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RESEARCH ARTICLE **OPEN ACCESS**

The Effect of Combinations of Antibiotics and Natural Products on the Antimicrobial Resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract:

Introduction/Background: The steadily increasing bacterial resistance to existing antimicrobial drugs is a significant issue, hence, it is imperative to look out for new approaches to bacterial therapy Occasionally, effective inhibitory action is not produced when antibiotics are used alone. To overcome this problem, a combination of drugs is often used. One approach to treat infectious diseases is the use of a combination of antibiotics together with plant extracts or phytochemicals. For patients with serious infections caused by pathogens resistant to drugs, combination therapy is beneficial and useful.

Materials and Methods: Seven antibiotics were obtained from a local pharmacy (gentamicin, ceftazidime, ciprofloxacin, doxycycline, amoxicillin, ceftriaxone, and azithromycin). Minimum inhibitory concentrations (MIC) were determined by broth micro-dilution method, and different antimicrobial combinations were studied on 20 Multidrug-resistant (MDR) clinical isolates (10 *S. aureus* and 10 *P. aeruginosa*). Moreover, the antibacterial activity of some volatile oils (limonene, rosemary, salvia, thymus, and black pepper), plant extracts (moringa seed, curcumin, and capsicum), and phytochemicals (thymol, and chitosan) was detected against *S. aureus* and *P. aeruginosa* isolates using broth micro-dilution method.

Results: According to our findings, ceftriaxone and ciprofloxacin or gentamicin together exhibited a substantial synergistic effect against *S. aureus*. Moreover, the combination of amoxicillin with ceftazidime was synergistic to reduce MIC by five to six times. Regarding MDR clinical isolates of *P. aeruginosa*, the combination of azithromycin with doxycycline exhibited a decrease of MIC of azithromycin by about five to sixfold. The combination of gentamicin with ceftriaxone was significant. For natural compounds, thymol, rosemary oil, curcumin, capsicum, and moringa seed extract exhibited the highest synergistic activity with the tested antibiotics against *S. aureus* and *P. aeruginosa*.

Conclusion: In conclusion, the lack of new antibiotics necessitates the improvement of existing ones. Our study shows that antibiotic combinations and antibiotic-natural plant combinations are very promising strategies for combating complex bacterial resistance.

Keywords: Antimicrobial resistance, Combination, Synergism, Plant extracts, Phytochemical structure-function relationship.

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1. INTRODUCTION

There are many factors contributing to the emergence and dissemination of antibiotic resistance [1]. A significant factor to consider is the use of antibiotics by humans. Not surprisingly, the level of antibiotic-resistant infections strongly correlates with the level of antibiotic consumption [2-4]. Self-medication certainly lacks the attributes of a successful therapy, such as proper diagnosis, suitable antibiotic choice, correct usage, and treatment efficiency monitoring, thus contributing to the mounting resistance problem [5].

Combination antibiotic therapy is frequently used to treat severe infections. Potential achievements with combinations as compared with monotherapy include a broader antibacterial spectrum, synergistic effects, and reduced risk for emerging resistance during therapy. Combinations are increasingly employed to enhance the antibacterial effects of available drugs against MDR strains [6].

In the treatment of infections caused by MDR pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, combinations of antibiotics have often been used [7]. Combinations of antibiotics with drugs that block resistance mechanisms demonstrate an *in vitro* activity against resistant clinical isolates, which are more likely to result in successful therapeutic results. Therefore, evidence of *in vitro* synergism could be useful in selecting the most favorable combinations of antimicrobials for the therapy of serious bacterial infections [8].

Furthermore, because of their bactericidal qualities, plants produce a wide variety of secondary metabolites that play a significant role in shielding the plant against microbial pathogens and predators [9]. It is estimated that there are more than 3,000 essential oils (EOs) among these secondary metabolites [10, 11]. For these reasons, they are extensively utilized in the food and medical industries. The scientific research community is working to discover new applications and uses for alternative natural compounds because of the growing interest in these substances. These substances have been studied *in vitro* and have demonstrated promising actions against a variety of food-borne diseases and spoilage microbes [12].

Plants can be prospective sources of natural MDR inhibitors that can modulate the performance of antibiotics against resistant strains [13]. It is anticipated that the screening of plant components for antibiotic synergy may open up new avenues for MDR inhibitor isolation. Many substances that have been extracted from plants have also been shown to have the ability to lower the MICs of antibiotics against resistant organisms *in vitro*. For example, Polyphenols (epicatechin gallate and catechin gallate) have been reported to reverse β-lactam resistance in MRSA [14-16]. Moreover, diterpenes, triterpenes, alkyl gallates, flavones, and pyridines have also been reported to have resistance-modulating abilities to various antibiotics against *S. aureus* resistant strains [17, 18].

The present study aimed to combat such resistance by different combinations of antimicrobial agents and natural products against resistant isolates and assess the *in vitro* interaction of antimicrobial combinations.

2. MATERIALS AND METHODS

2.1. Bacterial Isolates

20 clinical isolates were obtained from the culture collection of the department. All specimens were originally obtained from septic surgical wounds, diabetic foot, diabetic ischemia, bed sore, abscess, respiratory catheters, and urine. Samples were collected aseptically from patients attending Zagazig University Hospital and transported to the Microbiology laboratory at Faculty of Pharmacy, Zagazig University, and were immediately processed.

Identification of *S. aureus* was confirmed by growth on MSA (oxoid), and *P. aeruginosa* was confirmed by using cetrimide agar (Sigma-Aldrich, USA). The antibiotic resistance pattern of *S. aureus* and *P. aeruginosa* was determined to be multidrug resistance bacteria (MDR).

2.2. Antibiotic Drugs and Natural Products

Seven Antimicrobials were evaluated in the current study, including gentamicin (EIPICO) and ceftazidime (Glaxo, Smithkline Pharmaceuticals). Ciprofloxacin (EIPICO), doxycycline (Nile Co. for Pharmaceuticals and Chemical Industries-A.R. E), amoxycillin (ADCO), ceftriaxone (SANDOZ), and azithromycin (Pfizer).

The volatile oils of the aerial parts of thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), and rosemary (*Rosmarinus officinalis*); orange fruit`s rind (*Citrus sinensis*) and black pepper fruits (*Piper nigrum*) were obtained by hydrodistillation using Clevenger apparatus, according to Egyptian pharmacopeia [19]. The alcoholic extracts of morenga seeds (*Morenga oleifera*) and cayenne fruits (*Capsicum minimum*) were used, and the curcuminoid of turmeric rhizome (*Curcuma longa*) mixture was isolated from the alcoholic extract of Curcuma powder as described previously [20]. Thymol and VetoChitosan (hard gelatin capsule) were purchased from Sigma (Chemical Co, U.S.A).

2.3. Determination of Minimum Inhibitory Concentration (MIC) of Tested Antibiotics and Natural Compounds

For MIC determination, stock solutions of antibiotics were prepared by dissolving the drugs in DMSO (Sigma Aldrich). The stock solutions were serially diluted with Muller Hinton broth (Oxoid) in 96-well microtiter plates. Each antimicrobial agent received an addition of 100 µL bacterial suspension (equivalent to the volume of diluted antimicrobial solution). Plates were incubated at 37 °C for 16 hours, and the result was read. Inoculum density and preparation, incubation conditions, and determination of MIC endpoints followed the specifications given in Clinical and Laboratory Standard Institute (CLSI) guidelines [21, 22]. The amount of growth in each well compared with

positive growth control and the MIC was recorded [23]. Furthermore, the tested natural compounds were serially diluted two-fold in Muller Hinton broth (MHB). The samples were incubated for 18 hours at 37°C. After incubation, the last tube without any visible growth of the bacteria was taken to represent the MIC. Control samples (positive and negative) were incubated under the same conditions [24].

2.4. Synergy Testing Methods

The assay of combinations was designed to include antimicrobials from different classes in 2-fold serial dilutions [25, 26]. The broth microdilution method is used to assess antibiotic combinations *in vitro* using static antibiotic concentrations (1/4, 1/8 MIC). The data produced by the broth microdilution assay were analyzed in terms of the fractional inhibitory concentration index (FIC). The FIC index was calculated according to the previous formula [27]. Antimicrobial combinations that resulted in a 4-fold reduction in the MIC compared with the MICs of agents alone were considered synergistic (FIC ≤ 0.5). FICs in the 0.5 to 1.0 range are considered non-synergistic or additive. FICs from 1 to 4 are defined as indifferent, while those of >4 are defined as antagonistic.

3. RESULTS

3.1. Antibiotic-antibiotic Combinations

Our results revealed that the combination of ceftriaxone with either gentamicin or ciprofloxacin for *S. aureus* was significantly synergistic (Table **1**). The combination of azithromycin with doxycycline exhibited a decrease in MIC of doxycycline about five to six-fold. Moreover, the combination of amoxicillin with ceftazidime was synergistic and exhibited a decrease of MIC by 5-6 folds.

For MDR clinical isolates of *P. aeruginosa*, the combination of the antimicrobial agent gentamicin with ceftriaxone was significant as MIC of ceftriaxone decreased by fourfold. For *P. aeruginosa*, the combination of cefadroxil and gentamicin (β-lactam and amino-glycoside) was found to be the most effective (Table **2**) as it showed more than 80% inhibition.

Table 1. Antibiotic-antibiotic combinations against *staphylococcus aureus*.

Combination	MIC of Drug Alone	MIC in Combination	FIC
CTX+ 1/4 MIC of gentamicin	**2048**	**256**	**0.375 (S)**
Doxycycline+1/4 MIC of azithromycin	**128**	**4**	**0.28 (S)**
CTX+1/4 MIC of ciprofloxacin	**2048**	**64**	**0.28 (S)**
Amoxicillin+1/4 MIC of ceftazidime	**1024**	**32**	**0.28 (S)**

Abbreviations: CTX: Ceftriaxone antibiotic, **S:** synergistic.

Table 2. Antibiotic-antibiotic combinations against *pseudomonas aeruginosa*.

Combination	MIC of Drug Alone	MIC in Combination	FIC
Ciprofloxacin+1/4 MIC of gentamicin	**32**	**16**	**0.75 (A)**
CTX+1/4 MIC of gentamicin	**2048**	**256**	**0.375 (S)**
Ceftazidime+1/4 MIC of gentamicin	**2048**	**2048**	**1.25 (I)**
Ceftazidime+1/4 MIC of ciprofloxacin	**2048**	**2048**	**1.25 (I)**

Abbreviations: S: synergistic, **A:** additive, **I:** indifferent.

Table 3. The effect of sub-inhibitory concentration (1/4MIC) of the essential oils (thymus and limonene, rosemary, salvia, and black pepper) on the MIC of different antimicrobials.

Antibiotic	FIC in the Presence of 1/4MIC of				
	Thymus	Limonene	Salvia	Black Pepper	Rosemary
Doxycycline ^{S}	**0.3125 (S)**	**0.3125 (S)**	**0.3125 (S)**	**0.3125 (S)**	**0.375 (S)**
Ceftriaxone ^{S}	**0.28125 (S)**	**0.5 (S)**	**0.375 (S)**	**0.28125 (S)**	**0.5 (S)**
Amoxicillin ^{S}	**0.2539 (S)**	**0.2656 (S)**	**0.2578 (S)**	**0.28125 (S)**	**0.2578 (S)**
Azithromycin ^{S}	**0.28125 (S)**	**0.28125 (S)**	**0.2656 (S)**	**0.28125 (S)**	**0.28125 (S)**
Gentamicin ^{P}	**0.5 (S)**	**0.75 (A)**	**0.5 (S)**	**0.75 (A)**	**0.5 (S)**
Ceftazidime ^{P}	**0.75 (A)**	**0.75 (A)**	**1.25 (I)**	**1.25 (I)**	**1.25 (I)**
Ciprofloxacin ^{P}	**0.3125 (S)**	**0.75 (A)**	**0.75 (A)**	**0.75 (A)**	**0.75 (A)**

Abbreviations: FIC: fractional inhibitory concentration, **S:** synergistic, **A:** additive, **I:** indifferent, **S:***Staphylococcus aureus*, **P:***Pseudomonas aeruginosa*.

Table 4. The effect of plant extracts (capsicum, moringa seed extract and curcumin) and phytochemicals (thymol and chitosan) on the MIC of tested antimicrobial agents.

Antibiotic	FIC in the Presence of 1/4MIC of				
	Capsicum Extract)	Moringa seed Extract)	Curcumin Extract)	Thymol	Chitosan
Doxycycline ^{S}	0.2656 (S)	0.375 (S)	0.3125 (S)	0.2656 (S)	1.25 (I)
Ceftriaxone ^{S}	0.28125 (S)	0.375 (S)	0.2812 (S)	0.28125 (S)	1.25 (I)
Amoxicillin ^{S}	0.2578 (S)	0.2578 (S)	0.5 (S)	0.25195 (S)	1.25 (I)
Azithromycin ^{S}	0.5 (S)	0.28125 (S)	0.5 (S)	0.2656 (S)	1.25 (I)
Gentamicin ^{P}	0.3125 (S)	0.3125 (S)	0.5 (S)	0.28125 (S)	1.25 (I)
Ceftazidime ^{P}	1.25 (I)	1.25 (I)	0.75 (A)	0.5 (S)	1.25 (I)
Ciprofloxacin ^{P}	0.75 (A)	0.75 (A)	0.2812 (S)	0.3125 (S)	1.25 (I)

ABbreviations; FIC: fractional inhibitory concentration, **S:** synergistic, **A:** additive, **I:** indifferent, **S:***Staphylococcus aureus*, **P:***Pseudomonas aeruginosa*.

3.2. Antibiotics and Natural Plant Combinations

The tested essential oils, plant extracts, and phytochemicals showed varying degrees of antibacterial activity in combination with antibiotics (doxycycline, ceftriaxone, amoxicillin, and azithromycin) against *S. aureus* and (gentamicin, ceftazidime, and ciprofloxacin) against *P. aeruginosa* (Tables **3** & **4**). As shown in Tables **3** and **4**, all essential oils were synergistic with antibiotics used against MDR *S. aureus* strains. In addition, a few combinations were synergistic against *P. aeruginosa*, including the combination of thymus oil with gentamicin or ciprofloxacin and the combination of gentamicin with salvia oil and rosemary oil. Thymol was synergistic with all tested antibiotics, while chitosan showed indifferent effects.

4. DISCUSSION

The emergence of MDR pathogenic bacterial strains represents a significant global health threat. MDR *P. aeruginosa* and *S. aureus* strains, represent a large problem in therapy [5, 28, 29]. Hence, finding new therapeutic options is an urgent demand, and the use of different drug combinations may represent a solution to this resistance crisis [7, 8, 14, 30].

Our study and other previous studies reported that the combination of cefadroxil and amoxicillin (both are β-lactam drugs) was synergistic against *S. aureus,* inhibiting more than 80% of the isolates [31]. Synergism between β-lactam antibiotics against *P. aeruginosa* strains was also reported [32]. On the other hand, synergistic interactions of β-lactam/ aminoglycoside combinations have been reported in previous studies [33-35]. It is reported that the disruption of Gram-negative bacilli's cell walls by β-lactamases allows aminoglycosides to enter the periplasmic space [36, 37]. It was also observed that this combination was also synergistic against more than 75% of *S. aureus* isolates.

Our results showed that the efficacy of the combination of ceftazidime and gentamicin was non-significant. Dundar and Otkun detected synergy in ceftazidime tobramycin (67%) combination against resistant strains [38]. Moreover, the efficacy of the combination of ceftazidime with ciprofloxacin was not beneficial. Our results do not agree with the synergistic activity that has been reported for combinations of β-lactams and fluoroquinolones [39-42]. Reported results of *in vitro* synergy between β-lactams and fluoroquinolones against Gram-negative organisms ranged from 17-82% [39, 40, 43].

Other studies evaluated the ciprofloxacin /β-lactam combination against MDR *P. aeruginosa* isolates and demonstrated synergy with this combination [44]. An *in vitro* study found that the degree of synergy between β-lactam–aminoglycoside and β-lactam–fluoroquinolone combinations was shown to be the same in an *in vitro* investigation involving 12 clinical isolates of P. aeruginosa, with synergy percentages ranging from 58-79% [45].

There are many reports in the literature on quinolone and β-lactam interaction, with several rates observed [32, 46]. In a previous study, synergy with the imipenem/ ciprofloxacin combination was not demonstrated [38]. Moreover, the efficacy of combinations of ceftazidime/ ciprofloxacin and ceftazidime/gentamicin was not useful. In an *in vitro* study, synergy of ciprofloxacin with ceftazidime at rates of ≥50% was reported against ciprofloxacin-resistant *P. aeruginosa* isolates [47]. Pohlman and his colleagues evaluated the ciprofloxacin/ ceftazidime combination against different Gram-negative organisms [48]. They concluded that the synergy between ciprofloxacin and β-lactams was sporadic and was not consistent across drug concentrations or sampling times. Moreover, Tamma and coworkers reported that the synergistic potential of β-lactams and fluoroquinolones remains unclear [49].

On the other hand, the combination of gentamicin/ciprofloxacin decreases MIC about 3-fold with two isolates and 1-fold with one isolate. Combinations of aminoglycosides with fluoroquinolones were shown to be synergistic [46, 50, 51] and support our results. Dundar and Otkun observed an additive effect with the ciprofloxacin-tobramycin combination [38]. Furthermore, none of the antimicrobial combinations tested in the current study demonstrated antagonism against any of the tested isolates. The variability of the results obtained in several studies may be due to differences in methodology, definitions of synergy, and choice of strains.

It has been demonstrated that plants produce not only intrinsic antimicrobial chemicals but also antimicrobial inhibitors, which can increase action of the antimicrobial compounds. For example, studies have described the synergistic and additive interactions of whole essential oils (EOs) or their constituents and antibiotics with different mechanisms of resistance [52, 53]. The results from broth microdilution assays showed a decrease in antibiotic MIC in the presence of plant EOs and phytochemicals [54, 55]. The antibacterial activity of orange EO (limonene) showed lower activity when compared to other tested compounds, and this was in agreement with previous results [56]. Combinations of limonene against *P. aeruginosa* were not useful with all tested antimicrobial agents. This was in agreement with the previous results that lemon oil and orange oil did not show inhibition against *P. aeruginosa* [56].

In our study, thymol exhibited a significant effect with almost all tested antimicrobial agents against *Staphylococcus aureus* and exhibited a good potentiating effect with gentamicin and ciprofloxacin against *P. aeruginosa*. Thymus had a synergistic effect with doxycycline (3-4-fold decrease in MIC) and amoxicillin (7-8-fold decrease in MIC) against *S. aureus*. For *P. aeruginosa,* about a 4-5-fold decrease in MIC of ciprofloxacin was observed. Previous studies showed that the EO of *Thymus vulgaris* and its major component, thymol, had bacteriostatic and bactericidal activities against Gram-negative strains [57]. Nonetheless, the activity of the EOs was superior to the compound alone. Such finding was explained by the fact that the high antimicrobial activity of some EOs generally results from the synergism of their major components [58].

In the current study, thymol showed the highest antimicrobial activity among the 10 tested plant materials, with MIC 2048 µg/mL for *S. aureus* and 16384 µg/mL for *P. aeruginosa*, followed by moringa seed, rosemary EO, and curcumin. Ivanovic reported significant antimicrobial activity of the extract and EO of thyme against Gram-negative strains, with MIC of 640 µg/mL, however our MIC ranged from 32768 µg/mL to 262144 µg/mL [59]. Such activity was attributed to the high concentration of thymol in the extract (39.7%) and in EO (48.49%). Previous studies also reported an antimicrobial activity of the EO of thyme [60, 61]. The study found three times stronger inhibition of pure thymol against different organism species than thyme oil, which is constituted mainly of p-cymene (36.5%), thymol (33%), and 11.3% of 1,8-cineole [57].

In this study, the combination of curcumin with doxycycline, amoxicillin, and ceftriaxone against *S. aureus* and with ciprofloxacin against *P. aeruginosa* exhibited significant synergistic effects, while other curcumin combinations were not synergistic. Other studies showed that the antibacterial activities of cefixime, cefotaxime, vancomycin, and tetracycline were increased in the presence of curcumin against the test strains. Curcumin significantly improved antibiotic efficacy against *S. aureus* when combined with cefixime, cefotaxime, vancomycin, and tetracycline, as curcumin inhibited the efflux pump system [62]. The result demonstrated that curcumin as a safe natural product could also serve as a valuable probe to study the structure-function relationships of antibiotic resistance reversal agents [63, 64]. Therefore, this compound or its future derivatives have a good potential for combination therapy against *S. aureus* [11, 65].

Rosemary was also used in combination with different antimicrobial agents against *S. aureus*. The combination was synergistic with doxycycline, azithromycin, CTX, gentamicin, and amoxicillin as MIC decreased about three folds with doxycycline and about 7-8 folds (1024µg/ml to 4µg /ml) with amoxicillin. Other rosemary–antimicrobial combinations were not useful either against *S. aureus* or *P. aeruginosa.*

El Hosseiny and El-Shenawy showed that the anti-pseudomonal activities of antibiotics were enhanced by a range of 12-33.3% in the presence of rosemary oil [66]. The antibacterial activity displayed by EO, alone and in association with antibiotics, is probably related to the major components (eucalyptol) identified in this oil. The MICs of the ethanolic extract of rosemary in our study were 1.1- 4.6 mg/mL against *S. aureus* isolates. Jarrar reported MICs in the range of 0.39–3.13 mg/mL [67]. They also demonstrated a synergistic effect between rosemary and cefuroxime against MRSA isolates. A significant modulatory effect on drug resistance was verified when rosemary was used in association with aminoglycosides against *S. aureus* MDR strains [68].

The EOs of rosemary and thymol had the highest antimicrobial activity in our study. Rosemary gave synergistic action combined with amoxicillin, doxycycline, ceftriaxone, azithromycin, and gentamicin. Thymol had a synergistic effect with all studied antimicrobials. The EOs of rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*) gave valuable synergistic effects with antimicrobial agents [12]. attributed synergism effects to phenolic and alcoholic compounds. Phenols and terpenes were the major antimicrobial compounds [68]. In general, Gram-positive bacteria are known to be more susceptible to EO than Gram-negative bacteria [69]. The weak activity against Gram-negative bacteria was attributed to the presence of their cell wall. *P. aeruginosa* appeared to be the most resistant to EOs and active phytochemicals [24].

In our study, the salvia plant was combined with different antimicrobials against *S. aureus* and *P. aeruginosa*. The combination was synergistic with amoxicillin (about 7-fold reduction in MIC), doxycycline (about 4-fold reduction in MIC), ceftriaxone (about 3-fold decrease in MIC), and azithromycin (about 6-fold reduction in MIC). In the presence of subinhibitory concentration (1/4 MIC to 1/32 MIC) of sage, the MIC values of antibiotics were found to be decreased by 2- to 10-fold. The essential oils of *S. officinalis* exhibited the lowest antibacterial activity in the disc diffusion method and did not affect *P. aeruginosa* [69].

In our study, moringa seed extract gave antibacterial activity against Gram-positive (MIC 4-8.1mg/ ml) and negative bacteria (MIC 16.3-32.7 mg/ml). Alikwe and Omotosho revealed that high antibacterial activity was observed in *Moringa oleifera* seed extract [70], which is similar to the findings of others [71-73]. They found out that the ethanol extracts of the seed extracts of *M. oleifera* were active against all bacteria tested [70].

In this study, moringa seed extract had a great potentiating effect with doxycycline, amoxicillin, ceftriaxone, and azithromycin against *S. Aureus*, with no noticeable effect on *P. aeruginosa* except with gentamicin, where MIC decreased about 3-4 folds. Other studies revealed that moringa ethanol extract efficiently inhibited the growth of Gram positive and negative strains [74, 75]. Moreover, other studies [75-77] showed the ability of substances in moringa seeds to inhibit mainly *Bacillus subtilis*, *Mycobacterium phlei*, *Serratia marcescens*, *E. coli*, *Pseudomonas aeruginosa*, *Shigella* and *Streptococcus* sp.. The observation of both Gram-negative and Gram-positive effects in the same plant extract may be explained by the presence of a wide spectrum of bactericidal substances [77].

Capsicum extract exhibited a synergistic effect with amoxicillin (7 folds decrease in MIC), ceftriaxone (2-6 folds), and doxycycline (6-7 folds) but the other combinations with azithromycin against *S. aureus* and ciprofloxacin and ceftazidime against *P. aeruginosa* were not synergistic. The MIC of capsicum extract in our study ranged from 103125µg/mL to 412500 µg/mL. Other studies reported lower MIC ranged from 256 to 1024µg/mL [76]. Capsicum extract in other studies displayed a large spectrum of activity (73%) against the tested bacteria strains [78].

Piperine, a major plant alkaloid isolated from the family Piperaceae, including black pepper (*Piper nigrum*) and long pepper (*P. longum*), has been reported to increase the accumulation of antibiotics by *S. aureus* [79]. Moreover, our results showed that black pepper exhibited a synergistic effect with doxycycline, ceftriaxone, amoxicillin, and azithromycin against *S. aureus* but no synergistic effects against *P. aeruginosa*.

For chitosan, our study showed no antimicrobial activity nor any synergistic effect with any of the antibiotics studied. Studies showed that COS exhibited synergistic effects with azithromycin against *P. aeruginosa* infection in both wild-type and resistant strains [80]. It was also implied that macrolides have a more positive interaction with selected oligosaccharides than the other antibiotics (oxytetracycline, cefotaxime, and ampicillin). This is in agreement with our results except for azithromycin [81]. The addition of ADO and COS drastically decreased the MICs of AZM by 2.8 and 5-fold, respectively; however, there was no synergistic effect in the rest of the treatments [82, 83, 84].

CONCLUSION

A good approach for the development of successful combinations would be to aim for broad and potent activities against a wide range of pathogens, including *S. aureus* and *P. aeruginosa*. Our results revealed that the combination of ceftriaxone with either gentamicin or ciprofloxacin for *S. aureus* was significantly synergistic. Moreover, the combination of amoxicillin with ceftazidime was synergistic and exhibited a decrease of MIC by 5-6 folds. Regarding MDR clinical isolates of *P. aeruginosa*, a combination of azithromycin with doxycycline exhibited a decrease of MIC of azithromycin by about 5-6 folds. The combination of gentamicin with ceftriaxone was significant. For natural compounds, thymol, rosemary oil, curcumin, capsicum, and moringa seed extract exhibited the highest synergistic activity with the tested antibiotics against *S. aureus* and *P. aeruginosa*.

In conclusion, the lack of new antibiotics necessitates the improvement of existing ones. Our study shows that antibiotic combinations and antibiotic-natural plant combinations are very promising strategies for combating the complex bacterial resistance. However, future efforts should be made to conduct *in vivo* studies with the extracts on animal models to confirm the present *in vitro* findings that are not only affected.

LIST OF ABBREVIATIONS

MDR = Multidrug-resistant

MIC = Minimum Inhibitory Concentrations

EOs = Essential Oils

CLSI = Clinical and Laboratory Standard Institute

MHB = Muller Hinton Broth

FIC = Fractional Inhibitory Concentration Index

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data introduced in this study was a part of the results of the Master thesis of the first author, The datasets used /or analysed in the current study are available from the corresponding author [A-E] upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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