positive and Gram-negative bacteria, along with catalytic materials for the photocatalytic degradation of certain dangerous dyes or chemicals [16]. Furthermore, various bacterial and fungal groups exhibited high sensitivity to the combination of clove oil with different nanoparticles, such as silver. These agents demonstrated potent antimicrobial and antifungal properties [17,18].

The current study was explored the frequencies of bacteria associated with burn patients and evaluate the MIC for three forms of agents including: TiO2NPs, alcohol clove extract, and extract of alcohol clove -TiO2NPs against Vancomycin-Methicillin-Resistant Staphylococcus aureus (VMRSA).

Material and methods

Sample collection

Seventy transport media swabs were taken from the burn injury patients who were lying in Baghdad hospitals. The study started from January 2024 to May 2024. The patients were having two stages of burn, the samples collected under sterilization condition and also it toke under care doctorate specialized.

Isolation, identification, and antibiotic selectivity tests of bacteria

The swabs were cultured in brain broth and then incubated for 24 hours at 37 ºC to encourage the growth of bacteria. Subsequently, each activated culture broth was re-cultured using the ABC streak plating technique to isolate a single colony and conduct primary characterization. Next that were grown on three different types of media, including MacConkey agar, blood agar, and Mannitol salt agar. Following this, a series of biochemical tests were conducted on each bacterial isolate, including indole, methyl red, Voges Proskauer, catalase, oxidase, coagulase, and urease. Moreover, these isolates were confirmed by VITEK® 2 ID and estimation of drug sensitivity by AST cards (bioMérieux-Pioneering Diagnostics) [19].

Preparation of antimicrobial agents.

The buds of clove were collected from Baghdad Central Market, and they were sourced from the local market in Al-Hura city, Baghdad, Iraq. And their authenticity was verified and secured in the biology lab. It is choosing a dependent state by remaking physical characters like shape, color, odor, and smell. The flower buds were weighted at 10 grams and dissolved by de-ionized water, following, it dried at room temperature for 144 hours, and ground into a powder using a clean blender. The powder was then stored in the sterile polyethylene test bags until use. This powder was soaked in 20% ethanol solvent (v/v) in a glass reagent bottle with a screw cap and left for 96 hours.

After that, the mixture was filtered through a double-layered muslin cloth, and the liquid was recovered by centrifuging at 4000 rpm for 1 minute. The supernatant was then passed through Whatman No. 1 filter paper (0.22 Mm), and the resulting filtrate was considered the stock extract. [20].

Characterization and Preparation of TI2O nanoparticles

Characterize TI2O nanoparticles.

Titanium oxide nanoparticles are ready-made from the VCN (Iran) their inorganic compound appears as a white powder that is soluble in deionized water. The dimensions of the particle range from 19.00 to 33.27 nm. Additionally, the stock of TiO2NPs solution was prepared by dissolving 1.0 g of TiO2NPs in 10 ml of deionized water to a final concentration of 100 mg/ml.

Estimation of the minimum inhibitory concentration (MIC) for TIO2 nanoparticles, clove bunds, and clove extract mediated by TiO2NPs.

Since the extracts of clove bunds, TiO2NPs, and mixture reagents have shown antimicrobial activity against VMRSA isolates by the microplate dilution assay, and this was executed by dilution of the two-fold serial for their test's agents in MHB (2 ml volume). The aqueous extracts of the clove bunds and TiO2NPs were primed at concentrations from 50.0 to 0.001 mg/ml, three plate used to MIC for each agent. The first plate to evaluate the MIC for alcohol clove agents, following, the second plate for Alcohol clove - TiO2NPs. Finally, the mixture agent was prepared by mixing 50µl of aqueous extracts of the clove and 50µl of TiO2NPs stock solution in a well of plate three. Then 100µl refresh inoculum of bacterial isolates (1.5 x 10^8 CFU/ml) was added to wells of three plates and incubating for 24 hours at 370 °C[21]

Statistical analysis

The graphPad-8 (NBCC-USA) was used in the analysis of the results of the current study, which included percents and summations with the use of a chart to illustrate the results, and use Mann-Whitney U test to measure the p-vale at 0.05.

Results

Detection of frequencies of the isolated bacteria from wound infections associated with burn patients.

The different bacteria were isolated from 64 swabs out of the total of 70. In Figure 1, we present the bacterial isolation from the wounds of burn patients. Various medically significant bacterial species were isolated.

The data revealed that Vancomycin–Methicillin-resistance *Staphylococcus aureus* (VMRSA) had a high frequency in 30/70 bacteria isolates; it reached a percent (42.9%). The following bacteria was Methicillin - resistance *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* were scored as the second bacteria isolated (10/70); they reached 14.3%. Next, *Escherichia coli*, *Klebsiella pneumonia*, and *Streptococcus pyogenes* demonstrated frequency in 4,5, and 5 respectively, isolated out of 70 swabs, they reached (5.7-7.1 %). Finally, for another swab, no growth was reported.

Estimation of Minimum Inhibitory Concentrations (MIC) by the TiO2NPs, extract of clove buds, and extract of clove mediated by TiO2-NPs against Vancomycin–Methicillin-resistant *Staphylococcus aureus* (VMRSA).

Figure-2 presents the results of testing eight VMRSA isolates under the influence of three agents, each prepared at similar dilutions to determine their minimum inhibitory concentration (MIC). For the clove extract, the lowest concentration required to inhibit the growth of 5 out of 8 isolates (62.5%) was 0.75 mg/ml, while a concentration of 0.37 mg/ml served as the MIC for all isolates (100%) as shown in the fig3. The MIC of TiO2 nanoparticles was 0.15 mg/ml for all 8 isolates (100%) (see in fig4).

Finally, the antimicrobial efficacy of the combined extract of alcohol clove and TiO2 nanoparticles against Vancomycin–Methicillin-resistant Staphylococcus aureus (VMRSA) was observed, with a concentration of 0.18 mg/ml inhibiting 37.5% (3/8) of the isolates and 0.09 mg/ml inhibiting 100% (8/8) (see in fig5). We observed a highly significant difference ($p < 0.001$) in the effectiveness of the three agents against the bacterial isolates.

Figure 1. Frequencies of isolates bacteria from wound infection associated with burn patients.

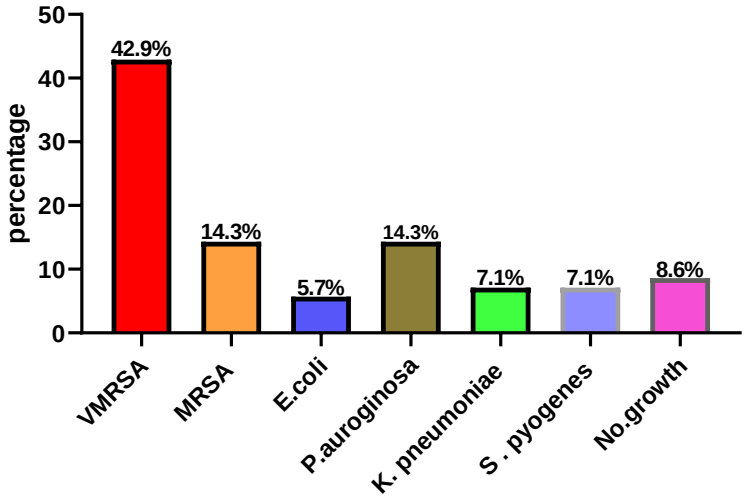


Figure 2. MIC of three agents and effect on growth the bacteria.

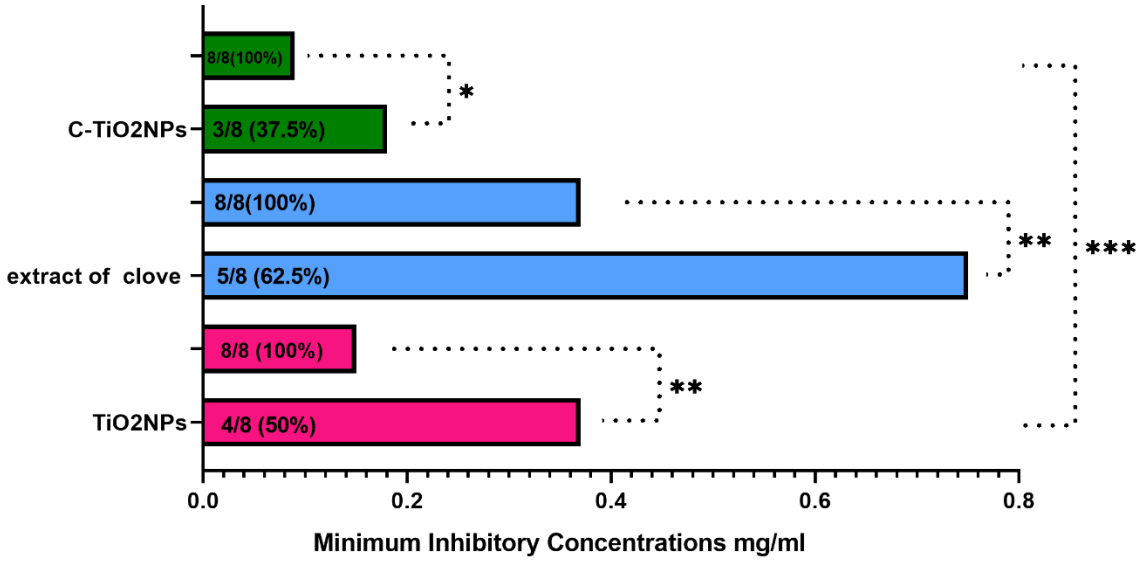


Figure 3. Microdilution method to tenacity of the MIC values of TIO2 to VMRSA. (C-)" Negative control (only both), (C+)" Positive control (only bacteria, broth). Wells with Blue color had no or inhibited growth, but, Wells with red color with growth.

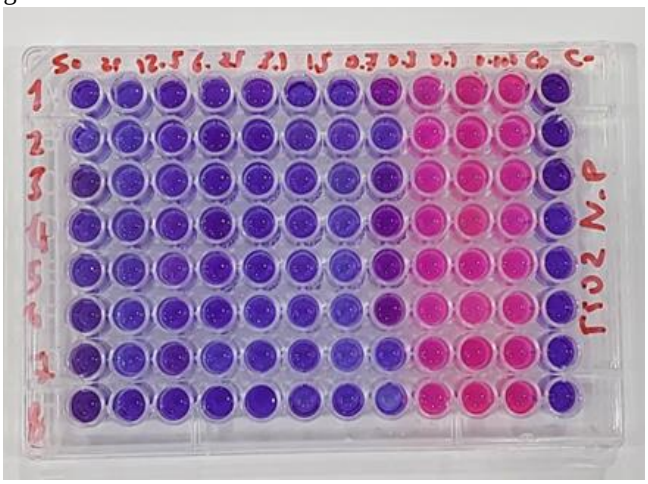
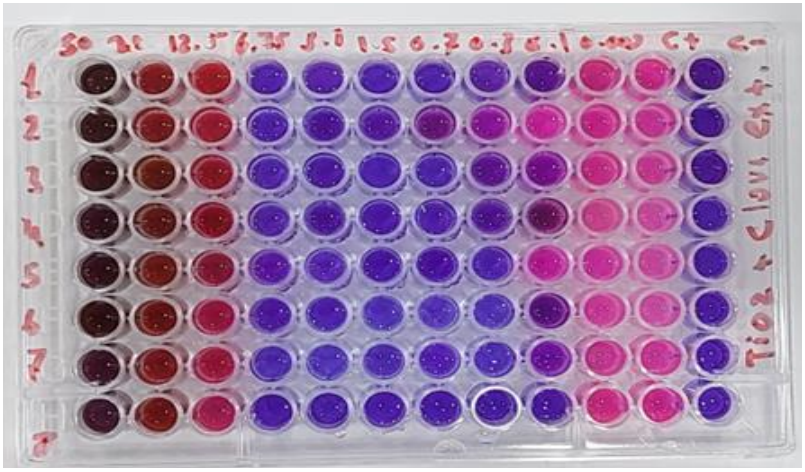


Figure 4. Microdilution method to tenacity of the MIC values of clove extract for VMRSA. (C-)" Negative control (only broth), (C+)" Positive control (only bacteria, broth), Wells with Blue color had no or inhibited growth, but, Wells with red color with growth.

Figure 5. Microdilution method to tenacity MIC values of clove extract mediated TIO2NPs for VMRSA. (C-)" Negative control (only broth), (C+)" Positive control (only bacteria, broth), Wells with Blue color had no or inhibited growth, but, Wells with red color with growth.



Discussion

Figure 1 presented the frequencies of the isolated bacteria. The different bacteria were isolated from 64 burn wound swabs out of the total 70, indicating that VMRSA was highly associated with the infection of burn wound patients; it reached 42.9% compared to other bacteria. These results agreed with several studies that demonstrated that *S. aureus* scored higher than other bacteria, following *P. aeruginosa* and *E. coli* [22,23]. Furthermore, patients with burn injuries often suffer from microbial contamination, with bacterial infections being particularly common the majority of these infections are caused by the pathogenic bacteria *multidrug resistance Staphylococcus aureus* and *P. aeruginosa* [24]. *Staphylococcus aureus* is the most pathogenic species within the Staphylococcus genus, responsible for both community-acquired and hospital-acquired infections. It often colonizes the skin and mucous membranes of healthy individuals without causing symptoms, particularly in the anterior nares (nostrils) [25,26]. In Iraq, a study found that Staphylococcus species were the most prevalent organisms in burn injuries, accounting for 75 out of 163 cases (46%), with 31 cases (41.3%) and 44 cases (58.7%) respectively[27]. Microbial agents that induce skin inflammation are classified as pyogenic bacteria. Additionally, colonization of the nasal passages and skin by Staphylococcus species and MRSA may serve as sources of contamination for wounds and burns [28–30] . Another study indicates that some resistant bacteria are the reason for malicious

infections of patients with burns, including MRSA which is particularly transmitted from hospitals [31].

According on the results of fig 2-5, about the effect of TiO2NPs alone on the growth of VMRSA, the current finding agreed with the study demonstrated that the TiO2NPs enhance the antimicrobial action of beta-lactams, cephalosporins, lacosamide, and tetracycline, a possible exertion of nano composition in combining effects against MRSA. Several studies have reported the MIC value of TiO2 nanoparticles at 2.0 mM [32].

Clove extract is also well-regarded as a traditional remedy, boasting a rich history of use in various folk medicines and a broad spectrum of pharmacological effects. Research has shown that clove oil exhibits antimicrobial and antioxidant properties even at very low concentrations, thanks to its active compounds like ergosterol and others [33]. Additionally, the phenolic compounds in clove have been used to facilitate the formation of stable mono- and bimetallic nanoparticle systems with various metal salts. These compounds have also been instrumental in enhancing the biological activities of the nanoparticles, including their antioxidant, antimicrobial, and anticancer properties [34–36].The major effect of essential oil was focused on three bacterial strains (*E. coli.*, *S. aureus*, and *S. typhi*) and several fungal species at 0.1 mg/ml and 0.3 ml/ml [37]. Furthermore, clove extract was found to be effective against almost all microorganisms in food transmission [38]. *Staphylococcus aureus* was established as sensitive to the extract of clove with inhibition, followed by *Staphylococcus epidermidis* [39].

However, according to the effect of microbial activity of a synergistic combination clove extract mediated by TiO2NPs. The result revealed that a concentration of 0.09 mg/ml inhibited of 100%, so that could be considered the lowest concentration inhibition of growth bacterium (MIC). In this context, our study uniquely demonstrates the effectiveness of clove extract-mediated TiO2 nanoparticles (TiO2NPs) against VMRSA. A similar study on a different plant, Capsicum annum, indicated that TiO2NPs mediated by its extracts significantly impacted microbial activity. Additionally, TiO2NPs mediated by ginger and rosemary have been recognized as potential antimicrobial agents against S. aureus [40–42]

Conclusion

Staphylococcus aureus is a significant pathogen associated with burn injury patients, particularly MRSA and VMRSA. In our study, 42.9% of the isolates were identified as either VMRSA or MRSA, making up 14.3% of the 70 swabs collected. Additionally, the study revealed that alcohol-based clove extract combined with TiO2 nanoparticles (TiO2NPs) is a potent antimicrobial agent, capable of inhibiting VMRSA growth even at low concentrations. Moreover, this combined agent demonstrates greater effectiveness than either the alcohol clove extract or TiO2NPs alone.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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