ANADOLU UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

European Journal of Life Sciences 2022; 1: 97-106

Research Article

Arum italicum Miller tuber extracts: evaluation of synergistic activities with ciprofloxacin against some pathogens

Hale Gamze Ağalar[1], Gözde Öztürk✉[1], Neşe Kırımer[1]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye.

✉ Gözde Öztürk
g.ozturkau@gmail.com

https://doi.org/10.55971/EJLS.1148283

Received: 27.07.2022
Accepted: 21.11.2022
Available online: 24.02.2023

ABSTRACT

Antibiotic misuse or overuse leads antibiotic resistance. Antibiotic resistant bacteria infections cause significant clinical problem. Recently, antibiotic resistant bacteria numbers have increased, this situation has become a global public health treat. To achieve these problems, development of new antibacterial compounds is still popular among researchers. The focus on natural compounds/plant extracts in combination with antibiotics increase their activities and decrease the doses of antibiotics and their side effects. Despite known as poisonous, *Arum italicum* Miller is used as food and/or is used for the treatment such ailments as furuncle, eczema, peptic ulcer, wounds, etc. This interesting species was found as anticancer, cytotoxic, apoptotic agent against some human cancers. In the present study, the fractions of *A. italicum* tuber extract against human pathogens (*Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 6538) were evaluated for their antibacterial activities by microdilution method. Each fraction was combined with ciprofloxacin and their synergistic activities were tested by checkerboard method. The MIC (minimum inhibitory concentrations) and FICI (fractional inhibitory concentration indexes) values were calculated. Totally, seven synergic interactions, ten additive interactions, and four indifferent interactions of tested fractions with ciprofloxacin were found.

Keywords: *Arum italicum* Miller, polar subfractions, antibacterial activity, checkerboard method, synergy

1. INTRODUCTION

Antibiotics has been used to prevent and treat bacterial infections for a long time. Since the beginning of the antibiotic era, antibiotic resistance has occurred when bacteria change the sensitivity to these medicines. These resistant bacteria can infect humans and animals, and the infections they cause are more difficult to treat than they are caused by resistant bacteria. In the world, the levels of antibiotic resistance are rising to dangerously. At early stages, the failure of antibiotic treatment was not accepted as an urgent clinical problem, since different groups of antibiotics with different targets were applicable. Nowadays, the number of resistance of bacteria has increased, hence, one of the global health threats has become as antibiotic resistance. This leads to increase medical costs, higher in-patient treatments, and increase the number of mortalities. The misuse and overuse of antibiotics are also accelerated the antibiotic resistant, as well as insufficiency of prevention and control of bacterial infections [1,2]. To prevent and control the spread of antibiotic resistance, one of strategies is to reduce the amount of antibiotics.

Antibiotics in combination with plant products may increase their antibacterial activity and decrease the doses of antibiotics and their side effects [3]. A potential strategy to combat resistance of bacteria is

positive interaction between antibiotic and natural products. If one agent enhances the effect of the other agent and together they act more efficiently than as individually, interaction between two agents has identified as "synergy". So, synergistic interactions between antibiotics and plant products motivate many scientists to evaluate of synergistic interactions and mechanisms of two agents [4]. Many plant products have shown antibacterial properties for a long time. Some of them enhance the activity of an antibiotic in combinations. Combinations with plant products resulted that bacteria became more sensitive to antibiotic or the antibiotic acted in lower concentrations than before. Those effects were due to the ability of plant active substances reflected in modification or blocked of resistance mechanism. With this approach, besides reducing the effective dose of antibiotics on one side, also reduces the side effects of antibiotics as medicine on the other. Synergistic effects of plant extracts and antibiotics with a significant reduction of Minimum Inhibitory Concentration (MIC) in antibiotics have been confirmed by *in vitro* studies [5-7]. As known, plants produce secondary metabolites such as polyphenols, terpenes, alkaloids. Crude extracts, complex mixtures of both secondary and primary metabolites, have been known to possess broad antibacterial activity [8-10].

Arum italicum (Araceae family) is an interesting plant and has gained popularity among Arum genus species. *A. italicum* is used as food from ancient times despite being poisonous. In traditional medicine, leaves, tubers, flowers, fruits and spate are used in different countries. Tubers are recorded as food (Bosna-Herzegovina, Iraq, Italy, Turkey) and are used to treat ailments such as hemorrhoid (Turkey), eczema (Turkey), rheumatic pains (Italy), muscle diseases (Spain, Turkey), hepatitis (Turkey), women diseases (Turkey), and to heal contusions (Italy) [11-16]. The tubers were characterized with lignan derivatives (lignan glucosides, 8-O-3' neolignan, 8-O-4' neolignan) and sterols [17-20]. Our group also published LC/MS-MS analysis results in detailed. According to our research, hydroxy-cinnamic acid-spermidine derivatives, flavones and lignans, their glucosides, and oxylipins were tentatively identified in tuber samples [21].

Arum italicum tuber extracts were evaluated for antibacterial [22], and antioxidant [23] activities. The study on the antibacterial activity of tuber extract against methicillin-resistant *Staphylococcus aureus* ATCC 33593 resulted that the extract showed no activity [22]. Recently, our group published that the tuber polar extract/fractions exerted notable cytotoxic activities against MCF-7 breast and A549 non-small lung cancer cell lines. The fractions also showed DNA synthesis inhibition and apoptotic effects [21]. These findings lead us to study these fractions for their antibacterial activities. In this present study, *Arum italicum* Miller tuber extracts/fractions were evaluated for their potential antibacterial activities against human pathogens such as *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 6538. Also, the combinations of the extracts with ciprofloxacin were evaluated by checkerboard method. To the best of our knowledge is to evaluate antibacterial activities against human pathogens and determine synergistic activities with ciprofloxacin.

2. MATERIALS AND METHODS

Plant material, extraction/fractionation and LC/MS-MS analysis parts of this manuscript belong to PhD thesis of Hale Gamze Ağalar, Anadolu University, Institute of Health Science, Pharmacognosy Department, Eskişehir-Turkey, 2016. The content such as plant material, extraction/fractionation and LC/MS-MS analysis of this manuscript was published in a refereed journal. Antibacterial activity and synergy combinations of the samples will be published for the first time.

2.1. Plant material

Arum italicum was collected from Bursa, Turkey in July, 2013. The aerial parts were separated from the tubers, then the tubers were sliced and dried in

the air-dried area. The voucher specimens are kept in Anadolu University, the Herbarium of Pharmacy Faculty with ESSE number 14620 [21].

2.2. Extraction and fractionation

The extract prepared from 792.7 g of dried and grounded *A. italicum* tubers by acetone:water (1:1, v:v) mixture using hot-continuous extraction (Soxhlet apparatus) procedure for 8 h. The extract was exhaustedly fractioned by *n*-hexane (Sigma-Aldrich), dichloromethane (Sigma-Aldrich), dichloromethane:methanol (1:1, v;v) and methanol (Sigma-Aldrich) by flash chromatography (Silicagel 60, 0.063-0.2 mm particle size, Merck), respectively. Then, the methanol fraction (E coded) was subjected to reverse-phase column chromatography (C18 column material, Macherel Nagel) under vacuum. The six subfractions were obtained by using water (E1 coded), methanol:water (20:80, v:v) (E2 coded), methanol:water (40:60, v:v) (E3 coded), methanol:water (60:40, v:v) (E4 coded), methanol:water (80:20, v:v) (E5 coded), and methanol (E6 coded), resp [21].

2.3. The LC/MS-MS analysis

The detailed information about mass spectrometry analysis was given by our previous study [21]. For identification, the UV spectra and total ion chromatograms were determined, and the molecular weights and fragmentation patterns of the molecules were screened by using previous studies.

2.4. Antibacterial activity

Bacteria strains used for the evaluation of biological activities were obtained from commercial sources (ATCC and NRRL) in the lyophilized form.

The antibacterial activity of the samples were evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute (CLSI) method as previously described [24-25]. *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* NRRL B3711 and *Salmonella typhimurium* ATCC 13311 were used as test microorganisms. The standard antibiotics ciprofloxacin (128-0.25 µg/mL) was used as standard control. Solvent and microbial controls were also added to the assay plate. Antibacterial assays were repeated at least three times for all the test samples and arithmetic means were reported.

2.5. Synergistic antibacterial activity

Interaction of the test samples were studied using the checkerboard microdilution assay in 96-well plates [26,27]. Checkerboard method was performed on a 96-well plate using an 8-by-8 well platform. Eight serial dilutions, two-fold dilutions of all tested *A. italicum* tuber samples and (20-0.019 mg/mL) and ciprofloxacin (128-0.25 µg/mL) were prepared. 25 µL aliquots of sample was added to the wells in a vertical orientation, and 25 µL aliquots of each antibiotics dilution were added in a horizontal orientation so that the plate contained various concentration combinations of the two compounds. Following this, each well was inoculated with a 50 µL ($5x10^3$ CFU/well) microorganism suspension (turbidometrically standardized), and was further incubated at 35 °C for 24 hours. After incubation 20 µL of resazurin was added to all wells and left at 35 °C for 2 h. Microbial growth was indicated by change in color from blue to pink. The broth microdilution checkerboard method was performed by using the fractional inhibitory concentration index (FICI), which is defined as the sum of the MIC of each sample, when used in combination divided by the MIC of the sample when used alone. Calculations were performed by following equations:

FICI = FIC X + FIC Y
FIC X= (MIC value of combined sample and antibiotic)/(MIC value of antibiotic alone)
FIC Y= (MIC value of combined sample and antibiotic)/(MIC value of sample alone)

Consequently, the activity was defined as follows:
FICI ≤0.5 = synergism;
FICI 0.5≤ 1 = additive effect;
FICI >1-4 = indifferent effect;
FICI ≥ 4 = antagonism
for more detailed information check references and references herein [26,27].

3. RESULTS AND DISCUSSION

Antibacterial activity of each sample against *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* NRRL B3711 and *Salmonella typhimurium* ATCC 13311 was evaluated by microdilution CLSI method. Ciprofloxacin was used as positive control. Minimum inhibitory concentrations (MIC) of tested samples were calculated.

In Table 1, individual MIC value of each sample was shown. The results revealed variability in the inhibitory concentrations of each sample for given bacteria. According to the results, subfractions except water subfraction (E1) were found more effective than E coded methanol fraction. *P. aeruginosa* among tested microorganisms was more sensitive to the samples. *Arum italicum* extracts ranging 0.12-20 mg/mL showed antibacterial activity against *P. aeruginosa*. The most effective subfractions were E3 (MIC, 0.12 mg/ml) and E5 (MIC, 0.12 mg/ml). But these effects were lower than ciprofloxacin (MIC, 0.0004 mg/mL). Against *B. cereus*, the most effective subfraction was E5 (MIC, 0.12 mg/mL), followed by E3 (MIC, 0.25 mg/mL), E6 (MIC, 0.28 mg/mL), E4 (MIC, 0.43 mg/mL), E2 (MIC, 0.87 mg/mL), E1 (MIC, 20 mg/mL) =E (MIC, 20 mg/mL). The MIC value of ciprofloxacin was 0.0016 mg/mL. When the effects of samples were compared, E3 and E5 were the more effective against *S. typhimurium*. The closest activity was found for E6 subfraction. To sum up, in general, fractionation procedure lead to the antibacterial activity better. Sugar-rich fraction coded E1 had the lowest antibacterial activity against tested all bacteria.

For the evaluation of synergism, all samples were tested by checkerboard method. As known, synergy is defined as a decrease in the viable organism as a result of the combination when compared with the most effective antibiotic when tested alone. Among the techniques employed in the evaluation of the combination of two antimicrobials potentially exhibiting synergism is the checkerboard technique. The checkerboard or fractional inhibitory concentration (FIC) technique employs a methodology similar to that utilized for the determination of the Minimum Inhibitory Concentration (MIC) [28]. A Fractional İnhibitory Concentration Index (FICI) was used to interpret the results. According to the Clinical Laboratory Standards Institute (2006) guidelines for broth microdilution, the MIC was defined as the lowest concentration of antibiotic that completely inhibited the growth of the organism as detected with the naked eye. Synergy is more likely to be expressed when the ratio of the concentration of each antibiotic to the MIC of that antibiotic was same for all components of the mixture [29].

When *Arum italicum* samples combined with ciprofloxacin, seven synergistic, ten additive, and four indifferent interactions were found while any antagonism interactions were occurred. The remarkable result was to observe any antagonism between *A. italicum* samples and ciprofloxacin. Table 2-4 show the results of the different combinations of ciprofloxacin with different *A. italicum* samples.

Among synergistic interactions, four of them (E, E2, E4, E5) against *P. aeruginosa*, one of them (E2) against *B. cereus* and two of them (E2 and E6) against *S. typhimurium* strains were found.

Surprisingly, despite no antibacterial activity of E sample against *P. aeruginosa* ATCC 27853, E sample showed very good synergic (FICI value, 0.257) activity with ciprofloxacin resulting in up to 8-fold reduction of MIC value and re-sensitization of *P. aeruginosa* strain. The association between E2 and ciprofloxacin against *P. aeruginosa* showed strong synergistic effect. E2 reduced the MIC value of

Table 1. Minimum inhibitory concentrations (mg/mL) of *Arum italicum* samples

	E	E1	E2	E3	E4	E5	E6	Cipro
Pseudomonas aeruginosa	10	20	0.43	0.12	0.43	0.12	0.28	0.0004
Bacillus cereus	20	20	0.87	0.25	0.43	0.12	0.28	0.0016
Salmonella typhimurium	20	20	0.87	0.25	0.43	0.25	0.28	0.0016

E, methanol fraction of %50 acetone extract; E1, water subfraction; E2, 20% methanol subfraction; E3, 40% methanol subfraction; E4, 60% methanol subfraction; E5, 80% methanol subfraction; E6, methanol subfraction; Cipro, ciprofloxacin.

Table 2. Synergistic antibacterial activities against *P. aeruginosa* strain (mg/mL)

	MIC sample	MIC sample COMB	FIC sample	MIC cipro	MIC cipro COMB	FIC cipro	FICI	RESULT
E	10	0.07	0.007	0.0002	0.00005	0.25	0.257	SYNERGISTIC
E1	20	0.07	0.0035	0.0004	0.0004	1	1.0035	ADDITIVE
E2	0.437	0.003	0.0068	0.0002	0.00005	0.25	0.2568	SYNERGISTIC
E3	0.125	0.0009	0.0072	0.0008	0.0008	1	1.0072	ADDITIVE
E4	0.437	0.003	0.0068	0.0008	0.0002	0.25	0.2568	SYNERGISTIC
E5	0.125	0.0009	0.0072	0.0002	0.0001	0.5	0.5072	SYNERGISTIC
E6	0.281	0.002	0.0071	0.0004	0.0016	4	4.0071	INDIFFERENT

E, methanol fraction of %50 acetone extract; E1, water subfraction; E2, 20% methanol subfraction; E3, 40% methanol subfraction; E4, 60% methanol subfraction; E5, 80% methanol subfraction; E6, methanol subfraction; Cipro, ciprofloxacin. FIC sample: MIC value of combined sample/MIC value of sample alone; FIC cipro: MIC value of combined ciprofloxacin/MIC value of ciprofloxacin.

Table 3. Synergistic antibacterial activities against *B. cereus* strain (mg/mL)

	MIC sample	MIC sample COMB	FIC sample	MIC cipro	MIC cipro COMB	FIC cipro	FICI	RESULT
E	20	0.07	0.0035	0.0008	0.0016	2	2.0035	INDIFFERENT
E1	20	0.07	0.0035	0.0008	0.0008	1	1.0035	ADDITIVE
E2	0.874	0.003	0.0034	0.0016	0.0008	0.5	0.5034	SYNERGISTIC
E3	0.25	0.0019	0.0076	0.0016	0.0032	2	2.0076	INDIFFERENT
E4	0.437	0.003	0.0068	0.0016	0.0016	1	1.0068	ADDITIVE
E5	0.125	0.0009	0.0072	0.0016	0.0016	1	1.0072	ADDITIVE
E6	0.281	0.002	0.0071	0.0016	0.0016	1	1.0071	ADDITIVE

E, methanol fraction of %50 acetone extract; E1, water subfraction; E2, 20% methanol subfraction; E3, 40% methanol subfraction; E4, 60% methanol subfraction; E5, 80% methanol subfraction; E6, methanol subfraction; Cipro, ciprofloxacin. FIC sample: MIC value of combined sample/MIC value of sample alone; FIC cipro: MIC value of combined ciprofloxacin/MIC value of ciprofloxacin.

ciprofloxacin up to 8-fold. A strong synergistic effect was also found between E4 and ciprofloxacin. The E4 + ciprofloxacin combination resulted the FICI value as 0.2568. One of the synergic combinations was also combined with E5 and ciprofloxacin against *P. aeruginosa* with FICI value, 0.5072.

According to Table 3, there was one synergistic combination between E2 and ciprofloxacin against *B. cereus* NRRL B3711 with FICI value 0.5034. Most samples (E1, E4, E5, E6) showed additive interactions with ciprofloxacin. E1 also ineffective sample against *B. cereus*, on the contrary, E1 + ciprofloxacin combination showed additive effect.

The combinations of *A. italicum* samples with ciprofloxacin resulted different interactions (additive, synergistic and indifferent) against *S. typhimurium* ATCC 13311 (Table 4). The FICI ranged from 0.5034 to 1.0072. The combinations of E2+ciprofloxacin and E6+ciprofloxacin were found to be best synergistic effects against *S. typhimurium* strain with FICI values 0.5034 and 0.5071, respectively.

As expected, the rapid emergence of resistant bacteria worldwide, increasing to sensitivity of bacteria, reducing the side effects of antibiotics, increasing the efficacy of antibiotics, that have modified medicine and saved millions of lives. The crisis of antibiotic resistance has been assigned to the misuse and overuse of these chemotherapeutics, as well as pharmaceutical industry have enough facilities to develop new drug because of the decrease in economic motivators and challenging regulatory requirements. Based on the CDC (Centers for Disease Control and Prevention) classification, bacteria are defined as urgent, serious, and concerning threats. These types of bacteria are responsible for significant clinical and financial charge on the health care systems as well as patients and their families. Generally, urgent or serious threats should

Table 4. Synergistic antibacterial activities against *S. typhimurium* strain (mg/mL)

	MIC sample	MIC sample COMB	FIC sample	MIC cipro	MIC cipro COMB	FIC cipro	FICI	RESULT
E	20	0.07	0.0035	0.0016	0.0016	1	1.0035	ADDITIVE
E1	20	0.07	0.0035	0.0016	0.0016	1	1.0035	ADDITIVE
E2	0.874	0.003	0.0034	0.0016	0.0008	0.5	0.5034	SYNERGISTIC
E3	0.25	0.0009	0.0036	0.0032	0.0032	1	1.0036	ADDITIVE
E4	0.437	0.003	0.0068	0.0016	0.0016	1	1.0068	INDIFFERENT
E5	0.125	0.0009	0.0072	0.0016	0.0016	1	1.0072	ADDITIVE
E6	0.281	0.002	0.0071	0.0016	0.0008	0.5	0.5071	SYNERGISTIC

E, methanol fraction of %50 acetone extract; E1, water subfraction; E2, 20% methanol subfraction; E3, 40% methanol subfraction; E4, 60% methanol subfraction; E5, 80% methanol subfraction; E6, methanol subfraction; Cipro, ciprofloxacin. FIC sample: MIC value of combined sample/MIC value of sample alone; FIC cipro: MIC value of combined ciprofloxacin/MIC value of ciprofloxacin.

Table 5. Synergistic antibacterial activities of *Arum italicum* samples with ciprofloxacin

	Pseudomonas aeruginosa **ATCC 27853**	***Bacillus cereus*** **NRRL B3711**	***Salmonella typhimurium*** **ATCC 13311**
E	Synergistic	Indifferent	Additive
E1	Additive	Additive	Additive
E2	Synergistic	Synergistic	Synergistic
E3	Additive	Indifferent	Additive
E4	Synergistic	Additive	Indifferent
E5	Synergistic	Additive	Additive
E6	Indifferent	Additive	Synergistic

E, methanol fraction of %50 acetone extract; E1, water subfraction; E2, 20% methanol subfraction; E3, 40% methanol subfraction; E4, 60% methanol subfraction; E5, 80% methanol subfraction; E6, methanol subfraction; Cipro, ciprofloxacin. FIC sample: MIC value of combined sample/MIC value of sample alone; FIC cipro: MIC value of combined ciprofloxacin/MIC value of ciprofloxacin.

be required more monitoring and prevention when compared with those considered concerning. Among serios threats by CDC classification, multidrug-resistant *P. aeruginosa*, drug-resistant nontyphoidal Salmonella, drug resistant *S. typhimurium* were listed [2]. The genus *Pseudomonas* (Gram-negative bacilli) are common inhabitants of soil, fresh water, and marine environments. *P. aeruginosa* is an opportunistic pathogen that is naturally resistant to many antibiotics. It is one of the causes of hospital infections [30]. *P. aeruginosa* is responsible for ventilator-associated pneumonia, contact lens keratitis, otitis externa, cystic fibrosis. It is a common cause of HAIs, including pneumonia and bloodstream, urinary tract, and surgical-site infections [31]. *Salmonella* species are the most common causes of foodborne illness worldwide and *S. typhimurium* can cause infection in humans. It is a Gram- negative, facultative anaerobe bacteria and the leading cause of gastroenteritis [32]. *Bacillus cereus*, a toxin-producing facultatively anaerobic Gram-positive bacteria, is often found in soil and vegetation, and can be present in foods. Because of the being ubiquitous microbe, it can contaminate foods easily and cause many gastrointestinal infections especially, food poisoning, vomiting and diarrhea [33].

Ciprofloxacin, a quinolone, is widely used in clinical practice. It inhibits a wide range of gram-positive as well as gram-negative bacteria such as *Pseudomonas aeruginosa, Streptococcus faecalis, Staphylococcus aureus* and *Enterobacter aerogenes*. For years, fluoroquinolones have been used exponentially due to their efficacy in treating common infections such as urinary tract, gastrointestinal tract, respiratory tract and skin infections [34]. The most common adverse effects are gastrointestinal disorders (nausea and vomiting) and central nervous system abnormalities. A rare adverse effect of it is renal failure [35]. For the

reduction of the use of ciprofloxacin, the combination studies have become more important for further clinical applications. *A. italicum* tuber extracts can be classified as promising natural sources. Table 5 summarizes the efficacies of the combinations with ciprofloxacin against *Pseudomonas aeruginosa*, *Bacillus cereus* and *Salmonella typhimurium* strains.

The E2 subfraction has strong synergistic interactions with ciprofloxacin against tested three pathogenic strains. According to our published data, E2 subfraction was rich in hydroxycinnamic acid-spermidine derivatives, phenolic acids, flavone glycosides, lignan derivatives [21]. The major groups were phenolic acids (ferulic acid and *p*-coumaric acid) and hydroxycinnamic acid-spermidine derivatives (N,N-dicaffeoyl-spermidine, caffeoyl-coumaroyl-spermidine, N,N-dicoumaroyl-spermidine, N-coumaroyl-N'-feruloyl-spermidine, N,N'-diferuoyl-spermidine).

Ferulic acid has broad-spectrum antibacterial activity against Gram-negative and Gram-positive bacteria. A recent study concluded that ferulic acid potentiates the antibacterial activity of quinolone-based antibiotics (ciprofloxacin and gemifloxacin) against *Acinebacter baumannii* AB5075 by increasing ROS generation, energy metabolism and electron transport chain activity with a concomitant decrease in glutathione [10]. These findings had good relation with previous studies [36-38]. Also, ferulic acid exerted antimicrobial activities against Gram (+) and Gram (-) bacteria such as *Listeria monocytogenes*, *Shigella sonnei, Campylobacter jejuni* [39-41].

p-Coumaric acid was tested previously for antibacterial potential against Gram-positive (*S. pneumoniae* ATCC49619, *B. subtilis* 9372, *S. aureus* 6538) and Gram-negative bacteria (*S. dysenteriae* 51302, *E. coli* ATCC25922, *S. typhimurium* 50013). The MIC values of *p*-coumaric acid were calculated 10-80 µg/mL against tested bacteria. The study resulted that *p*-coumaric acid killed pathogenic bacteria strain (*S. dysenteriae*, MIC 10 µg/mL) by provoking irreversible permeability changes in cell membrane, causing cells to lose the ability to maintain cytoplasm macromolecules, and binding to DNA to inhibit cellular functions [42]. Another study published that nisin/*p*-coumaric acid combination showed synergistic effects against planktonic cells of both the studied bacteria *B. cereus* MTCC1272 and *S. typhimurium* MTCC 3224. On the basis of FICI values, nisin/*p*-coumaric acid combination exhibited also synergistic antibiofilm activity [43]. Phenolic acids are well-known as antioxidative agents. It was recently reported that oxidative stress could contribute to the phenomenon of selection of pro-biofilm variants and H_2O_2-resistance, since ROS revealed to be an essential driving force for the selection of variants of *Pseudomonas aeruginosa* strain. New therapeutic strategies in targeting antioxidant pathways together with new antibacterial agents able to fight chronic infections caused by multidrug resistant bacterial strains [44]. Hence, ferulic acid and *p*-coumaric acid may act as antibacterial activity by exerting antioxidant effect.

Polyamines (PAs), mainly putrescine, spermidine, spermine, and its isomer thermospermine, are small polycationic molecules bearing amino groups. Some PA conjugates to hydroxycinnamic acids, and the products of PA oxidation (hydrogen peroxide and γ-aminobutyric acid) are required for different processes in plant development and participate in abiotic and biotic stress responses. The biological functions of PAs were initially associated with their ability to bind anionic macromolecules, and thus they were considered to be polycations with unique structural roles. Later studies showed that PAs also act as regulatory molecules in fundamental cellular processes, including cell division, differentiation, gene expression, DNA and protein synthesis, and apoptosis in many organisms. In plants, PAs are implicated in physiological processes, including organogenesis, embryogenesis, floral initiation and development, leaf senescence, pollen tube growth, fruit development and ripening, response to abiotic and biotic stresses [45,46]. Walters (2003) summarized some studies that hydroxycinnamic acid amides levels changed in plants responding to fungal infections [47]. In a dose-dependent manner, polyamines (cadaverine, putrescine, spermidine, and spermine) were reported that they increased the susceptibility of *P. aeruginosa* to 14 β-lactam antibiotics, chloramphenicol, nalidixic acid, and trimethoprim as demonstrated by a reduction in MIC of up to 64-fold [48]. A recent study results

concluded that phenolamines (di-*p*-coumaroyl-spermidine, *p*-coumaroyl-caffeoyl-hydroxyferuloyl-spermine, di-*p*-coumaroyl-hydroxyferuloyl-spermine, and tri-*p*-coumaroyl spermidine) showed protective effects on $HepG_2$ cells injured by AAPH. They could significantly reduce the reactive oxygen species, alanine aminotransferase and aspartate aminotransferase levels, and increase the superoxide dismutase and glutathione levels [49]. Hydroxycinnamic acid-spermidine derivatives in E2 subfraction may contributed antibacterial activity against tested bacteria.

4. CONCLUSION

For the health care systems as well as mankind, the infections of antibiotic-resistant bacteria are the most significant health and economic problem. This problem is growing, in the future, the use of antibiotics is still uncertain. Although a number of new chemotherapeutics have been produced, numerous antibiotic resistant bacteria have occurred. One the valuable sources of new and effective molecules as antibacterial agents is plants. Plant products were reported as directly antibacterial or as synergistic agents with antibiotics. In vitro combination studies have shown that plant products with different antibiotics have synergistic interactions. These findings encourage the possibility of development or designing new antibacterial agents for the prevention and treatment of infections.

Many studies have concluded that plant extracts including roots, stem, leaves, flowers and aerial parts have promising results against pathogenic microorganisms. This study is the first to report the synergistic antibacterial effects of *A. italicum* tuber extracts in combination with ciprofloxacin, a fluoroquinolone antibiotic. We obtained different interactions such as synergistic, additive, and indifferent between tuber extracts and ciprofloxacin. In the light of all findings obtained in this study, comments and assessments, it is considered that especially combinations of E2 with ciprofloxacin may be clarified in more detailed studies to assist in the discovery of new natural compounds that will encourage hope in terms of antibacterial treatment. When a number of scientific researches have confirmed the synergistic activity of plant extracts and antibiotics certainly, the next step was to investigate the mechanisms of the synergistic action. Further studies will be focused on the mechanisms of synergistic action.

Acknowledgements

Authors are very thankful to Prof. Dr. Hulusi Malyer (Uludag University, Faculty of Arts & Science, Department of Biology, 16059-Görükle, Bursa-Türkiye) for the identification of the plant material. Authors remember Prof. Dr. Malyer with respect, he lost his life because of Covid-19 infection.

This study was presented as an oral presentation at the 1st National Aroid Congress, Eskişehir, Türkiye (27-29 June 2019).

Author contribution

Concept: HGA, NK; Design: HGA, NK; Supervision: NK; Materials: HGA, NK; Data Collection and/or Processing: HGA, GÖ; Analysis and/or Interpretation: HGA, GÖ; Literature Search: HGA, GÖ; Writing: HGA, GÖ; Critical Reviews: HGA, NK.

Source of funding

This research was partially funded by Anadolu University Scientific Research Commission under grant no 1206S100 as a doctoral project.

Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. World Health Organization. Antibiotic resistance. Available at: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (Accessed on January 19, 2021).
2. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P&T. 2015;40(4):277.

3. Kuok CF, Hoi SO, Hoi CF, Chan CH, Fong IH, Ngok CK, Meng LR, Fong P. Synergistic antibacterial effects of herbal extracts and antibiotics on methicillin-resistant *Staphylococcus aureus*: A computational and experimental study. Exp Biol Med. 2017;242(7):731-743. https://doi.org/10.1177/1535370216689828

4. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomed. 2009;16(2-3):97-110. https://doi.org/10.1016/j.phymed.2008.12.018

5. Yam TS, Hamilton-Miller JM, Shah S. The effect of a component of tea (*Camellia sinensis*) on methicillin resistance, PBP2' synthesis, and *beta*-lactamase production in *Staphylococcus aureus*. J Antimicrob Chemother. 1998;42:211-216. https://doi.org/10.1093/jac/42.2.211

6. Nascimento GGF, Locatelli J, Freitas, FC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz J Microbiol. 2000;31:247-256. https://doi.org/10.1590/S1517-83822000000400003

7. Rafiq Z, Narasimhan S, Haridoss M, Vennila R, Vaidyanathan R. *Punica granatum* rind extract: Antibiotic potentiator and efflux pump inhibitor of multidrug resistant *Klebsiella pneumoniae* clinical isolates. Asian J Pharm Clin Res. 2017;10:1-5. https://doi.org/10.22159/ajpcr.2017.v10i3.16000

8. Bouarab-Chibane L, Forquet V, Lanteri P, et al. Antibacterial Properties of Polyphenols: Characterisation and QSAR (Quantitative Structure- Activity Relationship) Models. Front Microbiol. 2019;10:829. https://doi.org/10.3389/fmicb.2019.00829

9. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Front Microbiol. 2019;10:911. https://doi.org/10.3389/fmicb.2019.00911

10. Ibitoye OB, Ajiboye TO. Ferulic acid potentiates the antibacterial activity of quinolone-based antibiotics against *Acinetobacter baumannii.* Mic Path. 2019;126:393-398. https://doi.org/10.1016/j.micpath.2018.11.033

11. De Natale A, Pollio A. Plants species in the folk medicine of Montecorvino Rovella (inland Campania, Italy). J Ethnopharmacol. 2007;109:295-303. https://doi.org/10.1016/j.jep.2006.07.038

12. Cornara L, La Rocca A, Marsili S, Mariotti MG. Traditional uses of plants in the Eastern Riviera (Liguria, Italy). J Ethnopharmacol. 2009;125:16-30. https://doi.org/10.1016/j.jep.2009.06.021

13. Tuzlacı E, Alparslan DF. Turkish folk medicinal plants, Part V: Babaeski (Kırklareli). J Fac Pharm Ist Univ. 2007;39:11-23. Available at: https://dergipark.org.tr/tr/pub/iujfp/issue/577/5694

14. Genç GE, Özhatay N. An ethnobotanical study in Çatalca (European part of İstanbul) II. Turk J Pharm Sci. 2006;3(2):73-89.

15. Wright CA. Mediterranean Vegetables: A Cook's ABC of Vegetables and Their Preparation in Spain, France, Italy, Greece, Turkey, the Middle East, and North Africa with More Than 200 Authentic Recipes for the Home Cook. Harvard Common Press; 2001.

16. Luczaj L, Dolina K. A hundred years of change in wild vegetable use in Southern Herzegovina. J Ethnopharmacol. 2015;166:297-304. https://doi.org/10.1016/j.jep.2015.02.033

17. Della Greca M, Molinaro A, Monaco P, Previtera L. Two new lignan glucosides from *Arum italicum*. Heterocycl. 1993;36(9):2081-2086. https://doi.org/10.3987/COM-93-6424

18. Della Greca M, Fiorentino A, Molinaro A, Monaco P, Previtera L. Steroidal 5,6-epoxides from *Arum italicum*. Nat Prod Lett. 1993;2(1):27-32. https://doi.org/10.1080/10575639308043450

19. Della Greca M, Molinaro A, Monaco P, Previtera L. Dihydrobenzofuran neolignans from *Arum italicum*. Heterocycl. 1994;38(5):1099-1102. https://doi.org/10.3987/COM-94-6681

20. Della Greca M, Fiorentino A, Molinaro A, Monaco P, Previtera L. Hydroperoxysterols in *Arum italicum*. Nat Prod Lett. 1994;5(1):7-14. https://doi.org/10.1080/10575639408043928

21. Agalar HG, Akalın Çiftci G, Goger F, Kırımer N. Activity guided fractionation of *Arum italicum* Miller tubers and the LC/MS-MS profiles. Rec Nat Prod. 2018; 12(1):64-75. https://doi.org/10.25135/rnp.06.17.05.089

22. Quave CL, Plano LRW, Pantuso T, Bennett BC. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 2008;118:418-428. https://doi.org/10.1016/j.jep.2008.05.005

23. Yılmaz AD. Karaciğer ve safra rahatsızlıklarında kullanılan tıbbi bitkilerin antioksidan aktiviteleri üzerinde farmakognozik araştırmalar [master's thesis]. İstanbul: Marmara University; 2008.

24. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16. vol. 26–3; M7-A7, vol. 26–2; M2-A9, vol. 26–1. Wayne, PA: USA; 2006.

25. Demirci F, Güven K, Demirci B, Dadandı MY, Baser KHC. Antibacterial activity of two Phlomis essential oils against food pathogens. Food Cont. 2008;19(12):1159-1164. https://doi.org/10.1016/j.foodcont.2008.01.001

26. Van Vuuren SF, Suliman S, Viljoen AM. The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. Lett Appl Microbiol. 2009;48(4):440-446. https://doi.org/10.1111/j.1472-765X.2008.02548.x

27. Stanojevic D, Comic L, Stefanovic O, Solujic-Sukdolak S. *In vitro* synergistic antibacterial activity of *Salvia officinalis* L. and some preservatives. Arch Biol Sci. 2010;62(1):175-183. https://doi.org/10.2298/ABS1001167S

28. Jain SN, Vishwanatha T, Reena V, et al. Antibiotic synergy test: checkerboard method on multidrug resistant *Pseudomonas aeruginosa*. Int Res J Pharm. 2011;2(12):196-198.

29. Clinical and Laboratory Standards Institute. Clinical and Laboratory Standards Institute methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approve Standard M7-A7, CLSI, 7th ed, Wayne, PA: USA; 2006.

30. Goldberg JB, Pier GB. The role of the CFTR in susceptibility to *Pseudomonas aeruginosa* infections in cystic fibrosis. Trend Microbiol. 2000;8(11):514-520. https://doi.org/10.1016/S0966-842X(00)01872-2

31. Wolska K, Szweda P. Genetic Features of Clinical *Pseudomonas aeruginosa* Strains. Polish J Microbiol. 2009;58(3):255-260.

32. Fields PI, Swanson RV, Haidaris CG, Heffron F. Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. Proc Natl Acad Sci. 1986;83:5189-5193. https://doi.org/10.1073/pnas.83.14.5189

33. Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of Bacillus cereus infections. Microb Infect. 2000;2(2):189-198. https://doi.org/10.1016/S1286-4579(00)00269-0

34. El Demeiry M, Ali A, Abouleila Y, et al. Quantification and targeted detection of ciprofloxacin in dosage form and human urine by direct injection nano-electrospray ionization multi-stage mass spectrometry. Microchem J. 2020;153:104534. https://doi.org/10.1016/j.microc.2019.104534

35. Martin M, Boixeda R, Munoz A, Felip A. Ciprofloxacin as a cause of acute renal failure. Enferm Infect Microbiol Clin. 2020;38(8):402-403. https://doi.org/10.1016/j.eimc.2020.01.010

36. Wang X, Zhao X, Malik M, Drlica K. Contribution of reactive oxygen species to pathways of quinolone-mediated bacterial cell death. J Antimicrob Chemother. 2010;65:520-524. https://doi.org/10.1093/jac/dkp486

37. Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microb Drug Resist. 2013;19:256-265. https://doi.org/10.1089/mdr.2012.0244

38. Naz S, Ahmad S, Ajaz Rasool S, Asad Sayeed S, Siddiqi R. Antibacterial activity directed isolation of compounds from *Onosma hispidum*. Microbiol Res. 2006;161:43-48. https://doi.org/10.1016/j.micres.2005.05.001

39. Tsou MF, Hung CF, Lu HF, et al. Effects of caffeic acid, chlorogenic acid and ferulic acid on growth and arylamine N-acetyltransferase activity in *Shigella sonnei* (group D). Microbios. 2000;101:37-46.

40. Takahashi H, Kashimura M, Koiso H, Kuda T, Kimura B. Use of ferulic acid as a novel candidate of growth inhibiting agent against *Listeria monocytogenes* in ready-to-eat food. Food Cont. 2013;33(1):244-248. https://doi.org/10.1016/j.foodcont.2013.03.013

41. Ganan M, Martinez-Rodriguez AJ, Carrascosa AV. Antimicrobial activity of phenolic compounds of wine against *Campylobacter jejuni*. Food Cont. 2009;20:739-742. https://doi.org/10.1016/j.foodcont.2008.09.012

42. Lou Z, Wang H, Rao S, Sun J, Ma C, Li J. *p*-Coumaric acid kills bacteria through dual damage mechanisms. Food Cont. 2012;25(2):550-554. https://doi.org/10.1016/j.foodcont.2011.11.022

43. Bag A, Chattopadhyay RR. On the basis of fractional inhibitory concentration indices values, nisin/p-coumaric acid combination exhibited synergistic antibiofilm activity. Lett Appl Microbiol. 2017;65(5):366-372. https://doi.org/10.1111/lam.12793

44. Giacomini D, Musumeci R, Galletti P, et al. 4-alkyliden-azetidinones modified with plant derived polyphenols: antibacterial and antioxidant properties. Eur J Med Chem. 2017;140:604-614. https://doi.org/10.1016/j.ejmech.2017.09.048

45. Edreva AM, Velikova VB, Tsonev TD. Phenylamides in Plants. Russ J Plant Physiol. 2007;54(3):287-301. https://doi.org/10.1134/S1021443707030016

46. Tiburcio AF, Altabella T, Bitrian M, Alcazar R. The roles of polyamines during the lifespan of plants: from development to stress. Planta. 2014;240:1-18. https://doi.org/10.1007/s00425-014-2055-9

47. Walters DR. Polyamines and plant disease. Phytochem. 2003;64(1):97-107. https://doi.org/10.1016/S0031-9422(03)00329-7

48. Kwon DH, Lu CD. Polyamines Increase Antibiotic Susceptibility in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2006;50(5):1623-1627. https://doi.org/10.1128/AAC.50.5.1623-1627.2006

49. Zhang H, Liu R, Lu Q. Separation and Characterization of phenolamines and flavonoids from rape bee pollen, and comparison of their antioxidant activities and protective effects against oxidative stress. Molecules. 2020;25(6):1264. https://doi.org/10.3390/molecules25061264