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In vitro Treatment of Methicillin Resistant *Staphylococcus aureus* Using Natural Plant Extracts

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Abstract: The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community, hospitals and veterinary field had led to a demand for new antimicrobial agents that could be used to decrease the spread of these bacteria. Therefore, the current study was conducted to examine the antimicrobial and synergistic effects of clove and garlic extracts either alone or combined with oxacillin antibiotic. Moreover, the minimum inhibitory concentration (MIC) was determined. Twenty-four out of thirty-three swab samples were collected from pyogenic wound infection from different health care center from Taif governorate, Saudi Arabia. Additionally, nine nasal swabs were collected from sheep farms in Turrabah governorate, Saudi Arabia. All collected samples were cultured on suitable media for isolation of *Staphylococcus aureus*. MRSA was detected based on agar disc diffusion test. Three different extracts of clove and garlic were used (water, methanol and ethanol). The antimicrobial and synergistic effects of these extracts were achieved using agar well diffusion and MIC tests against clinical MRSA isolates. Results revealed that the highest inhibition zone ($P<0.05$) was detected with garlic water extract (GWE), followed by clove ethanolic extract (CEE), clove methanolic extract (CME) and clove water extract (CWE) respectively. The MIC was ranged from 3.125-6.25, 12.5-25, 12.5-25 and 25-50 mg/ml for GWE, CEE, CME and CWE respectively. The inhibition zone after synergism of GWE, CEE, CME and CWE with oxacillin was improved from 8 mm to 23 mm, 20 mm, 18 mm and 17 mm respectively by in conclusion, GWE was the most potent antimicrobial agent against MRSA followed by CEE, CME and CWE. The oxacillin sensitivity against MRSA was improved after synergism with GWE, CEE, CME and CWE respectively.

Key words: Clove · Garlic · Extracts · MRSA · Antibiotics · Synergism

INTRODUCTION

Staphylococcus aureus (*S. aureus*) had been identified as the most common causative agent of nosocomial infection [1]. Methicillin resistant *S. aureus* (MRSA) emerged soon after the introduction of methicillin into clinical practice. MRSA has become a public and veterinary pathogen. Strains that possess mecA gene are either heterogeneous or homogeneous in their expression of resistance. The heterogeneous expression occasionally results in minimal inhibitory concentrations that appear to be borderline and consequently the isolates may be interpreted as susceptible [2]. The problem of microbial resistance to antibiotics is fast growing particularly in the hospitals where MRSA has become a global threat to antimicrobial chemotherapy.

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The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [3]. With antibacterial drugs being widely used in clinical settings, many microorganisms, especially MRSA, *Pseudomonas aeruginosa* and *Candida albicans*, have adapted to synthetic antibiotics and become highly resistant to these drugs over time [4]. Microorganisms with multi-drug resistance now cause thousands of deaths throughout the world each year [5]. Little information is available on the preservative and antimicrobial role of spices and their oils and the role of various components of essential oils in the preservation of spoil of food [6].

Clove belongs to a tree *Eugenia caryophyllata* (*Syzygium aromaticum*), was used as a spice in almost all the world's fare. Bud oil of clove has natural behavior and the main properties include antioxidant, insecticidal, antifungal and antibacterial properties. By tradition, it has been used in food preservation as flavoring and antimicrobial substance [7]. Cloves consist of a significant amount of proteins, iron, carbohydrates, calcium, phosphorus, potassium, sodium and hydrochloric acid. They are also rich in vitamins A and C, manganese and dietary fiber [8]. Major antimicrobial component in clove has been reported to be eugenol which has been given special attention to find its antibacterial activity against food borne pathogens. Eugenol has been reported to inhibit the growth of *E. coli* O157: H7 and *Listeria monocytogenes* [9]. Considering the potent medicinal value of the plant, its antimicrobial sensitivity test was also carried out against few human pathogenic bacteria like Gram positive *S. aureus* MTCC -96 and Gram negative bacteria; *Salmonella typhi* MTCC- 98, *Klebsiella pneumoniae* MTCC -661, *Proteus vulgaris* MTCC - 744 and *Shigella flexneri* MTCC- 1457 along with fungus *Candida albicans*-183, were used with disc diffusion method. The results of antimicrobial sensitivity compared with the standard antibiotic like Ampicillin and Nystatin (10 µg/ml) [10]. The antioxidant properties of the aromatic clove or eugenia oil was extensively reviewed [11]. It is noteworthy the compound eugenol, having mold and bacterial inhibiting activity in bakery food items. Ground beef and cattle meat are generally spoiled by psychrotrophic bacteria but can be inhibited by the use of clove oil [12].

Garlic (*Allium sativum*), an essential food ingredient worldwide, is one of the extensively researched medicinal plants that has antibacterial, antifungal, antiviral, anthelmintic, antiseptic and anti-inflammatory effects depending on allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyase) on allin after crushing garlic [13, 14]. Garlic extract has been shown to be an effective agent for controlling methicillin-resistant *S. aureus* [15]. Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. Moreover, garlic extracts exhibited activity against both Gram negative (*E. coli*, Salmonella species and Citrobacter, Enterobacter, Pseudomonas, Klebsiella) and Gram positive bacteria (*S. aureus, S. pneumoniae*, Group A streptococcus and *Bacillus anthrax*). All of which are causes of morbidity worldwide[16]. The *in vitro* testing of garlic aqueous extract showed concentration dependent antibacterial activity against MRSA and Salmonella spp. [17]. The regular use of garlic may help to prevent cancer, to treat malaria and to raise immunity. Garlic has also proposed to treat asthma, candidiasis, colds, diabetes and antibacterial effect against food borne pathogens like Salmonella, Shigella and *S. aureus* [18]. The present study aimed at (i) determining the antimicrobial effect of clove and garlic extracts, (ii) evaluating the synergistic effect of those extracts with oxacillin antibiotic and (iii) detecting the minimum inhibitory concentration for those extracts.

MATERIALS AND METHODS

Sample Collection and Preparation: Thirty-three swab samples were collected over two months (July to September 2015). Twenty-four samples were collected from pyogenic skin infections from different health care centers in Taif, KSA. Additionally, nine swabs were collected from sheep suffering from respiratory symptoms in Tarrabah, KSA. These samples were inoculated onto mannitol salt broth media and incubated at 37°C for 24 h. Then inoculated onto mannitol salt agar media and blood agar and incubated at 37°C for 24-48 h [19]. All positive isolates on blood agar and mannitol salt agar were identified as *S. aureus* by conventional biochemical tests (Gram stain, DNase agar and tube coagulase) [20].

Sensitivity Test of Methicillin-Resistant *Staphylococcus aureus* (MRSA): All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method as previously described [21, 22]. Mueller Hinton agar (MHA) plates containing 4% NaCl were prepared. Plates were inoculated with 0.5 mL of 0.5 McFarland suspension of the isolate by Swabbing and then, plates were left to dry at room temperature. Antibiotics discs (Hi Media Laboratories, Pvt. Ltd.) were added to cultured plates. These antibiotic

discs were ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), oxacillin (1 µg) and vancomycin (30 µg). Plates were incubated at 35°C for 24 h and were observed carefully in transmitted light for any growth. The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less. Zones of inhibition are provided according to CLSI guidelines [23] to define susceptibility, intermediate resistance and resistance to specific antimicrobial agents.

Preparation of Garlic and Clove Water Extracts: The samples (garlic and clove) were purchased from local markets in Taif governorate, KSA. After wards, these were carefully washed under running tap water followed by sterile distilled water. They were air dried at room temperature (30°C) for two days, crushed to a fine powder using a sterilized mixer grinder and stored in sterile air-capped bottles. Three different solvents namely ethanol, methanol and aqueous cold were used for extraction. A 10 g amount of pulverized buds (clove and garlic) were separately soaked in 100 ml of ethanol, methanol and cold sterile distilled water for 24 h and allowed to stand for 30 min in a water bath with frequent shaking and kept undisturbed for 24 h. Filtering of each preparation was made through a sterilized Whatman filter paper No.1 following, the filtered extracts was concentrated under a vacuum below 40°C using rota evaporator [24, 25]. The obtained dried extracts thus were exposed to UV rays for 24 h and checked for sterility on nutrient agar plates and stored in labeled sterile bottles in a freezer until further use.

Determination of Antimicrobial Effect of Clove and Garlic Extracts: Antimicrobial sensitivity test was done using agar well diffusion method as previously reported [26]. The Mueller Hinton agar plates were spread with either 0.5 ml of the bacterial inoculum equal to 0.5 McFarland standards. Wells (6 mm in diameter) were cut from agar plates using a sterilized stainless steel borer and were filled with 25, 50 and 75 µl of each of different aqueous, methanolic and ethanolic clove and garlic extracts. The plates were incubated at 37°C for 24 h and the resultant diameter zone of inhibition was measured for each volume and type of the extracts and indicated by mm. furthermore, synergism was achieved by mixing of 50 µl of different clove and garlic extracts with methicillin antibiotic disc (1µg/disc) purchased from Hi Media Laboratories Pvt. Ltd. Diameters of inhibition zones were also measured and expressed by mm.

Determination of Minimum Inhibitory Concentration (MIC) of Clove and Garlic Extracts: The test was performed by micro-dilution technique [27] using ninety-six well microtiter plates with some modifications. Serial dilutions of each clove and garlic extracts (aqueous, methanolic and ethanolic) were made at 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml. Each well was filled with 100 µL Muller broth containing aqueous garlic and clove extracts and 100 µL inoculated broth of 0.5 McFarland standards and incubated overnight at 37°C. The same procedures for methanolic and ethanolic garlic and clove extracts were repeated but the initial dilutions were done using DMSO reagent. The same volumes of different methanolic and ethanolic (clove and garlic) extracts (100 µL) were also used and finally, 100 µL of bacterial inoculum previously adjusted to 0.5 McFarland standard was added. Vancomycin MIC was also calculated as positive control. The last well showing no visible bacterial growth was reported as MIC of each extracts.

Statistical Analysis: Statistical analysis was done using ANOVA and Fischer's post hoc test, with $p < 0.05$ being considered as statistically significant.

RESULTS

Analysis of results revealed that the identified *S. aureus* was 29 (87.87%) out of 33 swab samples and four (12.13%) samples from 33 were not *S. aureus* depending on culturing on mannitol salt agar and blood agar (Table 1). Moreover, twenty swabs out of twenty-nine were collected from patients and nine from twenty-nine were collected from sheep farms. Six from twenty human swabs were identified as MRSA and three from nine sheep samples identified as MRSA. Twenty-nine out of thirty-three was confirmed as *S. aureus* (87.87%) depending on biochemical tests as catalase, coagulase and DNase activity. The number of negative samples was 4 (12.13%) which only were catalase test positive but the coagulase test and DNase activity were negative (Table 1).

Concerning of human samples, twenty swab samples that previously confirmed as *S. aureus* were tested against six different antimicrobial agents using Bauer-Kirby disk diffusion test (Table 2). The reported results indicated that 14 (70%) *S. aureus* isolates were sensitive to ampicillin, while these tested against amoxicillin/clavulanic was 17 (85%) samples. Moreover, ciprofloxacin was effective against all tested samples (100%). Gentamycin was effective for nineteen (95%)

Table 1: Morphological, culture and biochemical characters of the isolated bacteria.

Sample type	No of samples	Gram staining	Mannitol salt agar	Blood agar	Catalase test	Coagulase test	DNase agar
Positive isolate	29	Gram positive	Yellow colour	β-hemolysis	+	+	+
Negative isolate	4	Gram positive	No change in colour	α-haemolysis	+	-	-

No: number; β: beta; α: alpha

Table 2: Percent of sensitivity and resistance of the isolated *S. aureus* against six different antibiotics.

Antibiotic concentration /disc	Number of human isolates/Percent of sensitivity	Number of sheep isolates/Percent of sensitivity
Ampicillin [10 µg]	14/70	6/70
amoxicillin/clavulanic acid [20/10 µg]	17/85	7/77
Ciprofloxacin [5 µg]	20/100	9/100
Gentamicin [15 µg]	19/95	8/88.8
Oxacillin [2µg]	14/70	6/70
Vancomycin [5 µg]	20/100	9/100

Table 3: Antimicrobial effect of clove extracts [water, methanol and ethanol) and oxacillin on human and sheep isolated MRSA.

	Inhibition zone diameter (mm)									
	Clove water extract			Clove methanolic extract			Clove ethanolic extract			Oxacillin
Sample code	25µl	50 µl	75 µl	25µl	50 µl	75 µl	25µl	50 µl	75 µl	1 µg
Human isolates	6.3± 1.03	12.3±1.8	15±1.7	13.3±1.3	15.3±0.8*	17.3±1.3*	14.2±1.3	17±0.8*	19±0.81*	7.6±0.05
Sheep isolates	6.3±1.5	11±1.7	14±1.5	13.6±1.1	15±1*	16.6±0.9*	14.3±0.6	16.6±0.5*	19.8±0.01*	8.3±0.6

Values are means ± standard error (SEM) for independent isolates. Values are statistically significant at *$p<0.05$* Vs oxacillin inhibition zone.

Table 4: Antimicrobial effect of garlic extracts (water, methanol and ethanol) and oxacillin on human and sheep isolated MRSA.

	Inhibition zone size (mm)					
	GWE			GME	GEE	Oxacillin
Sample code	25µl	50 µl	75 µl	75 µl	75 µl	1µg
Human isolates	9.3±1.9	14.1±0.9*	20.6±0.9*	NZ	NZ	7.6±0.05
Sheep isolates	6.3±1.5	15±0.9*	21.6±0.8*	NZ	NZ	8.3±0.6

Values are means ± standard error (SEM) for independent isolates. Values are statistically significant at *$p<0.05$*Vs oxacillin inhibition zone.

Table 5: Minimum inhibitory concentration [MIC) of clove and garlic extracts [water, methanol and ethanol) and vancomycin of isolated MRSA.

MIC values by mg/ml for clove and garlic extracts and by µg/ml for vancomycin							
Sample code	CWE	CME	CEE	GWE	GME	GEE	Vancomycin
Human isolates	37.5±13.6	20.8±6.4	16.6±6.4*	3.6±.95*	NT	NT	0.75±0.27
Sheep isolates	41.6±14.4	20.8±7.2	20.8±7.2	3.1±0.85*	NT	NT	0.83±0.28

CWE: clove water extract, CME: clove methanolic extract, CEE: clove ethanolic extract, GWE: garlic water extract, GWE: garlic methanolic extract, GWE: garlic ethanolic extract.Values are means ± standard error (SEM) for independent isolates. Values are statistically significant at *$p<0.05$*Vs vancomycin MIC values

Table 6: Synergistic effect of either clove or garlic extracts (water, methanol and ethanol) with oxacillin antibiotic against isolated MRSA.

	Inhibition zone size (mm)						
Sample code	CWE with Oxacillin	CME with Oxacillin	CEE with Oxacillin	GWE with Oxacillin	GME with Oxacillin	GEE with Oxacillin	Oxacillin 1µg
Human isolates	16.6±1.3	19.3±1.3	19.3±1.3	23±2.1	7.6±1.03	7.6±1.03	7.6±1.03
Sheep isolates	16.3±0.57	18±1	19.6±1.5	22.6±2.3	8.3±1.1	8.3±1.1	8.3±1.1

CWE: clove water extract, CME: clove methanolic extract, CEE: clove ethanolic extract, GWE: garlic water extract, GWE: garlic methanolic extract, GWE: garlic ethanolic extract. Values are means ± standard error (SEM) for independent isolates. Values are statistically significant at *$p<0.05$*Vs oxacillin alone inhibition zone

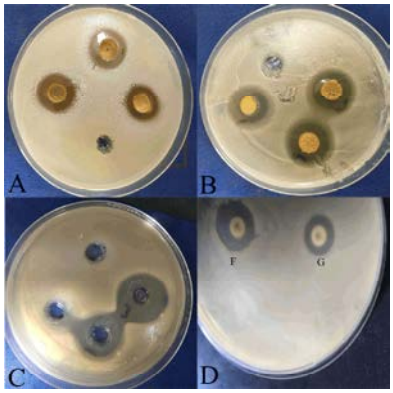

Fig. 1: Exemples of Sensitivity Test and the Resulted Inhibition Zone
A. Sensitivity test and inhibition zone of isolate H1 using methanolic clove extract.
B. Inhibition zone of isolate H3 using ethanolic clove extract.
C. Inhibition zone of isolate H3 using aqueous garlic extract.
D. Synergistic effect of oxacillin antibiotic and aqueous garlic extract.

samples. Oxacillin, which is responsible for MRSA was effective against 14 (70%) tested samples. Finally, vancomycin showed 100% sensitivity against all *S. aureus* isolates as illustrated in Table 2. The veterinary samples also showed six (70%) samples from nine were sensitive to ampicillin. While these tested against amoxicillin/clavulanic was seven samples by (77%). Moreover, six (70%) samples from nine were sensitive to oxacillin. By testing of nine nasal swabs against gentamycin antibiotic, eight (88.8%) samples were sensitive. Finally, all (100%) tested samples were sensitive to vancomycin and ciprofloxacin.

Results in Table 3 indicated that six human isolated MRSA strains in addition to three MRSA isolates from sheep were tested against water, methanolic and ethanolic clove extracts using different volumes (25, 50 and 75µl) and oxacillin antibiotic. The obtained results revealed that clove water extract was effective against all tested MRSA strains at concentration of 75µl per well and the mean of inhibition zone was (15±1.7, 14±1.5 mm) for human and sheep MRSA isolates respectively and the P value was statically significant ($p<0.05$). While volume (50 µl) of the same extract was effective against five isolates only from nine and the inhibition zones of the sensitive isolates were (12.3±1.8 mm, 11±1.7) for human and sheep MRSA isolates respectively. Volume of 25 µl showed no effect against all tested strains and the mean inhibition zones was (6.3± 1.03, 6.3±1.5mm) for human and sheep MRSA isolates respectively. When the same MRSA strains were tested using the same volumes of clove methanolic extract showed high inhibition zone of all tested strains with increasing volume of extract. Finally, ethanolic extract of clove was the most effective compared by water and methanolic extracts and the inhibition zones of (75µl) were the higher ($p<0.05$) than 50 and 25 µl and the zones measured were (19±0.81, 19.8±0.01mm) of human and sheep MRSA strains respectively.

Recorded results in Table 4 indicated that testing of six human MRSA isolates and three sheep isolates for detection of antimicrobial effect of three different extracts of garlic revealed that the ethanolic and the methanolic give no inhibition zones for all tested isolates. Volume of (75 µl) water garlic extract was the most effective on sheep MRSA isolates (21.6±0.8mm) followed by human isolates (20.6±0.8mm) and the results were statically significant ($p< 0.05$). Decreasing volume of the extract (50 µl) decreased the inhibition zone diameter (15±0.9, 14.1±0.9) for sheep and human MRSA respectively. Finally, volume of 25 µl of the same extract gave weak inhibition zone and the mean (9.3±1.9, 6.3±1.5) for human and sheep MRSA isolates repectively.

Positive MRSA isolates on agar well diffusion test were subjected to the MIC test for determinations of the lowest concentrations of each clove, garlic and vancomycin that inhibit visible bacterial growth (Table 5). Tabulated results showed that the water garlic extract was the most effective and the MIC values were [3.6±.95, 3.1±0.85 mg/ml) for human and sheep MRSA isolates respectively ($p<0.05$) followed by ethanolic clove extract (16.6±6.4, 20.8±7.2 mg/ml) for human and sheep isolated MRSA isolates respectively. Methanolic clove extract showed MIC values ranged from (20.8±6.4, 20.8±7.2 mg/ml) for human and sheep isolated MRSA. While water clove MIC was the least between the test garlic and clove extracts (37.5±13.6, 41.6±14.4 mg/ml) for human and sheep isolated MRSA. Finally, garlic methanolic and ethanolic did not test, as they give no inhibition zone on agar well diffusion test. All data was compared by vancomycin antibiotic that give MIC values (0.75±0.27, 0.83±0.28) for human and sheep isolated MRSA.

After mixing of volumes of 50 µl of six different clove and garlic extracts (water, methanol and ethanol) with oxacillin antibiotic and after that, the sensitivity test was performed using Bauer Kirby method against nine MRSA isolates (Table 6). The obtained results illustrated that there were improvement in diameter of inhibition zones in all tested MRSA isolates and the best synergisms were

obtained with water garlic extract (23±2.1, 22.6±2.3 mm), followed by clove ethanolic extract (19.3±1.3, 19.6±1.5 mm), clove methanolic extract (19.3±1.3, 18±1 mm) and clove water extract (19.3±1.3, 18±1 mm) for human and sheep MRSA isolates respectively. On the contrary garlic methanolic and ethanolic extract did not show any synergism and the measured inhibition zone was (7.6±1.03, 8.3±1.1 mm) for human and sheep MRSA isolates respectively.

DISCUSSION

Methicillin-resistant *S. aureus* (MRSA) become a major nosocomial pathogen worldwide [28]. It is a popular cause of hospitals, community-acquired and veterinary infections [29]. The current findings showed that MRSA could be isolated and diagnosed used culture and biochemical methods in addition to sensitivity testing using Kirby Bauer technique. Moreover, the resistance to beta lactam antibiotics was detected [30]. The current results showed that the percent of MRSA in clinical isolates was 30% in both human and sheep samples while the 29 out of 33 isolates (87.87%) were identified as *S. aureus* not MRSA. This approximately agree with other study [31]. Clove (*Syzygium aromaticum*) was used in treatment and controlling of MRSA [32]. The antimicrobial effect of clove was reported due to the eugenol phytochemicals [33]. Results denoted that the ethanolic extracts of clove was the most effective and give the highest inhibition zone against nine clinical isolates of MRSA and the mean inhibition zone diameter (19±0.81 mm, 19.8±0.01 mm) for human and sheep isolates MRSA respectively. The obtained zone increased with increasing volume and concentration of the extract (75 µl). Parallel, the methanolic extract showed efficacy against all MRSA isolates but the mean size of the inhibition zone was less than ethanolic clove extract (17.3±1.3, 16.6±0.9 mm) for human and sheep isolates MRSA respectively. The water extract had no activity against five isolates from nine and the inhibition zone ranged from 8-10 mm at volume of 25 µl. While the last four isolates showed intermediate zone from 11-12 mm. This finding indicates that increasing in the volume of extract improves the antibacterial activity with increasing inhibition zone for all tested MRSA isolates. These results were parallel with the antimicrobial findings of clove against MRSA [33]. Sensitive isolates in agar well diffusion test, using the three different clove extracts (water, methanol and ethanol) were subjected to minimum inhibitory concentration test (MIC) to determine the minimum concentration of each type of extract that inhibits the visible bacterial growth [34].

The obtained results of MIC of clove extracts (water, methanol and ethanol) showed that ethanolic extract was the most potent one among tested clove extracts and the values of MIC were (16.6±6.4 and 0.8±7.2 mg/ml) for nine MRSA isolates. The methanolic extracts showed high MIC values compared by ethanolic one for all tested MRSA isolates except for isolate number H12 at 12.5 mg/ml and for other isolates at 25 mg/ml. Finally, water clove extracts showed the high MIC values compared by methanolic and ethanolic extracts and the values ranged from 25 to 50 mg/ml. It has been reported that the ethanolic extracts showed the highest sensitivity against food borne staphylococci and the MIC folds were ranged from 2.5 mg/ml to 5mg/ml [35]. The little variations in results are due to difference in the original stock concentration of the extracts. Another study [36] stated that the higher MIC folds ranged from 5-10%, this difference was attributed to the presence of proteins and fat in foods contaminated by staphylococci that decrease the effect of clove extracts and need more concentration.

The use of two antibiotics in expectation for rapid bacteriologic response is known as synergism [37]. Nowadays, several researchers try to make synergism between plants extracts and several antibiotics to treat the rapidly developing bacterial resistance. Our work was included the studying of synergism between clove extracts (water, methanolic and ethanolic) and oxacillin antibiotic. The obtained results revealed that there was synergistic effect between clove extracts and oxacillin antibiotic with varying degrees and the best action was found with ethanolic clove extract with oxacillin. The inhibition zone was improved from 8 mm in case of oxacillin alone to 16-20 mm when using of ethanolic extract with oxacillin. Moreover, the inhibition zone also was increased in size with methanolic clove extract and oxacillin and ranged from 15-19 mm for all nine MRSA isolates. The synergistic effect was also noticed in eight from nine of tested bacterial isolates with water clove extracts and oxacillin and the zone size was ranged from 12-17 mm. Synergistic effects resulting from the combination of antibiotics with extracts were documented in the literature [38]. They studied the association of different clove extracts and ampicillin antibiotic with methicillin to inhibit strains of *S. aureus* resistant to methicillin (MRSA).

Garlic (*Allium sativum*) has numerous active components that work together and produce antimicrobial properties against nosocomial MRSA in complex ways. Of all the novel ingredients, allicin, a sulfur compound, is regarded as the paramount antibacterial agent in garlic extracts and exhibits protective effects against nosocomial

infections caused by MRSA [15]. Our results denoted that fresh aqueous garlic extract had pronounced antimicrobial effect against nine isolates of MRSA depending on volume and concentration of the extract. The highest inhibition zone diameter (20.6±0.8 and 21.6±0.8 mm) was found with 75 µl of water garlic extract for human and sheep isolated MRSA. The inhibition zones using water garlic extract (WGE) with diameter of 21.6 mm was matched with our results [39]. Antimicrobial effect of garlic methanolic (GME) and garlic ethanolic extracts (GEE) was also studied in our work. The results showed no antimicrobial activity against all nine MRSA isolates used in our work. The reason for these results might be due to allicin that is rapidly oxidized, unstable and volatile, meaning it rapidly breaks down after raw garlic is cracked. It has been reported that garlic extract has more potent anti-staphylococcal activity than an equal amount of allicin. This may be because a water-based extract of garlic stabilizes allicin, compared to methanolic and ethanolic extracts. At least partially, due to the hydrogen bonding between water and the reactive oxygen atom in illicit that lessens its instability and/or there may be water-soluble ingredients in cracked garlic that destabilizes the molecule [40].

The GWE that had antimicrobial activity against MRSA was subjected to MIC test to determine the lowest concentration of GWE that inhibits visible bacterial growth using broth micro dilution method. MIC results using GWE were showed lowest MIC values (3.6±.95 and 3.1±0.85 mg/ml) for human and sheep obtained MRSA while the GME and GEE extracts were not subjected to MIC test because they showed no antimicrobial activity against all MRSA isolates. Comparable results were previously recorded [15] and obtained MIC of thirty clinical MRSA isolates (16-32 µg/ml). The GWE combining with oxacillin antibiotic in agar disc diffusion test improved the antibiotic sensitivity of these pathogens to oxacillin [40]. In this work, we tried to make synergism between GWE and oxacillin antibiotic by mixing of 50 µl of fresh GWE and oxacillin and the obtained results revealed that inhibition zones were improved after combinations compared to those obtained with oxacillin alone and zones ranged from (23±2.1 and 22.6±2.3 mm) with combinations compared to (7.6±1.03 and 8.3±0.28 mm) with oxacillin alone. These results confirmed similar results [41] that mentioned that the inhibitions zones were improved after using of combination of oxacillin and fresh garlic extracts and the zone size was 23±2.1 mm.

CONCLUSIONS

The present study discovered that clove and garlic extracts have great potential antimicrobial components against microorganisms especially MRSA. Thus, they can be used as alternative method in the treatment of infectious diseases caused by MRSA. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

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