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Original Article

A COMPUTATIONAL STUDY OF CIPROFLOXACIN METABOLITES AND SOME NATURAL COMPOUNDS AGAINST RESISTANT METHICILLIN *STAPHYLOCOCCUS AUREUS* (MRSA)

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ABSTRACT

Objective: In this paper, a computational study, including molecular docking, was accomplished for ciprofloxacin metabolites and some natural compounds, then a practical study of that compounds alone and in combination was applied against resistant methicillin *STAPHYLOCOCCUS AUREUS* (MRSA) isolates.

Methods: A docking software was used for molecular docking of the enzyme isomerase (3UWZ from protein data bank PDB) with ciprofloxacin (CIP) and its metabolites like sulfo-ciprofloxacin (Sulfo-CIP), oxo-ciprofloxacin, desethylene-ciprofloxacin, acetyl-ciprofloxacin, and natural compounds such as flavonoids (rutin, quercetin, hesperidin), phenols (thymol, gallic acid), phenolic acids (salicylic acid), terpenoids (menthol, eucalyptol) and coumarins (7-hydroxy coumarin). An antibacterial application for the highest binding energy of metabolites and natural compounds alone and in combination by using well diffusion method applied to ten of (MRSA) isolates.

Results: Docking results revealed that rutin, CIP, and Sulfo-CIP were the highest binding energy values of-106.76,-104.64, and-102.23 K/cal, respectively. The diameter of the inhibition zone pointed to the antibacterial activity against MRSA isolates, and it showed a range from 16-18, 18-22, and 18-19 mm in order. But the inhibition zone diameter in the combination of rutin with Sulfo-CIP ranged from 28 to 35 mm.

Conclusion: Metabolite Sulfo-CIP showed up high antibacterial activity close to CIP theoretically and *in vitro*; also, the relationship with natural compound rutin showed a synergistic effect.

Keywords: Methicillin-resistant *staphylococcus aureus* (MRSA), Natural compounds, Ciprofloxacin (CIP), Rutin, Sulfociprofloxacin (Sulfo-CIP), 3UWZ

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INTRODUCTION

Computational studies include employing computer-aided software to carry out the protein-ligand simulations of drug molecules to a given target which means molecular docking that is widely used in drug discovery and drug design [1]. It can be used to suppose the predominant binding models of a ligand with a protein of known three-dimensional structure [2], perform virtual screening on large libraries of compounds, rank the results according to their binding affinities, and propose structural hypotheses of how the ligands inhibit the target [3].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of positive gram bacteria that has developed or acquired multiple drug resistance to beta-lactam antibiotics, including methicillin [4]. They can cause various invasive infections, especially skin infections, pneumonia (lung infection), and other deeper diseases such as endocarditis, septic arthritis, osteomyelitis, and septicemia [5, 6]. As this bacteria is resistant to beta-lactam antibiotics, the glycopeptides and linezolid are recommended as first-line therapy for serious MRSA infections, then quinolones antibiotics like ciprofloxacin which have been proposed as a possible alternative to parenteral vancomycin therapy [7, 8].

Ciprofloxacin is a fluoroquinolone antibiotic that inhibits bacteria by affecting isomerase enzymes, especially DNAgyrase [9]. The use of fluoroquinolones is now limited only to those strains that show laboratory confirmation of their susceptibility [10]. Nowadays, many researchers demonstrated that the metabolism of a drug can have important consequences on its therapeutic effect or its toxicity [11]. Ciprofloxacin is partially metabolized in the liver by modification of the piperazinyl group to at least four metabolites which are N-acetyl ciprofloxacin, Oxo-ciprofloxacin, desethylene ciprofloxacin, and sulfo-ciprofloxacin [12]. Some of these metabolites had been revealed to have antibacterial activity [13].

Natural compounds are organic substances produced by living organisms and they have various chemical structures, including flavonoids, phenolics, alkaloids, glucosinolates, and organic acids [14]. They play a predominant role in the development of new therapeutic agents and possess pharmacological activity as an antiviral antioxidant and antibiotic effects [15]. At present, antibiotic combinations are widely studied as an alternative strategy to combat resistant microbes [16]. Most recent studies showed that natural compounds have explored a synergistic, additive, or antagonistic activity against bacteria when they were used in combination with antibiotics [17].

MATERIALS AND METHODS

In this work, molecular docking of 14 compounds was carried out into isomerase enzyme, then *in vitro* tests were applied to ten isolates of MRSA to compare *in silico* results.

Protein preparation

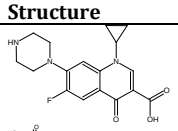
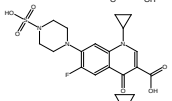
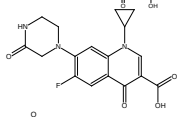
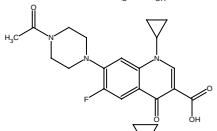
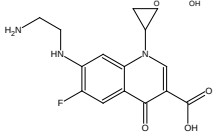
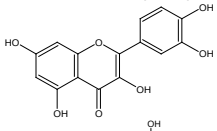
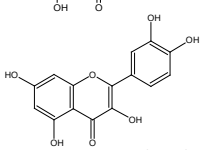
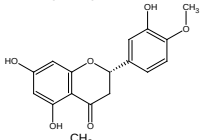
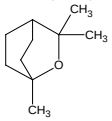
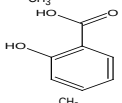
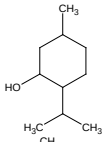
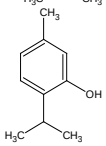
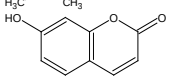
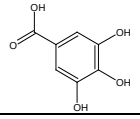
PDB is a fundamental repository site for 3D structure data of large molecules [18]. The isomerase enzyme required for the docking study related to MRSA has been retrieved from PDB with ID-3UWZ and had a resolution factor of 2.50 A° [19]. The enzyme was downloaded and then saved in PDB file format and the 3D of it was shown in (fig. 1). We defined the active site of (3UWZ) based on the x-ray complex structure of protein and binding ligand glycerol as shown in fig. 2 [20].

Ligands preparation

In this study, natural compounds were chosen depending on the antibacterial effect where the flavonoids like rutin, quercetin, and hesperidin were sorted as antibacterials, antioxidants, and antitumors [21]. While phenols, phenolic acids, and coumarins

compounds like (gallic acid, thymol, eucalyptol, menthol, salicylic acid, and 7-hydroxy coumarin, respectively) had weak to mild antibacterial effects but they were studied here to compare their activity [22]. Oxo-ciprofloxacin, N-acetyl-ciprofloxacin, and deseythylene-ciprofloxacin were metabolites of ciprofloxacin and they were separated and studied for their antibacterial action [23]. Whereas sulfo-CIP has few studies for its antibacterial efficacy but not on MRSA. For molecular docking, all structures were downloaded from the ZINC database site with MOL2 form [24] and collected in table 1 with the source of supply.

Table 1: Chemical group and source of studied CIP metabolites and natural compounds

S. No.	Name	Structure	Source
1	Ciprofloxacin		Himedia
2	Sulfo-ciprofloxacin		Toronto research chemicals
3	Oxo-ciprofloxacin		-
4	N acctyl-ciprofloxacin		-
5	Desethylene-ciprofloxacin		-
6	Rutin		Aldrich
7	Quercetin		Aldrich
8	Hesperidin		Sigma-Aldrich
9	Eucalyptol		Himedia
10	Salicylic acid		Himedia
11	Menthol		Himedia
12	Thymol		Himedia
13	7-hydroxy coumarine		Himedia
14	Gallic acid		Himedia

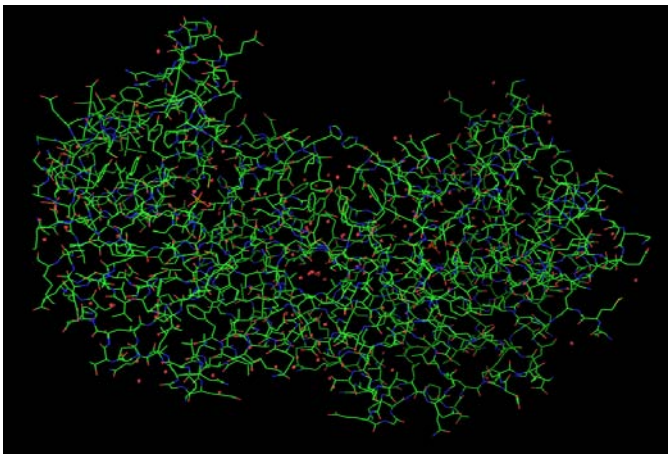


Fig. 1: The 3D of isomerase enzyme 3UWZ

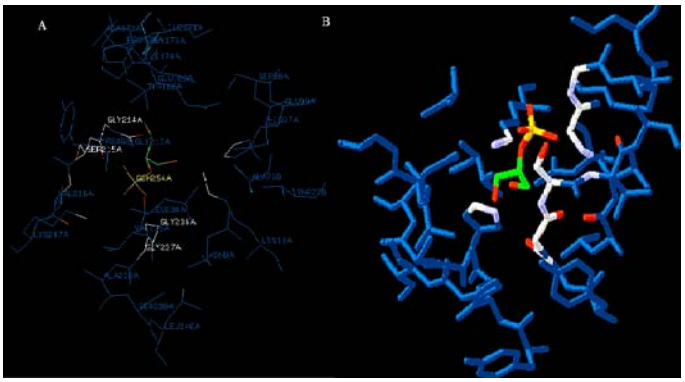


Fig. 2: The binding site of 3UWZ, A=wireframe mode of amino acids in the binding site, B= binding site in sticks mode, glycerol compound showed in green color

Protein-ligand docking

In this study, ciprofloxacin, its metabolites, and natural compounds were docked to the enzyme (3UWZ) by using iGemdock v2.1 software, which is available for free and was used in various previous research [25].

Docking software

This tool has been developed for virtual screening, preparations of the target protein and the compound library, docking, and post-screening analysis [26]. It generates protein-compound interaction profiles by providing interactive interfaces to prepare both the binding site of the target enzyme and the screening compounds library [27, 28]. The docking consisted of protocol "accurate docking" by setting a population size of 800 is set with 80 generations and 10 solutions. After the completion of the docking, the post-docking analysis was performed to find the docking pose and its energy values [29].

Practical study

MRSA isolates collection

About 10 clinical strains were collected from patients of Aleppo University Hospital. Bacterial culturing medias such as nutrient agar (NA; CM0003B), and Muller Hinton agar (MHA; CM0337B), were from Oxoid, UK. Mannitol salt agar (MSA; LAB007) was obtained from Lab M Limited, UK. Isolates identification was performed using Gram-staining, catalase test, coagulase test, and MSA differentiation. Isolated colonies of S. aureus from MSA plates were aseptically inoculated in sterile nutrient broth and incubated overnight at 37 °C [30]. Thereafter, the turbidity of the inoculum was adjusted to 0.5 McFarland using 0.9% (w/v) sterile normal saline and was used to prepare bacterial lawns on sterile MHA plates [31]. Methicillin discs were applied on seeded plates and incubated overnight at 37±1 °C. Following incubation, plates with zones of inhibitions were measured.

Antibacterial activity test

Antibacterial activity was determined by using a well diffusion method according to National Committee for Clinical Laboratory Standard [31]. Briefly, Petri plates containing approximately 25-30 ml of Mueller Hinton agar medium were inoculated using a cotton swab to cultivate a 4-6 h-old culture of bacterial isolates. Wells (6 mm diameter) were punched in the agar and filled with 5 µg/50 µl of sulfo-CIP and 500 µg/50 µl of each natural compound [32]. The sensitivity antibacterial tests were repeated three times and then the mean diameter of the inhibition zone (mm)±standard deviation (SD) was calculated by the SSPS software and the tested substance was considered to have antibacterial activity if the mean diameter of the inhibition zone was>10 mm, while the diameter≤ 10 mm of inhibition zone was regarded as inactive [33].

Natural compounds and sulfo-cip sensitivity tests

Susceptibility tests of clinical strains to sulfo-CIP+natural compounds combinations were tested using the well diffusion method on MHA plates. Every well was injected with 500 µg/50 µl of every natural compound with 5 µg/50 µl of sulfo-CIP. Following overnight incubation at 37 °C, diameters of inhibition zones were recorded. The antibacterial activity was assessed by measuring the inhibition zone diameter (mm) around the well. The synergism effect was considered when combinations exhibited with enlargement of combined inhibition zone size by 5 mm [34].

RESULTS AND DISCUSSION

Molecular docking results

Computational study of molecular docking is the best approach to check the utility of any chemical compound as a drug before going through any *in vitro* or *in vivo* analysis to shorten the experiments

and cost-cutting. In this work, about 14 compounds were docked against the isomerase 3UWZ to evaluate the theoretical antibacterial activity against MRSA. The objective of this current work is to estimate the antibacterial efficacy of metabolites of ciprofloxacin, especially the Sulfo-CIP; then a practical experience was done to examine the feasibility of the combination between natural compounds and chemicals as an effective antibacterial step in facing the resistance of MRSA.

Post-screening analysis

All the compounds in the post-screening analysis of PDB ID-3UWZ, in comparison to the CIP that is considered as the reference, were potential antibacterial drugs of MRSA with the target enzyme as the correlations had high values of energy [35]. The results displayed that the values of binding energy ranged between (-106.76) to (-44.79) Kal/mol as it showed in table (2) ranked from high to low value. The metabolite Sulfo-CIP exhibited a value of binding energy (-102.23) Kal/mol, which is very close to CIP (-104.64) Kal/mol. While the N-acetyl-CIP, desethylene-CIP, and Oxo-CIP had approximate binding energy values of (-86.25), (-78.21), and (-75.49) Kal/mol in order. So, All metabolites of CIP exhibit antibacterial effects theoretically as they had binding energies close to CIP value and some studies confirmed that *in vitro* by isolating them and studying the activity in which they exhibited efficacy very close to norfloxacin which is considered a derivative of CIP [36]. Sulfo-CIP was a very minor metabolite of CIP, and it was synthesized in 2006 by (Emami S and his colleagues) and evaluated as a new N-piperazinyl fluoroquinolone that had been investigated for many bacterial species but not on MRSA [37]. Otherwise, the natural compound rutin had a high value of binding energy (-106.76) Kal/mol but hesperidin and quercetin revealed values of (-84.93) and (-71.08) Kal/mol in order. These results of natural compounds are comparable with a lot of studies that took up flavonoids like quercetin and rutin, which had expressed influence in molecular docking and *in vitro* against bacteria [38].

Molecular docking with iGemdock interfered with the pharmacological interactions and clusters the screening compounds for the post-screening analysis based on profiles of electrostatic (E), hydrogen-bonding (H), and Van der Waal's (V) interactions and compound structures [39], then ranks and visualizes the screening compounds by combining the pharmacological interactions and energy-based-scoring functions [40]. Table 2 showed these energies where Sulfo-CIP showed VW, H Bond, and Elec energies as the reference CIP and rutin showed a VW binding energy only. This association o that compounds in the binding site candidate them as effective antibacterial agents [41].

Table 2: The binding energies of CIP, CIP metabolites, and Natural compounds

Name of structure	VW force (kcal/mol)	H Bond (kcal/mol)	Elec. energy (kcal/mol)	Total binding energy (kcal/mol)
Rutin	-106.76	0	0	-106.76
CIP	-73.11	-29.49	-2.04	-104.64
Sulfo-CIP	-71.06	-27.91	-3.26	-102.23
N-acetyl-CIP	-82.75	-3.5	0	-86.25
Hesperidin	-84.93	0	0	-84.93
Oxo-CIP	-70.81	-7.41	0	-78.21
Desethylene-CIP	-71.99	-3.5	0	-75.49
Quercetin	-71.08	0	0	-71.08
Eucalyptol	-65.69	0	0	-65.69
Salicylic acid	-41.09	-19.87	0	-60.96
Menthol	-57.91	0	0	-57.91
Thymol	-57.65	0	0	-57.65
coumarin	-47.96	0	0	-47.96
Gallic acid	-40.43	-4.36	0	-44.79

VW= vander valce energy, H Bond= hydrogen bonding energy, Elec= electricity energy.

The previous energies are produced by the interactions of compounds and the amino acid residues in the protein binding pocket and the associated correlations shown in table 3 for Sulfo-CIP, rutin, and CIP, and fig. 3 illustrated the linkages and fitness scores of that compounds with protein pocket. These results could suppose the most potent isomerase inhibitors for the prevention and treatment of infections caused by MRSA.

Antibacterial results

All the studied compounds have binding energy and good affinity toward the active site; thus, they may be considered good antibacterial agents in the practical experience, but the *in vitro* study displayed that compounds possessed different activities against the MRSA strains. The means and SD of the diameters of the inhibition zones presented the antibacterial activity [42], as shown in table 4.

Table 3: Pharmacological interactions and residues involved in the binding site

PDB-ID	Predicted pharmacologic interactions	Rutin	CIP	Sulfo-CIP
3UWZ	V-M GLU 169	-4.1	-0.8	0
	V-M ILE 171	-3.4	-1.5	0
	V-M ILE 174	-9.2	-0.7	-5
	V-S ILE 174	-11.5	-7.1	-5.5
	V-M GLY 213	-5.3	-1	-6.2
	V-M GLY 214	-11.6	-8.4	-8.3
	E-S HIS 97	0	0	-11.4
	H-S SER 98	0	0	0
	H-S GLU 169	0	0	-3.4
	H-M ILE 174	0	-2.5	-1.5
	H-S SER 215	0	0	0
	H-M VAL 216	0	0	0
	H-S LYS 217	0	0	0
	H-M GLY 237	0	0	0
	H-M ALA 238	0	0	0

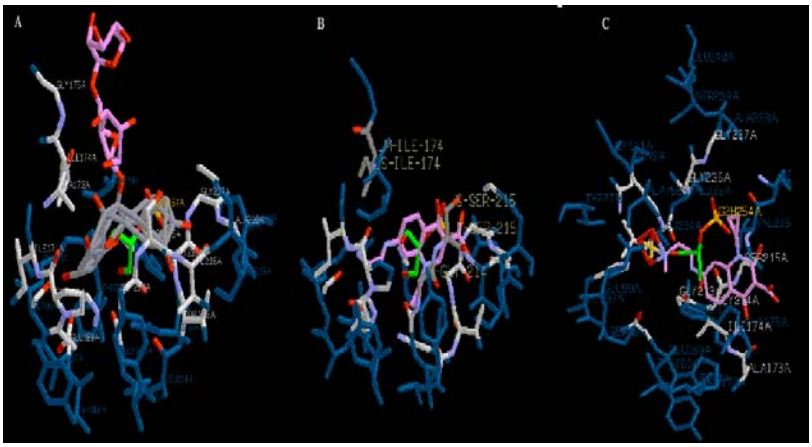


Fig. 3: The interactions of the amino acids with compounds in the binding site. Pink structures=docked compounds, A=rutin, B=CIP, C=Sulfo-CIP, Green structure =GLY

Table 4: Antibacterial activity of natural compounds and sulfo-cip

MRSA	Diameters of inhibition zones (mm) mean±SD										
	R	H	Q	EU	SAl	MEN	T	C	G	CIP	Sulfo-CIP
1	17.3±0.5	13±0.1	17±1.7	9±1.5	N	10.7±1.7	N	N	N	22±1.1	19±0.9
2	16±0.1	12±0.1	14.3±1.7	9.5±0.7	N	15±0.4	N	N	N	20±1.1	19±0.9
3	18±0.6	14±0.3	N	9±1.5	N	N	N	N	N	21±0.9	18±0.5
4	19±0.6	N	16±1.5	10±1.7	10±1.6	N	N	N	N	20.5±0.7	18.3±0.4
5	17.8±0.5	12±0.2	16.5±0.5	N	N	N	N	N	N	21±1.1	17.5±0.5
6	17.8±0.5	11±0.1	15.5±0.6	N	N	N	N	N	N	18±0.9	17.8±0.6
7	17±0.6	N	14.7±1.6	9±1.5	N	9±1.5	N	N	N	18.3±0.9	17.5±0.6
8	16.5±0.1	N	N	9.3±0.7	N	N	N	N	N	20±1.1	17.7±0.7
9	16±0.7	14±0.3	16.5±0.7	10±1.7	9±0.7	N	N	N	N	20±1.1	17±0.9
10	N	N	N	N	N	N	N	N	N	19±0.5	N

R=rutin, H=hesperidin, Q=quercetin, EU=eucalyptol, SAL=Salicylic, MEN=menthol, T=thymol, C=7-hydroxy coumarin, G=gallic acid, N=no antibacterial sensitivity. SD=standard deviation, Values are mean±SD, n=3, active value>10.

The flavonoids rutin, quercetin, and hesperidin were ranged from (16±0.7) to (18±0.6) mm, (14.3±1.7) to (17±1.7)mm, and (11±0.1) to (14±0.3) mm in order. While MRSA strains had different responses to the other natural compounds as the eucalyptol, salicylic acid, and menthol were active against some strains, thymol, gallic acid, and 7-hydroxy coumarin had no antibacterial activity. Many studies proved that natural components differ in response to bacteria where flavonoids rutin, quercetin, and hesperidin activity related to the structure and chemical groups [43]. Our results clarified that rutin and quercetin activities were approximate *in vitro* because of the similar chemical structures and that close to many numerous studies [44]. The compound hesperidin showed moderate activity in contrast to the *in silico* results, and some observations indicated its sensitivity related to the used concentrations [45]. Results of phenols, phenolic acids, and coumarins compounds like (gallic acid, thymol, eucalyptol, menthol, salicylic acid, and 7-hydroxy coumarin, respectively) demonstrated that they were practically inactive against bacterial strains as many studies showed that antibacterial activity likely depends on interactions between phenols and their derivatives and bacterial cells surface[46]. The inhibition zones of CIP and Sulfo-CIP were estimated from (18±0.9) to (22±1.1) mm and (17.5±0.5) to (19±0.9) mm respectively, and these values were compatible with CLSI standards of antibiotics inhibition zone diameter measurement [47]. These results reflect the corresponding binding energy values as the mechanism of CIP action is the correlation and inhibition of topoisomerase enzymes, especially DNAgyrase [48]. The metabolite Sulfo-CIP is produced from CIP by the addition of the sulfo group on the piperazine ring during the metabolism; then it execrates as a fecal metabolite [49]. Many studies covered the antibacterial activity of metabolites in plasma, serum, and urine which exhibited different antibacterial activity, while Sulfo-CIP as a minor product had not enough investigations [50]. In this research, Sulfo-CIP was active *in silico* and the experience exhibited inhibition zones similar to CIP, which suggests a mechanism of inhibition as CIP and the binding forces.

The growing and sustained resistance to antibiotics is becoming the most recent major health issue worldwide, and an emerging option to fight such pathogens is combination therapy, such as combinations of two antibiotics or antibiotics with adjuvants which is play a promising therapeutic approach [51]. In the investigation for the combination, an *in vitro* work had activated CIP and its metabolite Sulfo-CIP with natural compounds, especially rutin that had high binding energy. The combination showed antibacterial activity against MRSA isolates and the results were shown in table 5. The mean inhibition diameter of Sulfo-CIP with rutin was increased to (35±1.1)mm compared to the inhibition zone of each compound alone, and the combination of CIP and rutin were up to (29±0.6), while the inhibition zone of CIP with quercetin ranged to (28±0.6)mm which mentioned here to compare the results. Recently studies showed up that rutin enhances antibacterial activity in combination with other flavonoids, which suggests the increased inhibition zones [52]. A review study performed by Lindsay K. *et al.* pointed to synergism, antagonism, and additive activity of natural components and studied the standard of that effects where the synergism may occur if combined constituents are greater than the expected additive effect [53]. The combination results proved increased inhibition zones that may point to synergistic or additive effects, but *in vivo* investigation should applicate to confirm the effects. The present research was *in vitro* studies only because of the non-availability of animal model facilities, which remained the major limitation of this study.

Table 5: Antibacterial activity of rutin in combination with sulfo-CIP

10	9	8	7	6	5	4	3	2	1	MRSA	Diameters of inhibition Zones (mm) mean±SD
N	30±1.4	28±0.9	35.7±1.1	30±1.5	33±0.9	32.7±0.9	30.7±1.5	35±1.1	30.3±1.5	R+Sulfo-CIP (500+5)µg	
N	28±0.6	25±1	25.3±1.4	25.7±1.5	25±1	27±0.6	28±0.5	28±0.5	29±0.6	R+CIP (500+5)µg	
N	25±1.1	24±1.1	20±1.4	24±1.1	28±0.6	25±1	20±1.5	23±1.1	25.7±0.6	Q+Sulfo-CIP (500+5)µg	

R+CIP=rutin+ciprofloxacin. Q+sulfo, CIP=quercetin+sulfociprofloxacin. SD=standard deviation. N=no antibacterial sensitivity. Values are mean±SD, n=3, active value>10.

CONCLUSION

From the antibiotic sensitivity tests, it is evident that natural compounds rutin, CIP, and its metabolite Sulfo-CIP had a strong antibacterial effect and it was synergistic in case of combination. Therefore, the prescription of two compounds together should be taken into consideration to confront the advanced resistance of bacteria.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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