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In Vitro Analysis of Single and Combined Antibiotic Combination in Alone and Plant Extract against Escherichia Coliand Staphylococcus Aureus

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Abstract: We compared in this study the in vitro potency of some antibiotics when used singly and in combination with another antibiotics and plant extracts against thirty-seven isolates of Staphylococcus aureusand fifty-five isolates of Escherichia coliusing disk diffusion method. Imepenem, Tobramycin, Nitrofurantoin, Ampicillin, Ciprofloxacin, Vancomycin, and Amikacin were randomly selected for this study. The isolates of E. coli and S. aureuswere previously identified from clinical specimens of human infections which included burn, wound, urine, and stool admitted to Internal Lab of Teaching Hospital. The samples were cultured on different culture media in order to test, isolate and identify of bacterial genera, according to the cultural characteristics, morphological features and biochemical reactions. Tremendous potencies were observed when the antibiotics used in combination compared with each antibiotic alone. The inhibition zone diameter increased significantly with combination between either two antibiotics or more in compare to monotherapy of antibiotics, and also there was recorded the significant different among antibiotics in alone status with combination status when saturated with plant extract against S. aureus only.

Keywords: E. coli, S. Aureus, Antibiotic Susceptibility, Plant Extract, Synergistic Therapy.

1. INTRODUCTION

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind. Different antibiotics exercise their inhibitory activity on different pathogenic organisms. The development and spread of resistance to currently available antibiotics is a worldwide concern. The increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs. Today, clinically important bacteria are characterized not only by single drug resistance, but also by multiple antibiotic resistances–the legacy of past decades of antimicrobial use and misuse. Drug resistance presents an ever-increasing global health threat that involves all major microbial pathogens and antimicrobial drugs. These are difficult to treat and are responsible for a variety of infectious diseases. For over a decade, the pace of development of new antimicrobial agents has slowed down while the prevalence of resistance has grown at an astronomical rate. The rate of emergence of antibiotic resistant bacteria is not matched by the rate of development of new antibiotics to combat them. Antibiotics that work today may not work tomorrow. It is essential to investigate newer drugs to which there is lesser resistance. As resistance to old antibiotics spreads, the development of new antimicrobial agents has to

be expedited if the problem is to be contained. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy.

S. aureus is the most clinically significant species of staphylococci. It is responsible for numerous infections ranging from relatively mild to life–threatening. *S. aureus* can be recovered from almost any clinical specimen and is an important cause of nosocomial infections. *S. aureus* continues to increase in importance as a community–acquired pathogen, and increasing drug resistance continues to be a concern with this common isolate (Mahon et al., 2018).

E. coli is the most common cause of infections by gram negative bacilli and it is a frequent cause of outpatient urinary tract infections in women worldwide, septicemia, diarrhea and meningitis. Resistance to recommended first and second line agents, such as penicillin, cephalosporin, sulfa drugs and fluoroquinolones(Carroll et al., 2016).

The antibacterial efficiency of plant compounds depends on several factors: (i) characteristics of target microorganism (the type, genus, species, strain), (ii) characteristics of plant material (botanical source, composition of the bioactive compounds as well as time of harvesting, stage of development or method of extraction) and (iii) chemical properties (hydrophilicity, lipophilicity, concentration, pH value). It is widely accepted that plant extracts, because of complex nature, possess multiple mechanisms of action. Plant extracts and their main components may exhibit activity by: (i) inhibiting bacterial growth or viability, (ii) targeting bacterial virulence factors or (iii) potentiating effectiveness of antibiotics as resistance modifying agents (Stefanović, 2018).

In phytotherapy, there are potentially significant advantages associated with the synergistic interactions which may be of different antibiotics, or plant extracts or the synergy may be of antibiotic and plant extract. The advantages are (Fluit et al., 2000) increased efficiency (Sahm et al., 2001) reduction of undesirable effects (Stelling et al., 2005) increase in stability or bioavailability of the free agents and (Blondeau, 2004) obtaining an adequate therapeutic effect with relatively small doses, when compared with a synthetic medication. Plant antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs, they enhance the effect of that drug.

Definitive combination therapy including two or three antibiotics to which the bacteria are susceptible has been suggested to recover clinical outcome as compared with monotherapy for critically unpleasant or patients and severe infections with bacterial isolates. Supplementary, it has been argued that combinations should be used to prevent advent of resistance during therapy. Nevertheless, recent analyses conclude that the existing clinical evidence is insufficient to support the use of definitive combination therapy for these reasons and that combination therapy is associated with an increased risk for ototoxicity, nephrotoxicity, bacterial super infections, and selection of resistant strains. It has therefore been recommended to de-escalate antibiotic treatment to the most appropriate single agent as soon as the antibiotic susceptibility profile of the causative pathogen is known (Chanda and Rakholiya, 2011).Synergistic interaction between two agents, in which one agent enhances the effect of the other and together they act more efficiently than as individual agents, motivated many scientists to examine and assess the significance of synergistic acting of plant-derived compounds and traditional antibiotics (Wagner and Ulrich-Merzenich, 2009). It is well known that plant extracts possess antibacterial properties but, also, the ability to enhance the activity of an antibiotic in combination with it. That ability of plant active substances reflects in modification or blocking of resistance mechanism so that bacterium becomes sensitive to antibiotic or the antibiotic acts when in lower concentrations. Such an approach, besides reducing the effective dose of antibiotics on one side, also reduces the side

effects of antibiotics as medicine on the other. Numerous in vitro researches have confirmed synergistic effects of plant extracts and antibiotics with a significant reduction of minimum inhibitory concentration (MIC) in antibiotics. Scientists have tested various types of extracts of numerous plants in combination with different antibiotics. These were primarily antibiotics from the group of inhibitors of cell wall synthesis and protein synthesis. The tests included both Gram-positive and Gram-negative bacteria (Stefanović, 2018).

2. MATERIALS AND METHODS

Specimen's Collection

A total of thirty-seven isolates of *S. aureus* and fifty-five isolates of *E. coli* were isolated from different clinical specimens include; urine, stool, blood and burn. The samples were taken with sterile cotton swab and cultured on nutrient agar for 24 hrs. at 37°C, then transfer single colonies to Blood, MacConkey and Mannitol salt, Eosin methylene blue, Simmons citrate agar directly and incubated for 24 hrs. for screening of *S. aureus* and *E. coli*.

Identification of the Isolates

Identification of these isolates was carried out using morphology, cultural and biochemical characteristics, and biochemical tests were performed like; catalase, coagulase, oxidase, indole, and motility tests(Power and Johnson, 2009), VITEK II system and molecular analysis through specific ID genes.

Genomic DNA Extraction

Genomic DNA was extracted from pure cultures *via* the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) according to the manufacturer's instructions; extract was eluted with 100 µLof elution buffer. Extracts were stored at -20°C before PCR run. DNA concentration measurements and purity assessment were performed using NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA).

DNA Electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 1X TAE buffer solution. The gels were allowed to cool down to about 45°C and 10 µL of 5 mg/mL RedSafe dye was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µL of the DNA with 2 µL of 6X loading dye were mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 hrs. The integrity of the DNA was visualized and photographed on UV light source. The extracted genomic DNA was used as template DNA for amplification of the 16S rRNA gene(Hamasalih and Abdulrahman, 2019).

PCR for Amplification of IDGenes

The extracted genomic DNA from all isolates was tested to detect the presence of IDgenes. The amplification of the ID genes was done using specific primers for 16S rRNA gene in *E. coli*:F5'-GAC CTC GGT TTA GTT CAC AGA–3' andR5'-CAC ACG CTG ACG CTG ACC A-3' (Gamal et al., 2017)and for *nuc* gene in *S. aureus*: F5'-AGCCAAGCCTTGACGAACTAAAGC-3' and R5'-GCG ATT GAT GGT GAT ACG GTT-3' (Hamasalih and Abdulrahman, 2019)yielding a PCR product of 585 base pairs and 279 bps, respectively. PCR amplification for detection of IDgenes was conducted using 1 µL of elute in a 25 µL as a final volume. Following the addition of 12.5 µL of 2× HotStart Taq Master Mix (RED AMPLICON, Germany), then 1 µL of each primer in the tube, the volume completed with free nuclease water to 25 µL. The thermal cycling protocol for PCR was

comprised as following: for 16S rRNA gene (Initial denaturation step (2 min at 94°C), 30 cycles of: denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec, elongation at 72°C for 1 min, final extension at 72°C for 3 min), and for *nuc* gene (5 min at 95°C to denature DNA initially, followed by 35 cycles of denaturation at 94°C for 2 min, primer annealing at 55°C for 2 min, standard extension for 1 min at 72°C and a final extension at 72°C for 7 min). After amplification, 5µl of PCR product was analyzed by electrophoresis in 1.2% agarose gel stained with ethidium bromide and visualized under UV trans–illuminator. The gene ruler 100 bp DNA ladder was used as a DNA size marker. A positive PCR product yielded a band of 585 bp and 279 bp, while a negative one did not produce any visible band on the gel.

Antibiotic Susceptibility Studies

The antibiotic sensitivity of the five isolates of *E. coli*and eight isolates of *S. aureus* were isolated from clinical specimens to five antibiotics which include Imepenem (2µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg), Ampicillin(10µg), Vancomycin (30µg), Tobramycin (10µg), and Amikacin (30µg)was determined according to Clinical and Laboratory Standards Institute standards(CLSI, 2017). 20 ml of Muller Hinton agar (MHA) melted and cooled at 45 °C was poured into sterile petri dishes and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 100 µl inoculums ($1.5*10^8$ CFU/ml) according to 0.5 McFarland standard solutions(Yasir et al., 2019)with the help of a sterilized swab onto the entire surface of Mueller Hinton Agar plate. The plates were allowed to dry before applying antibiotic disc. The discs were firmly applied to the surface of agar plates within 15 minutes of inoculation.

Preparation of Plant Extracts

For the preparation of *Juglans regia*, a solid–liquid extraction was performed in triplicate with ethanol. Two grams of sample were weighed in duplicate into screw cap tubes, followed byethanol, at a ratio of 1:20 (v/v). The flasks were placed in a water bath with stirring at 70°C during 30 min. Then, the extracts were filtered and centrifuged at 9000 ×*g*. The supernatants were collected and the precipitates discarded. ethanol extract was submitted to complete evaporation in a rotary evaporator at 40°C. The dry extracts were resuspended in 10% dimethyl sulfoxide (DMSO) in order to perform a final concentration of 4 mg/ml. This procedure was performed in triplicate(Freitas et al., 2013).

Combination Assay

The synergistic activity of the antibiotics and extracts was determined by using disk diffusion method which is based on the saturation of antibiotics with extract in solid medium. The Mueller–Hinton agar was poured in sterile petri dishes. The paper discs (6 mm diameter) that were impregnated with 2 µL of each extract and tested standard discs were placed on the inoculated agar surface. Petri dishes were allowed to stand for 30 min at room temperature before incubation at 37 °C for 24 hrs. The effect of essential oils was reflected by the appearance around disc with a transparent circular zone corresponding to the absence of growth. The diameter of inhibition zone was measured in mm. To evaluate the synergistic effect of the combination of the extract and antibiotics which are in the form of ready to use discs, 2 µL of extract was saturated to the antibiotic disc to determine the zones of inhibition. The obtained results were compared with those of the antibiotics tested on the same strains alone and by the same method.

Statistical Analysis

Statistical evaluation of the data was performed by Student's t-test using the Graph Pad Prism v.8 for Windows. The results were considered to be statistically significant at $p<0.05$. The results of antibacterial activity of plant extracts were statistically analyzed.

3. RESULTS AND DISCUSSION

Specimens collection and identification of bacterial isolates

The research findings are related to isolate of *S. aureus* and *E. coli* from several samples taken from Teaching hospital in Erbil city. The results were summarized in Table 1 which describes the sampling data that consists of various numbers of clinical specimens include; urine, stool, blood and burn, analyzed and confirmed as *S. aureus* and *E. coli*.

Table 1: Distribution of Both Genera among Clinical Specimens

Clinical sources	***E. coli* *n.* (%)**	***S. aureus* *n.* (%)**
Urine	17 (30.91)	7 (18.92)
Stool	20 (36.36)	8 (21.62)
Blood	7 (12.73)	5 (13.51)
Burn	11 (20.00)	17 (45.95)
Total	**55 (100)**	**37 (100)**

Out of 125 isolates, only 37 isolates were confirmed as *S. aureus*; 55 isolates were confirmed as *E. coli* on the basis of morphological, biochemical characterization, VITEK II compact system and molecular approach (Table 2). According to these results a high infection with *E. coli* and *S. aureus* was found in samples collected from different clinical sources. All the isolates of *E. coli* had given positive test for indole, methyl–red test, motility and negative biochemical test are citrate, and oxidase, there we confirmed that these organisms are *E. coli*. Confirmation of all isolates of the *S. aureus* was done by gram staining and various biochemical tests including catalase, coagulase positive and hemolysis on Blood agar, and negative for motility, and oxidase tests.

Table 2: Biochemical Characteristics of *E. Coli* and *S. Aureus*

Colonies and tests	***E. colin.* (%)**	***S. aureus n.* (%)**
Gram reaction	Negative	Positive
Eosin methylene blue agar	Metallic sheen colonies	NA
Indole	55 (100)	NA
Citrate utilization	0 (0.00)	NA
Oxidase	0 (0.00)	0 (0.00)
Motility	Motile	Non-motile
Methyl red	55 (100)	NA
Mannitol salt agar	NA*	Ability growth on salt medium
Hemolysis	NA	Variable hemolysis
Catalase	NA	37 (100)
Coagulase	NA	37 (100)
DNase	NA	37 (100)
VITEK II system	55 (100)	37 (100)
16S rRNA gene	55 (100)	NA
nuc gene	NA	37 (100)

*: NA: not applicable.

To endorse the identification of all isolates of both *S. aureus* and *E. coli* by a conventional method, the VITEK II system was conducted,andthis system reidentified all isolates as *S. aureus and E. coli*. The present results of the VITEK II system show that all isolates were identified confirmed by conventional methods. Conventional methods used for identification and confirmation of *S. aureus* and *E. coli* are consuming time. Molecular techniques, such as PCR have been used extensively for several years for identification and characterization of pathogens in clinical samples. Here we tried to use PCR method based on 16S rRNA and *nuc* genes as fast and accurate method for Identification and confirmation of *S. aureus* and *E. coli*. All isolates of *E. coli* and *S. aureus* were examined for the presence of the 16S rRNA and *nuc* genes to characterize and confirm the staphylococcal species, respectively. All of the strains were confirmed as *E. coli* and *S. aureus* by the occurrence of 16S rRNA and *nuc*genes (Figure 1).

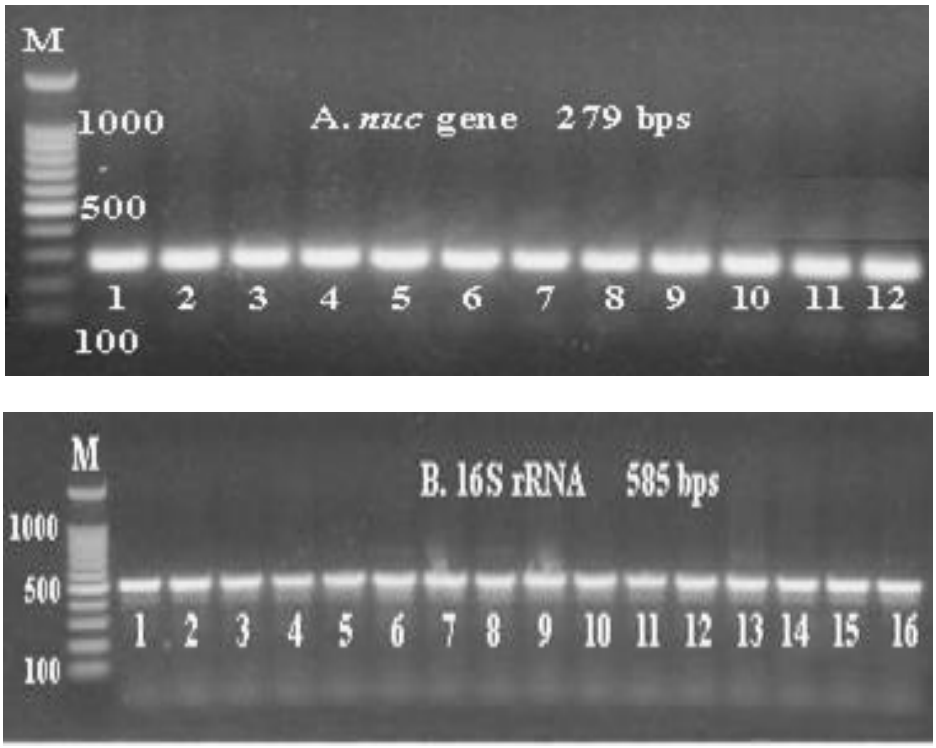
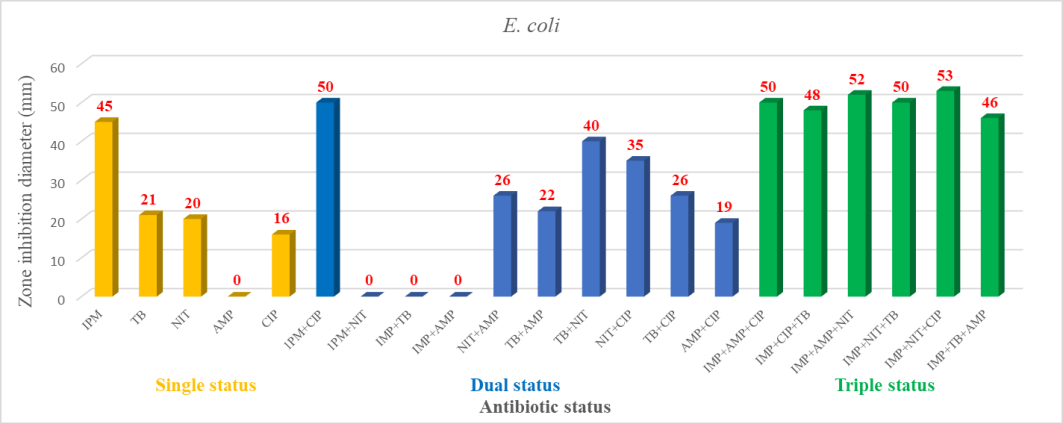


Figure 1 Agarose Gel Electrophoresis Graphic of PCR Amplification for the ID Gene in *S. Aureus* and *E. Coli*. A. *nuc* Gene for *S. Aureus*; Lane M: DNA Ladder 100 bps, Lane (1-12) Positive fornuc Gene at 279 bp Band. B. 16S rRNA Gene for *E. coli*; Lane M: DNA Ladder 100 bps, Lane (1-16) Positive for 16S rRNA Gene at 585 bp Band.

Synergistic Efficacy of Antibiotics

Figure (2) had shown the results of combination of Imepenem with Nitrofurantoin and Ciprofloxacin. Each of Imepenem, Nitrofurantoin and Ciprofloxacin alone were the inhibition zone 40, 39, and 19 mm respectively, while the inhibition zone significantly increased up to 45 and 53 mm. in diameter when these antibiotics were combined together, which indicates for synergistic effect of these antibiotics against *E. coli*.



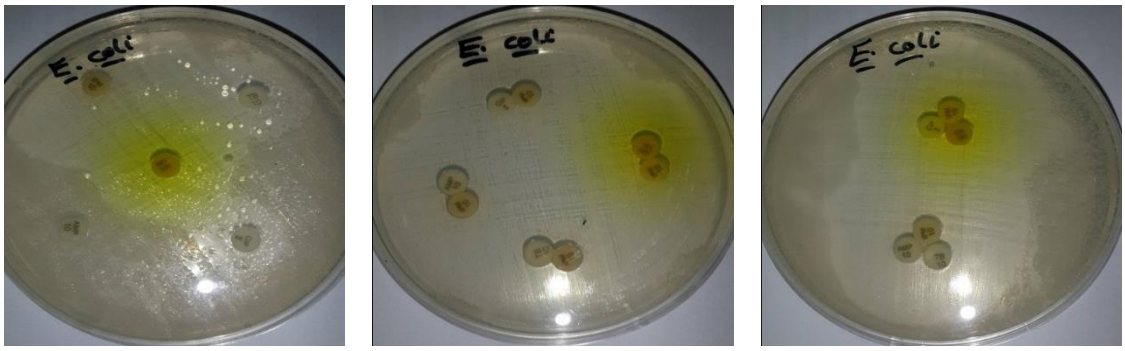


Figure 2 Comparison of Antibiotic Cases Against *E. coli*

Using antibiotics in in combination with other antibiotics can increase the efficacy and effects of antibiotics against bacterial growth. Statistically, there were a significant different in inhibition zone diameter among antibiotics in alone, dual and triple status. The inhibition zone diameter of Imepenem was 45 mm while the diameter of inhibition increased (50 mm) when combined with Nitrofurantoin (in dual combination, *p*-value=0.0328*) and with both Ampicillin and Ciprofloxacin (in triple combination, *p*-value= 0.0182*) against *E. coli* (Table 3), while in comparison between dual and triple combination, there is no revealed any significant difference (*p*-value=0.0821).

Table 3: Statistical Analysis among Synergistic Effects of Antibiotics against *E. Coli* and *S. Aureus*

Compare status	***E. coli***		***S. aureus***	
	***p*-value (<0.05)**	**Significant**	***p*-value (<0.05)**	**Significant**
Single vs. Dual	0.0328*	Yes	0.0328*	Yes
Single vs. Triple	0.0182*	Yes	0.0091**	Yes
Dual vs. Triple	0.0821	No	0.0582	No

From another line, also the combination of antibiotics had positive effects against *S. aureus*, and the results were clarified in Figure (3). The significant differences were clearly found between antibiotics against *S. aureus* in both single and triple antibiotic combination (*p*-value=0.0091**), while the differences was shown in low pick between single and triple antibiotics combination (*p*-value=0.0328*). Also, there is no any differences was observed between dual and triple combination of antibiotics (*p*-value=0.0582).

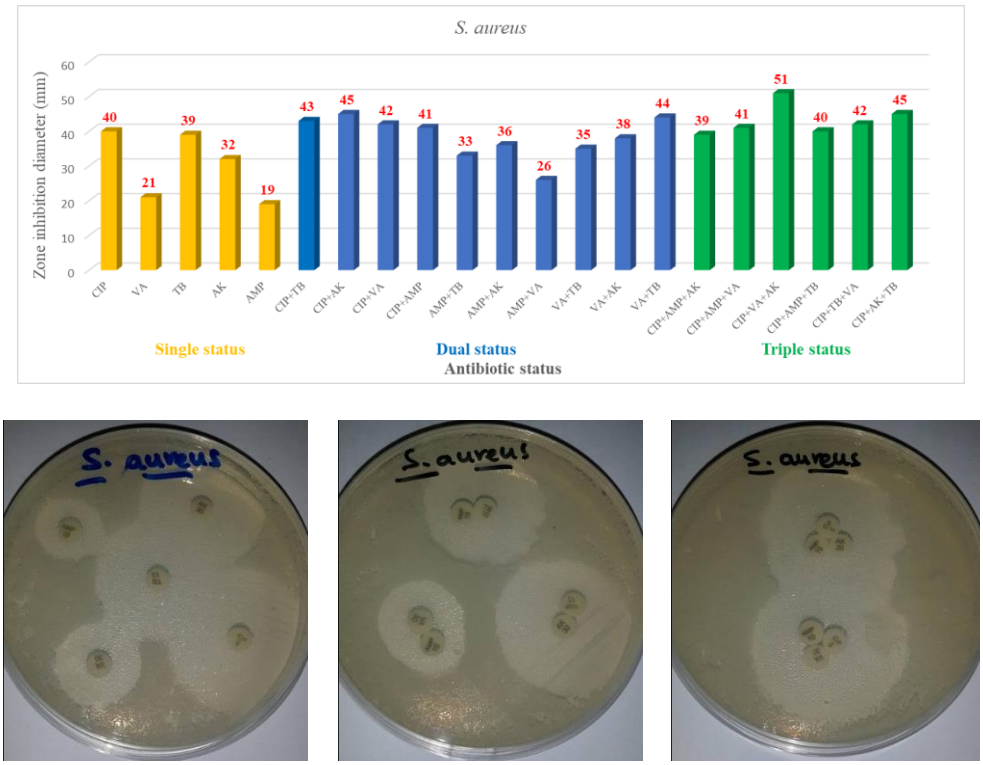


Figure 3 Comparison of Antibiotic Cases against *S. Aureus*

This result strongly confirmed the result obtained by Habeeb and Rabab (2011) who stated that Imepenem was found to enhance the activity of both Nitrofurantoin and Ciprofloxacin against *E. coli* and in other hand, Ciprofloxacin increase the heighten potency of Vancomycin and Amikacin against *S. aureus*. Both, Ciprofloxacin and Nitrofurantoin, Vancomycin and Amikacin were had low effectiveness against *E. coli* and *S. aureus* respectively, and the inhibition zone went up to 53 mm. in diameter when these antibiotics were combined together. The effects of these antibiotics against both bacterial isolates are seemed to be synergy. This can be attributed in part to Imepenem, which has number of characteristics that make it potentially effective when used in combination with other antibiotics, including its potent bactericidal activity, modest activity against non–growing cells and ability to penetrate cells(Deresinski, 2009).

Effect of Antibiotics with Plant Extract

In phytotherapy, there are potentially significant advantages associated with the synergistic interactions which may be of different antibiotics, or plant extracts or the synergy may be of antibiotic and plant extract. The advantages are (1) increased efficiency (2) reduction of undesirable effects (3) increase in stability or bioavailability of the free agents and (4) obtaining an adequate therapeutic effect with relatively small doses, when compared with a synthetic medication. Plant antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs, they enhance the effect of that drug.

This study investigated the antimicrobial activities of the phytochemicals extracted from *J. regia* in combination with antibiotics against isolates of *S. aureus* and *E. coli*. Antibiotic resistance profile in alone and with combination of tested isolates that presented in Figure 4 and 5 revealed that clinical isolates of *S. aureus* and of *E. coli* were multidrug-resistant to tested antibiotics.

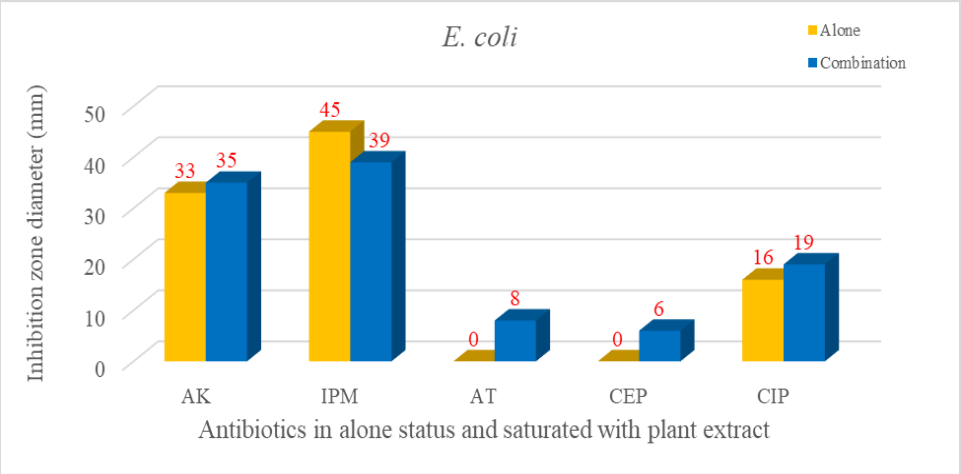


Figure 4A Effects of Antibiotic alone and Saturated Antibiotic Discs with Water Extract against *E. coli*.

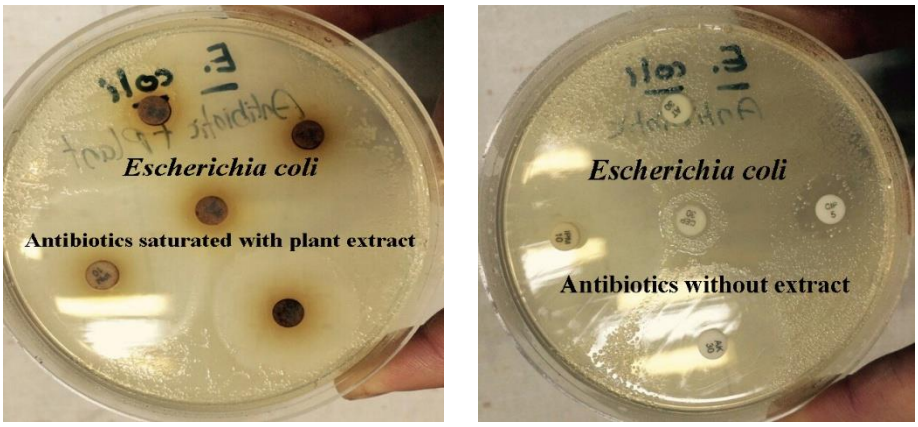


Figure 4B Effects of Antibiotic alone and Saturated Antibiotic Discs with Water Extract against *E. Coli*.

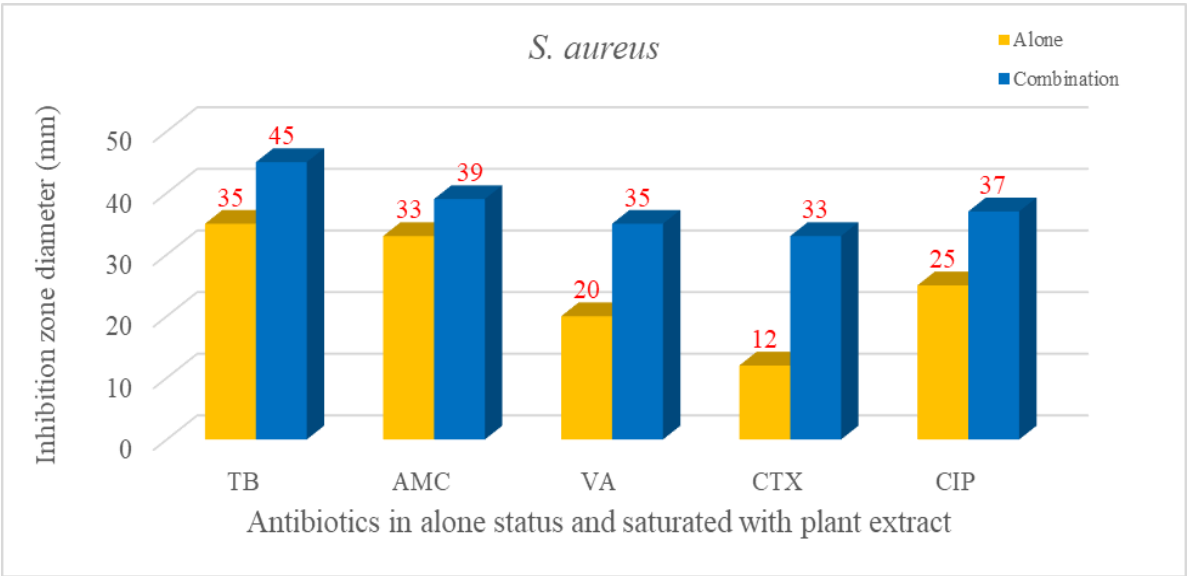


Figure 5A Effects of Antibiotic alone and Saturated Antibiotic Discs with Water Extract against *S. Aureus*

Figure 5B Effects of Antibiotic alone and Saturated Antibiotic Discs with Water Extract against *S. Aureus*

Statistically, the impact of the extract on the effect of AK, IMP, AT, and CIP against *E. coli* was not significant (p=0.1698), in spite of increasing of the diameters of inhibition zones (Table 4), but surprisingly the effect of extract on TB, AMC, VA, CTX and CIP against the *S. aureus* were observed clearly(p=0.0035**).An extract is considered to exhibit synergy with antibiotic if the combinations of the antibiotic with the plant extract (¼ MIC) lead to up to 5 mm increase in inhibition zone diameter of the discs or if such combination leads to up to 2-fold decrease in the MIC of the antibiotic (Adwan and Mhanna, 2009).

Table 4: The Inhibition Diameter Zone (mm) for Antibiotic alone and Antibiotic Plant Extract Combination against Both *E. Coli* and *S. Aureus*

Antibiotics status	***E. coli***			***S. aurous***		
	Antibiotics	Inhibition zone diameter (mm)	*p*-value (<0.05)	Antibiotics	Inhibition zone diameter (mm)	*p*-value (<0.05)
Antibiotics alone	AK*	33	0.1698 ns	TB	35	0.0035**
	IPM	45		AMC	33	
	AT	0		VA	20	
	CEP	0		CTX	12	
	CIP	16		CIP	25	
Combination of Antibiotics with Plant Extract	AK	35		TB	45	
	IPM	39		AMC	39	
	AT	8		VA	35	
	CEP	6		CTX	3337	
	CIP	19		CIP		

*: AK: Amikacin, AMP: Ampicillin, CIP: Ciprofloxacin, IMP: Imepenem, NIT: Nitrofurantoin, TB: Tobramycin, VA: Vancomycin.

Oliveira et al. (2011) investigated the synergistic activity of norfloxacin, tetracycline and erythromycin with ethanol extract of *Mangifera indica* L. peel against *S. aureus* strains. Individual extract did not display significant antibacterial activity (MIC ≥ 2048 μg/ml), but it modulated the activity of antibiotics (MIC = 512 μg/ml), i.e. in combination with antibiotics, a four-fold reduction in the MIC values for tetracycline and erythromycin was observed. The

study indicated that mango peel could serve as a source of potential adjuvant of antibiotics, which adds value to this mango by-product.

Toroglu (2011) investigated *invitro* synergistic effects of different spices and herbs (*Rosmarinus officinalis*, *Coriandrum sativum*, *Micromeria fruticosa* L., *Cumium cyminum*, *Mentha piperita*) with gentamicin, cephalothin, ceftriaxone and nystatin against 13 microbial species. This study suggested that essential oils of tested spices and herbs could protect some bacterial strains and the combination of plant extract with antibiotics further reduced drug resistance. The synergistic effects obtained could lead to new choices for the treatment of infectious diseases.

Adikwu et al. (2010)investigated the *in vitro* combined effects of erythromycin and methanol extract of leaves of *Euphorbia hirta* against clinical isolates of *S. aureus* using the Checkerboard technique. The organism was susceptible to the extract with MIC of 25 mg/ml, while erythromycin had MIC of 0.005 mg/ml. Synergistic effect was obtained by a combination of erythromycin and *E. hirta* against *S. aureus* in the ratios (9:1, 8:2, 7:3, 6:4, 3:7, 2:8,1:9) while others (5:5, 4:6) showed indifference. Combined drug use is recommended to prevent resistance emerging during treatment and to achieve higher efficacy in the treatment of infections and other diseases.

Odunbaku et al. (2008) reported synergistic activity between standard antibiotics and ethanolic extract of *Ficus exasperata* leaf on *E. coli* and *S. albus*. In this study, antibiotics were selected in such a way that the different antibiotics have different targets on bacteria (protein synthesis, nucleic acid, cell wall synthesis). The MIC of the plant extract against *E. coli* was 300 mg/ml while that of *S. albus* was 700 mg/ml. The study revealed that the combination of the crude plant extract and the protein synthesis inhibitors had the highest inhibitory activity.

Combinations of antibiotics activity against the cell wall (CWAs) plus aminoglycosides or possibly Vancomycin exert bactericidal synergy against both coagulase positive and coagulase negative strains; however, the mechanisms of such synergy are poorly defined. The addition of Vancomycin to regimens of CWAs for synergy remains controversial: there are conflicting data on synergy as well as antagonism in such combinations. Some investigators believe that the main impact of CWA–Vancomycin combinations for staphylococcal infections relates to the prevention of the emergence of rifampin resistance. Moreover, some studies have suggested that the *in vivo* synergistic mechanism of CWA–aminoglycoside combinations involves the diverse metabolic and growth characteristics of the bacterial population in the cardiac vegetations. The high titers of organisms in vegetations (sometimes reaching 10^9–10^{10} of viable bacteria per gram) suggest that bacteria have reached maximum population densities and a state of reduced metabolic activity and cell division within these lesions. Thus, an additional factor that may play a role in the *in vivo* mechanism of synergy is the potential for the individual CWA and aminoglycoside to act in different phases of bacterial metabolic activity and cell division within the vegetations (Goldstein et al., 2003).

4. CONCLUSIONS

In conclusion, in despite of successful findings being reported in this study, antagonism of antibiotics was also observed (data are not shown), which represents the main disadvantage of antimicrobial combinations, but it still takes advantages of different mechanisms of action and/or toxicities profiles as indicated above. However, advantages and disadvantages are closely related with the selection of agents which should be dependent in part upon the type of antibiotic, the nature of action, the susceptibility patterns, the site of infection, and the type of microbe.

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