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Contents

Arum italicum **Miller tuber extracts: evaluation of synergistic activities with ciprofloxacin against some pathogens 97**
Hale Gamze Ağalar, Gözde Öztürk, Neşe Kırımer

Volatile compositions of *Tilia platyphyllos* Scop. infusions by headspace-solid-phase microextraction (HS-SPME), antioxidant activity 107
Damla Kırcı, Gözde Öztürk, Betül Demirci

Essential oil composition and antimicrobial activity of *Glaucosciadium cordifolium* (Boiss.) Burtt. & Davis 112
Nagehan Saltan, Ayla Kaya, Gökalp İşcan, Betül Demirci

Focusing on the moderately active compound (MAC) in the design and development of strategies to optimize the apoptotic effect by molecular mechanics techniques 118
Asaf Evrim Evren, Demokrat Nuha, Leyla Yurttaş

Latest research about active pharmaceutical ingredient loaded Poly Lactic Acid-co-Glycolic Acid (PLGA) based drug delivery system in Türkiye........ 127
Berna Kaval, Fatma Dilara Şen, Kemal Kaya Batmaz, Meliha Ekinci, A. Alper Öztürk

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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

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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.

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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.

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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

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Research Article

Volatile compositions of *Tilia platyphyllos* Scop. infusions by headspace-solid-phase microextraction (HS-SPME), antioxidant activity

Damla Kırcı[1], Gözde Öztürk[2], Betül Demirci[2]

[1]Selçuk University, Faculty of Pharmacy, Department of Pharmacognosy, Konya, Türkiye.

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

Damla Kırcı
damla.kirci@selcuk.edu.tr

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ABSTRACT

The genus *Tilia* (Tiliaceae) represents 45 species, of which six species are European. *Tilia* sp. are simple, cordate or deciduous trees with long, silicate shaped, 5-valve fruits and fragrant flowers. It is used as a medicinal tea in traditional medicine for colds, coughs, and hypertension and as an antioxidant.

Within the scope of this research, *Tilia platyphyllos* Scop. in the culture form from Eskişehir was obtained, and infusions were prepared at different times (including 5 min., 10 min., 15 min., and 30 min., respectively). Headspace-solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used to examine the volatile components in the infusion extracts. Major volatile compounds of infusion extracts determined as *E)-β*-ocimene (15.7-45.9%) and limonene (11.7-33.4%), respectively. Also, terpinolene (26.0%) were identified as the main compounds for 30 min infusion. After then, the infusion extracts were lyophilized, and the antioxidant activity of the infusion extracts were performed by DPPH· radical scavenging effect. It was determined that inhibition percentages were relatively high in the concentration range of 10-0.02 mg/mL (5.60-72.45%).

Our first research was the chemical composition and biological activity of the time-dependent *T. platyphyllos* infusions.

Keywords: *Tilia platyphyllos* Scop., volatile compounds, infusion, antioxidant activity

1. INTRODUCTION

Tilia sp. (Tiliaceae) are trees with simple, cordate leaves or very tall, silicate-shaped, deciduous trees with 5-valve-blooming fruits and fragrant flowers. The genus *Tilia*is represented by 45 species, 6 of which are European. It is distributed in Central-Southern Europe, especially in Ukraine, France, Sweden and Northern Iran [1]. In Turkey, it grows in provinces such as Bolu, Zonguldak, Kastamonu, Çanakkale, Eskişehir, Isparta, Trabzon, İzmir, Kırklareli, Sakarya, Tekirdağ [2]. Generally, these species are widely grown in parks and gardens [3].

Due to its pleasant taste, linden or dried flower states of the linden tree (*T. cordata* Miller or *T. platyphyllos* Scop.) are traditionally often used in the form of herbal tea. It has been determined that *Tilia* species contain essential oil, mucilage, condensed tannins, procyanidin dimers, flavonoids, phenolic acids, amino acids, carbohydrates and saponins [4]. Thanks to these compounds it carries, Commission

E has approved the use of linden flower in coughs associated with colds and colds [5]. The British Herbal Compendium reported that it is used in upper respiratory tract diseases, colds, cough, hypertension and anxiety [6].

In this study, *Tilia platyphyllos* Scop. in the culture form from Eskişehir was obtained, and infusions were prepared at different times (including 5 min., 10 min., 15 min. and 30 min., respectively). Headspace-solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used to examine the volatile compounds in the infusion extracts.

2. MATERIALS AND METHODS

2.1. Plant material

The *Tilia platyphyllos* material was collected in 2019 from Eskisehir province, Turkey. The plant was identified by Prof. Dr. Yavuz Bülent KÖSE (Anadolu University, Department of Pharmaceutical Botany). The sample is kept in Anadolu University, the Herbarium of Pharmacy Faculty with archive number 52. The Inflorescences of the plant was dried in the shade.

2.2. Extraction

Distilled water boiled at 80°C was added to the dried flowers. It was kept in different brewing times (5 min., 10 min., 15 min. and 30 min., respectively), filtered and infusions were prepared. After lyophilization, the infusions were used in biological activity studies.

2.3. Chemical composition

2.3.1. Headspace-Solid Phase Microextraction (HS-SPME)

Headspace-solid phase microextraction (HS-SPME) is a simple to use and solvent-free extraction technique. It is a sensitive, fast and inexpensive method that is widely used in the analysis of volatile components. It is a method based on the adsorption of volatile components onto the fiber coated with a polymeric stationary phase and the heat desorption of the components attached to the fiber at the gas chromatography injection port. Volatile components in the sample are adsorbed onto the fiber from the headspace of the samples, either directly or in a closed environment. When equilibrium is reached, usually after a period of 2-30 minutes, the fiber containing the adsorbed components is removed from the sample. These components are recovered by thermal desorption at a GC injector port and analyzed with the appropriate column and detector [7].

2.3.2. Gas Chromatography (GC)/Mass Spectrometry (MS)

GC/MS conditions were described previously. Identification of the volatile components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC-MS Library, MassFinder 4.0 Library), and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data was used for the identification as also previously reported [7,8].

2.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

Serial dilutions were carried out with stock solutions (10 mg/mL) of the infusions to obtain the concentrations of 0.5-0.001 mg/mL. Diluted solutions were mixed with DPPH· and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm at room temperature using a microplate spectrophotometer. The experiment was performed three times and average absorption was noted for each concentration. Ascorbic acid was used as a positive control. The percentage of inhibition was calculated using equation [9].

$A_{control}$: Absorbance of control

A_{sample}: Absorbance of sample

$$\text{Percentage Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The chemical compositions of the infusions were analyzed by gas chromatography/mass spectrometry (GC/MS) methods. The results of the analyzes were listed in Table 1.

(*E*)-*β*-Ocimene was determined as the major compound found in common in each sample (43.0%, 45.9%, 15.7%, 18.7%, 41.2%). Benzene acetonitrile (10.7%) was found as major component for 5 min. infusion of *T. platyphyllos*, Limonene (11.7%) and benzene acetonitrile (7.7%) were found as major components for 10 min infusion. Limonene (22.5%) was found as major constituent for 15 min. infusion. Limonene (34.4%) and terpinolene (26.0%) were defined as the main compounds for 30 min infusion. The percentage of limonene and terpinolene increased in the 30 min infusion, and their structure is cyclic monoterpenes. Also, ocimene, which is an acyclic monoterpene, was decreased depending on the time in the infusions.

In previous research, the essential oils of the inflorescences and leaves of *T. platyphyllos* were obtained from five different provinces in Kosovo. *n*-Heneicosane (3.59-13.50%), *n*-pentacosane (13.90-19.61%), *n*-nanocosane (1.49-13.98%), and *n*-nonanal (3.30-8.97%) were identified as the main components [10]. In the other research, tricosan (18,12%), heneicosan (10,06%), and pentacosan (6,08%) were found to be the main components of essential oil of *T. platyphyllos* linden blossoms [11]. A high amount of hydrocarbons (47.5–66.5%) was found in the essential oil of *T. platyphyllos* flowers [12].

The essential oil and the infusion of *T. platyphyllos* flowers were extracted with dichloromethane and were analyzed by GC/MS. The main component of essential oil was 2-phenyl ethanol with 26.07%. In the infusion of *T. platyphyllos*, 2-phenyl ethanol and 2-phenylethyl butanoate represented 29.48% and 12.11%, respectively [13].

A high amount of hydrocarbons was identified in the essential oils of *T. platyphyllos* flowers, while major components of monoterpenes were found in the infusions of *T. platyphyllos* flowers.

3.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) radical scavenging activity

The antioxidant capacity of infusions was tested. The % inhibition values of the samples and positive control ascorbic acid were given in Figure 1. It was

Table 1. Volatile components of *Tilia platyphyllos* Scop.

RRI[a]	**Component**	**A %**[b]	**B %**	**C %**	**D %**
1203	Limonene	tr[c]	**11.7**	**22.5**	**34.4**
1266	(*E*)-*β*-Ocimene	**43.0**	**45.9**	**15.7**	**18.7**
1290	Terpinolene	-	-	-	**26.0**
1360	1-Hexanol	3.8	3.5	-	-
1452	*α*-*p*-Dimethyl styrene	5.3	2.7	-	2.8
1553	Linalool	-	-	1.5	1.4
1719	Borneol	-	-	0.5	-
1957	Benzene acetonitrile	**10.7**	**7.7**	-	-
2091	*cis*-Methyl isoeugenol	2.3	-	-	-
2400	Tetracosane	-	1.8	-	-
2500	Pentacosane	-	2.5	-	-
2600	Hexacosane	-	2.9	-	-
2700	Heptacosane	-	3.6	-	-
2900	Nonacosane	-	3.7	-	-
	Total	**85.6**	**92.6**	**75.9**	**96.5**

[a]RRI: Relative retention indices calculated against n-alkanes; [b]%, calculated from the TIC; chromatograms; [c]tr: Trace amount (<0.1%);
A: 5 min. infusion; B: 10 min. infusion; C: 15 min. infusion; D: 30 min. infusion.

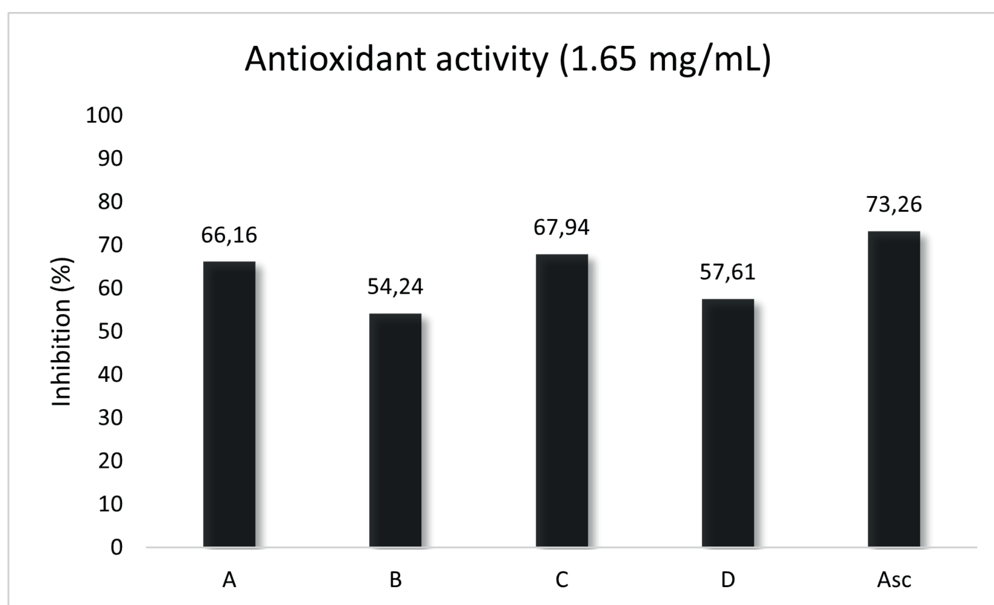

Figure 1. % Inhibition values of *Tilia platyphyllos* Scop. infusions
A: 5 min. infusion; B: 10 min. infusion; C: 15 min. infusion; D: 30 min. infusion; Asc.: Ascorbic acid.

studied in the concentration range of 10-0.02 mg/mL. According to the antioxidant activity results, all infusions showed as high effects as ascorbic acid.

In previous study, the antioxidant effects of *T. platyphyllos* infusions and methanol extract were determined by the DPPH· radical scavenging method. It has been reported that there is lower antioxidant activity in infusions (0.03 mmol/g) compared to the extract and essential oil [14]. In another study, it was determined that the antioxidant activity of ethanol extracts (IC_{50} =105±1 µg/mL) was high [15].

4. CONCLUSION

According to our results, the volatile components of *T. platyphyllos* infusions were rich in monoterpenes. The infusion of *T. platyphyllos,* 15 min., was found to have high antioxidant activity. To the best of our knowledge, the volatile components of *T. platyphyllos* infusions were evaluated for the first time by using the HS-SPME method. Also, the changes in the contents depending on the infusion times were revealed.Our research was the first on the biological activity of time-dependent infusions.

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Author contribution

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

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Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Mabberley DJ. The Plant-Book. Cambridge: Cambridge University Press; 1997. ISBN: 0521414210.

2. Turkish Plants Data Service. Available at: https://www.tubives.com (Accessed on November 17, 2022)

3. Browicz K. Flora Europaea. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, editors. *Tilia* L. Cambridge: Cambridge University Press; 1976. p. 247-248. ISBN: 0521087171.

4. Yayalaci Y, Celik I, Bati B. Hepatoprotective and antioxidant activity of linden (*Tilia platyphyllos* L.) infusion against ethanol-induced oxidative stress in rats. J Membr Bio. 2014;247(2):181-188. https://doi.org/10.1007/s00232-013-9622-z

5. Blumenthal M, Goldberg A, Brinckmann J. Herbal Medicine. Expanded Commission E Monographs. Austin, TX: American Botanical Council; 2000. ISBN: 0967077214.

6. Bradley P. British Herbal Compendium. Bournemouth: British Herbal Medicine Association; 1992. ISBN: 9780903032124.

7. Demirci B, Demirci F, Başer KHC. Headspace-SPME and hydrodistillation of two fragrant *Artemisia* sp. Flavour Fragr J. 2005;19:395-398. https://doi.org/10.1002/ffj.1426

8. Demirci B, Kırcı D, Öztürk G, Demirci F. The Effect of extraction time on Origanum onites L. infusions and essential oils–biological evaluation, statistical principal component and hierarchial cluster analyses. Chem Biodiversity. 2022;19(12):e202200482. https://doi.org/10.1002/cbdv.202200482

9. Demirci F, Demirci B, Gürbüz İ, Yeşilada E, Başer KHC. Characterization and biological activity of *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek essential oils. Turk J Biol. 2009;33(2):129-136. https://doi.org/10.3906/biy-0808-1

10. Kelmendi N, Mustafa B, Zahiri F, Nebija D, Hajdari A. Essential oil composition of *Tilia platyphyllos* Scop. collected from different regions of Kosovo. Rec Nat Prod. 2020;14(5):366-371. http://doi.org/10.25135/rnp.171.19.11.1490

11. Prączko A, Góra J. The chemical composition of essential oil distillated from Linden blossoms. Żywność. 2000;4(25):90-94.

12. Toker G, Baser KHC, Kürkçüoğlu M, Özek T. The composition of essential oils from *Tilia* L. species growing in Turkey. J Essent Oil Res. 1999;11(3):369-374. https://doi.org/10.1080/10412905.1999.9701158

13. Rădulescu V, Oprea E. Analysis of volatile compounds of officinal Tiliae flos by gas–chromatography coupled with mass spectrometry. Farmacia. 2008;56(2):129-138.

14. Pogačnık L, Ulrih NP. Determination of antioxidants in Medicinal herbs. Bulletin of the Transilvania University of Braşov Series VI: Medical Sciences. 2011;4(53):95-102.

15. Jabeur I, Martins N, Barros L, et al. Contribution of the phenolic composition to the antioxidant, anti-inflammatory and antitumor potential of *Equisetum giganteum* L. and *Tilia platyphyllos* Scop. Food Funct. 2017;8:975-984. https://doi.org/10.1039/C6FO01778A

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Research Article

Essential oil composition and antimicrobial activity of *Glaucosciadium cordifolium* (Boiss.) Burtt. & Davis

Nagehan Saltan[1], Ayla Kaya[1], Gökalp İşcan[2], Betül Demirci[2]

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

✉ Nagehan Saltan
ndagdeviren@anadolu.edu.tr

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ABSTRACT

Glaucosciadium cordifolium (Boiss.) Burtt. & Davis, which is a monotypic species naturally grown in Turkey belonging to the Apiaceae (Umbelliferae) family. In this study, the essential oil obtained from *G. cordifolium* was analyzed and evaluated for its antimicrobial effects. In chemical studies, the analyzes of essential oil compounds obtained from the aerial parts of plants by hydrodistillation were carried out with GC-FID and GC-MS. In total, 23 compounds were identified making up 98.7% of the total volatile constituents. Sabinene (42.1%), α-pinene (17.1%), and α-phellandrene (10.1%) were found as the main constituents in the oil.

In antimicrobial studies, the anticandidal and antibacterial effects of essential oils were tested against 14 pathogenic microorganisms according to the standard protocols of the Clinical Laboratory Standards Institute (CLSI). It has been determined that essential oils have a very weak inhibitory effect when compared with standard antibacterial agents. MIC values of 1800, 3600 µg/Ml, and higher were determined. In addition, it was determined that it showed inhibitory effects, especially against *C. krusei* at a concentration of 250 µg/mL, with MIC values ranging from 250 to 2000 µg/mL.

Keywords: Antimicrobial activity, Apiaceae, essential oil, GC-MS, *Glaucosciadium cordifolium*

1. INTRODUCTION

The Flora of Turkey states that there is just one taxon of the genus *Glaucosciadium* Burtt & Davis in Turkey and two taxa worldwide [1]. *G. cordifolium*, known as “çağşır otu, sakar otu” is used as an aphrodisiac in traditional medicine by crushing its roots and chewing with honey [2]. It has also been reported that the roots and leaves are crushed and used for stomach ailments [3]. Regarding the ethnobotanical use of the plant, there are no known reports of toxicity and/or side effects. In Turkey, it spreads in Central Anatolia, the Mediterranean region, and Cyprus. Creeping, perennial, glaucous, erect, 34-180 cm long and 3-10 mm diameter, glabrous plant. Stem branched and pronounced stripes, with an acrid smell when bruised [4]. Başer et al. (2000) stated that there was 0.7% essential oil in the aerial parts of the samples taken from the Konya and that the main components that make up the essential oil were limonene (39.7%), α- pinene (12.3%) and β-pinene (10.3%) [5]. In the study conducted by Karadoğan et al. (2015), the essential oil components of the aerial parts of *G. cordifolium* grown in different locations (Isparta and Burdur) were defined. In essential oils, 1-limonene, α-pinene, and cis-ocimene were determined as the main components [6]. In another study, the main components of the essential oil

prepared from the aerial parts of the plant collected from Karaman-Ermenek were found to be α-pinene (27.7%), β-pinene (15.7%), (Z)-β-ocimene (14%), sabinene (7%) has been reported [7]. In previous studies, it has been reported that there are significant differences between the components that make up the essential oil of *G.cordifolium* and the proportional distributions of the components, depending on the locations where they were collected [5-7].

Our study deals with the analysis of essential oil isolated from the aerial parts of *G. cordifolium* growing in Kütahya, Turkey. Additionally, using microdilution techniques, the antibacterial properties of the aforementioned volatile oil were examined.

2. MATERIALS AND METHODS

2.1. Plant Material

G. cordifolium was collected at the flowering stage in July, in Uşak-Gediz road 3rd km by the roadside (Figure 1). A voucher specimen is also deposited at the Herbarium of Faculty of Pharmacy of the Anadolu University, Eskişehir, Turkey (ESSE No: 15001).

2.2. Isolation of Essential Oil

A Clevenger apparatus was used to hydrodistilled the air-dried aerial components after they had been roughly crushed. Before analysis, the obtained oil was kept at a low temperature in a dark vial.

2.3. GC-MS Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 m film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60ºC for 10 min and programmed to 220ºC at a rate of 4 ºC/min, and kept constant at 220 ºC for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted at 40:1. The injector temperature was set at 250ºC. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

2.4. GC Analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300ºC. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 1.

2.5. Identification of the essential oil components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to the series of nalkanes. Computer matching against commercial [8,9] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [10,11] was used for the identification.

2.6. Antimicrobial Activity

The minimum inhibitory (MIC) concentrations of the essential oil was determined using standard protocols [12,13]. Anticandidal effects of essential oil obtained from *G. cordifolium* on 7 *Candida* species (*C. albicans, C. utilis, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis,* and *C. zeylanoides*) were identified. Moreover, the antibacterial activity of *G. cordifolium* essential oil against 7 bacteria (*Serratia marcescens*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus, Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus epidermidis*) was evaluated. Positive controls for anticandidal testing included ampicillin and chloramphenicol, whereas, for antibacterial tests, ketoconazole and amphotericin were employed**.**

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the essential oil

GC and GC/MS analyses were carried out simultaneously in the essential oil obtained after

distillation. As a result of the analysis of the essential oil obtained from the aerial parts of *G. cordifolium* with a yield of 0.4% (v/w), 23 volatile components were determined, corresponding to 98.7% (Table 1). It was determined that the main components of the essential oil obtained were sabinene (42.1%), α-pinene (17.1%), and α- phellandrene (10.1%). Sabinene is a monoterpene isolated from the essential oil of many plants including medicinal herbs [14-16]. Increasing data suggest that sabinene may be used as a treatment for a number of illnesses [17-20]. Sabinene possesses biological characteristics like anti-inflammatory and anti-fungal capabilities [21,22]. It has been shown that sabinene may have antioxidant [23-25] and anti-radical properties in respect to DPPH radicals [26]. Furthermore, sabinene decreases the increased level of reactive oxygen species (ROS) in myotubes under starvation [27].

Figure 1. *G. cordifolium*

On the other hand, it is also known that essential oils of popular plants can reveal toxic properties that many people are unaware of. Studies reporting that Savin oil (50% sabinyl acetate) obtained from *Juniperus sabina* L. is embryo-fetotoxic, abortifacient, and hepatotoxic recommend that caution should be taken when using the plant. [28].

Table 1. The chemical components of *G. cordifolium* essential oil

RRI	Compounds	%
1032	α-Pinene	17.1
1035	α-Thujene	0.4
1118	β-Pinene	0.6
1132	Sabinene	42.1
1174	Myrene	4.1
1176	α- Phellandrene	10.1
1188	α-Terpinene	1.1
1203	Limonene	2.4
1218	β-phellandrene	4.3
1255	γ-Terpinene	2.3
1280	p-Cymene	4.3
1290	Terpinolene	0.9
1474	trans-Sabinene hydrate	0.9
1483	Octyl acetate	1.9
1556	cis-Sabinene hydrate	0.7
1571	trans-p-Menth-2-en-1-ol	0.4
1611	Terpinene-4-ol	4.8
1638	cis-p-Menth-2-en-1-ol	0.2
1690	Krypton	0.3
1706	α-Terpineol	0.2
1823	p-Mentha 1(7),5-dien-2-ol	0.3
2209	T-Muurolol	0.2
2239	Carvacrol	0.2
	Monoterpenes	89.7
	Oxygenated terpenes	8
	Oxygenated sesquiterpenes	0.2
	Others	1.9
	Total	98.71

RRI: Relative retention indices calculated against n-alkanes; %: calculated from the FID chromatograms; tr: Trace (<0.1 %). Identification method (IM): tR, identification based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

In the study conducted by Maral (2022), the major components of the essential oil obtained from the aerial parts of *G. cordifolium* collected from Ermenek district of Karaman were found to be 1-phellandrene (9.23-34.08%), α-pinene (10.23-31.95%), dl-limonene. (10.39-22.21%) and cis-ocimene (6.84-12.45%) have been reported [29]. In another study (2019), a total of 62 volatile compounds were detected in *G. cordifolium* essential oil collected from Karaman-Ermenek. The three main ingredients in the essential oil of the aerial part are β-pinene (15.7%), (Z)-β-ocimene (14%), and sabinene (7%) while the three principal constituents in the essential oil of fruits are sabinene (10.1%), β-pinene (10.1%) and α-phellandrene (5.3%). Furthermore, in the essential oil obtained from the root, hexadecane (12.2%), tetradecane (11.9%), and octadecane (7.4%) were reported, respectively [7]. The main elements of the essential oil extracted from the aerial parts and those reported in other studies have similarities, according to the findings. The possible differences between the volatile components are thought to be due to the location and ecological differences of the plants.

3.2. Antimicrobial activity

The antibacterial effect of the essential oil obtained from *G. cordifolium* on seven different bacteria strains were investigated. It has been determined that essential oils have a very weak inhibitory effect when compared with standard antibacterial agents. MIC (minimum inhibitory concentration) values of 1800, 3600 µg/mL, and higher were determined (Table 2).

In anticandidal effect studies, *G. cordifolium* essential oil is effective at lower concentrations, especially against *C. krusei* (125 µg/mL), *C. parapsilosis*, *C. tropicalis* and *C. zeylanoides*, with MIC values ranging from 250 to 2000 µg/mL. was determined (Table 3).

In a study in which the antimicrobial effect of the essential oil of *G. cordifolium* was tested against some pathogenic bacteria, it was reported that *Listeria monocytogenes* (0.156 mg/mL) was more sensitive to essential oils. Sabinene, one of the main components of essential oil obtained from *G. cordifolium*, has been reported to inhibit the growth

Table 2. Antibacterial effects of *G. cordifolium* essential oil (MIC, µg/mL)

Microorganisms	EO	Ampicillin	Chloramphenicol
Serratia marcescens NRRL B-2544	>3600	32	16
Pseudomonas aeruginosa ATCC 10145	>3600	32	8
Salmonella typhimurium ATCC 14028	>3600	0.5	8
Staphylococcus aureus ATCC 43300	>3600	1	16
Escherichia coli ATCC 8739	>3600	1	2
Listeria monocytogenes ATCC 19111	1800	1	8
Staphylococcus epidermidis ATCC 14990	1800	1	1

EO: Essential oil

Table 3. Anticandidal effects of *G. cordifolium* essential oil (MIC, µg/mL)

Microorganisms	EO	Ampicillin	Chloramphenicol
Candida albicans ATCC 10231	2000	0.5	0.5
C. utilis NRRL Y-900	2000	0.5	0.125
C. zeylanoides NRRL Y-1774	500	1	0.5
C. glabrata ATCC 66032	500	0.5	0.5
C. tropicalis ATCC 750	500	1	0.5
C. parapsilosis ATCC 22019	500	0.5	2
C. krusei ATCC 6258	250	0.25	2

EO: Essential oil

of various fungi *in vitro*, including several *Candida*, *Trichophyton,* and *Aspergillus* species (MIC = 0.16-5 µl/ml) [22]. Among the tested bacteria in this present study, *L. monocytogenes* and *S. epidermidis* were the more sensitive to the essential oils, while the others appeared to be more resistant. Additionally, it has been established that the essential oil possesses anti-*Candida* effects, particularly against *C. krusei*. The chemical composition of the plant is assumed to be significantly influenced by climatic and geographic factors. The results imply that shifting plant locations may affect biological activity by changing the phytochemistry of plants.

In conclusion, to the best of our knowledge, this is the first report of volatiles and *in vitro* antimicrobial activities of *G. cordifolium* collected from this locality.

4. CONCLUSION

In previous studies, it has been reported that plants in the Apiaceae family are mostly used for digestive system diseases, hemorrhoids, diabetes, aphrodisiac and sedative [3]. *G. cordifolium* is one of the species used for these purposes. Our research revealed that the essential oil isolated from the aerial parts of *G. cordifolium* had a significant amount of monoterpene hydrocarbons. The major class of secondary metabolites in plants, monoterpenes are present in a wide range of plants, including those in the Apiaceae family. The compounds have antioxidative, antibacterial, sedative, and anti-inflammatory properties, which is the reason they are frequently used in pharmaceuticals and medicine. Nevertheless, long-lasting studies have revealed their toxic properties. Although the majority of monoterpene compounds are safe for use in food and medicine for humans, some of them have the potential to be harmful in certain doses or under specific conditions (such as pregnancy). Numerous monoterpenes can exhibit a variety of hazardous features, including genotoxic, allergic, neurotoxic, and embryotoxic effects.

Additionally, the antibacterial activity of the essential oil was moderate. The findings of this study also suggested that this plant's essential oil might be employed as an antimicrobial component to ensure the safety of foodborne pathogens.

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Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: NS, AK; Design: NS, AK; Supervision: AK; Materials: NS; Data Collection and/or Processing: NS, AK; Analysis and/or Interpretation: NS, Gİ, BD; Literature Search: NS; Writing: NS, AK, Gİ, BD; Critical Reviews: AK.

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Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Davis PH. Flora of Turkey and the East Aegean Islands. Vol. 4. Edinburgh, UK: Edinburgh University Press; 1982. p. 514.
2. Ozhatay N, Kocak S. Plants used for medicinal purposes in Karaman Province (Southern Turkey). Istanbul J Pharm. 2011;41:75-89.
3. Bulut G, Tuzlacı E, Doğan A, Şenkardeş İ. An ethnopharmacological review on the Turkish Apiaceae species. J Fac Pharm Istanbul. 2014;44(2):163-179. Available at: https://dergipark.org.tr/tr/pub/iujfp/issue/585/5875
4. Saltan N, Kaya A, Dinc M, Doğu S. Morpho-anatomical and palynological studies on *Glaucosciadium cordifolium* (Apiaceae) from Turkey. Istanbul J Pharm. 2021;51(3):403-410. https://doi.org/10.26650/IstanbulJPharm.2021.909570

5. Baser KHC, Özek T, Demirci B, Duman H. Composition of the essential oil of *Glaucosciadium cordifolium* (Boiss.) Burtt et Davis from Turkey. Flavour Fragr J. 2000;15(1):45-46. https://doi.org/10.1002/(SICI)1099-1026(200001/02)15:1<45::AID-FFJ867>3.0.CO;2-L
6. Karadoğan T, Şanlı A, Tosun B, Özçelik H. Essential Oil Content and Composition of *Glaucosciadium cordifolium* (Boiss.) Burtt & Davis from Türkiye/Lakes Region. BIBAD. 2015;8(1):35-39.
7. Karadağ AE, Demirci B, Çeçen Ö, Tosun F. Chemical characterization of *Glaucosciadium cordifolium* (Boiss.) B. L. Burtt & P. H. Davis essential oils and their antimicrobial, and antioxidant activities. Istanbul J Pharm. (2019);49 (2): 77-80. https://doi.org/10.26650/IstanbulJPharm.2019.19013
8. McLafferty FW, Stauffer DB. The Wiley/NBS Registry of Mass Spectral Data. New York: Wiley and Sons; 1989.
9. Koenig WA, Joulain D, Hochmuth DH. Terpenoids and related constituents of essential oils. MassFinder 3. Convenient and Rapid Analysis of GC-MS. 2004.
10. Joulain D, Koenig WA. The Atlas of Spectra Data of Sesquiterpene Hydrocarbons. Hamburg: EB-Verlag; 1998.
11. ESO 2000. The complete database of essential oils. The Netherlands: Boelens Aroma Chemical Information Service (BACIS); 1999.
12. CLSI (NCCLS) M27-A2, 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, second edition.
13. CLSI (NCCLS) M7-A7, 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, seventh edition.
14. Maya KM, Zachariah TJ, Krishnamoorthy B. Chemical composition of essential oil of nutmeg (*Myristica fragrans* Hout) accessions. J Species Aromat Crops. 2004;13:135-139.
15. Rouatbi M, Duquenoy A, Giampaoli P. Extraction of the essential oil of thyme and black pepper by superheated steam. J Food Eng. 2007;78:708-714. https://doi.org/10.1016/j.jfoodeng.2005.11.010
16. Sieniawska E, Swiatek L, Rajtar B, Kozioł E, Polz-Dacewicz M, Skalicka-Wozniak K. Carrot seed essential oil-Source of carotol and cytotoxicity study. Ind Crops Prod. 2016;92:109-115. https://doi.org/10.1016/j.indcrop.2016.08.001
17. Sieniawska E, Sawicki R, Swatko-Ossor M, et al. The effect of combining natural terpenes and antituberculous agents against reference and clinical *Mycobacterium tuberculosis* strains. Molecules. 2018;23:176. https://doi.org/10.3390/molecules23010176
18. Sruthi D, John ZT, Leela NK, Jayarajan K. Correlation between chemical profiles of black pepper (*Piper nigrum* L.) var. Panniyur-1 collected from different locations. J Med Plants Res. 2013;7:2349-2357. https://doi.org/10.5897/JMPR2013.4493
19. Viña A, Murillo E. Essential oil composition from twelve varieties of basil (*Ocimum* spp) grown in Colombia. J Braz Chem Soc. 2003;14:744-749. https://doi.org/10.1590/S0103-50532003000500008
20. Zhao T, Solheim H, Langström B, Borg-Karlson AK. Storm-induced tree resistance and chemical differences in Norway spruce (*Picea abies*). Ann For Sci. 2011;68:657-665. https://doi.org/10.1007/s13595-011-0049-3
21. Yamasaki Y, Kunoh H, Yamamoto H, Akimitsu K. Biological roles of monoterpene volatiles derived from rough lemon (*Citrus jambhiri* Lush) in citrus defense. J Gen Plant Pathol. 2007;73:168-179. https://doi.org/10.1007/s10327-007-0013-0
22. Valente J, Zuzarte M, Gonçalves MJ, et al. Antifungal, antioxidant and anti-inflammatory activities of *Oenanthe crocata* L. essential oil. Food Chem Toxicol. 2003;62:349-354. https://doi.org/10.1016/j.fct.2013.08.083
23. Quirog, PR, Asensio CM, Nepote V. Antioxidant effects of the monoterpenes carvacrol, thymol and sabinene hydrate on chemical and sensory stability of roasted sunflower seeds. J Sci Food Agric. 2015;95:471-479. https://doi.org/10.1002/jsfa.6744
24. Zheljazkov VD, Astatkie T, Jeliazkova EA, Heidel B, Ciampa L. Essential oil content, composition and bioactivity of *Juniper* species in Wyoming, United States. Nat Prod Commun. 2017;12:201-204.
25. Zheljazkov VD, Astatkie T, Jeliazkova EA, Schlegel V. Distillation time alters essential oil yield, composition, and antioxidant activity of male *Juniperus scopulorum* trees. J Oleo Sci. 2012;61:537-546. https://doi.org/10.5650/jos.61.537
26. Bua-in S, Paisooksantivatana Y. Essential oil and antioxidant activity of *Cassumunar ginger* (Zingiberaceae: *Zingiber montanum* (Koenig) Link ex Dietr.) collected from various parts of Thailand. Kasetsart J. 2009;43:467-475.
27. Ryu Y, Lee D, Jung SH, et al. Sabinene Prevents Skeletal Muscle Atrophy by Inhibiting the MAPK-MuRF-1 Pathway in Rats. Int J Mol Sci. 2019;20(19):4955. https://doi.org/10.3390/ijms20194955
28. Pages N, Fournier G, Chamorro G, Salazar M, Paris M, Boudene C. Teratological evaluation of *Juniperus sabina* essential oil in mice. Planta Med. 1989;55(2):144-146. https://doi.org/10.1055/s-2006-961908
29. Maral H. Composition of essential oil and antioxidant properties of *Glaucosciadium cordifolium* (Boiss.) B.L. Burtt and PH. Davis plant organs growing wild in Turkey. Bangladesh J Bot. 2022;51(3):573-579. https://doi.org/10.3329/bjb.v51i3.62004

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Research Article

Focusing on the moderately active compound (MAC) in the design and development of strategies to optimize the apoptotic effect by molecular mechanics techniques

Asaf Evrim Evren[1,2], Demokrat Nuha[1,3,4], Leyla Yurttaş[1]

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

[2]Bilecik Seyh Edebali University, Vocational School of Health Services, Department of Pharmacy Services, Bilecik, Türkiye.

[3]Eskişehir Technical University, Faculty of Science, Department of Chemistry, Eskişehir, Türkiye.

[4]University of Business and Technology, Faculty of Pharmacy, Prishtina, Kosovo.

✉ Asaf Evrim Evren
asafevrimevren@anadolu.edu.tr

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ABSTRACT

Today, chemotherapeutic agents are mostly used to fight cancer in clinics. But even though they have selectivity for cancer cells, their mechanism of action could result in necrosis. Therefore, we aimed to suggest new design strategies using a moderately active compound (MAC) to get better activity and increase the apoptotic effect in this study. Although MAC, previously synthesized and evaluated for its anticancer properties, has been marked as a moderately active compound, it has let us develop new molecules using its molecular core supported by molecular docking and molecular dynamics simulation. The caspase-3 enzyme was subjected to density functional theory (DFT), docking, and molecular dynamics simulation studies, and the results were analyzed to better understand the structure-activity relationship (SAR); thus, new design strategies were proposed.

Keywords: Apoptosis, DFT, molecular dynamics simulation, thiazole, piperazine

1. INTRODUCTION

Drug research and development studies are a series of sensitive processes that involve time-consuming and expensive studies [1]. The main goal is, of course, to find an effective compound that can be used clinically. Therefore, many pharmaceutical and medicinal chemists first focus on determining potential compounds that can be applied to organisms [2-5]. This process can be performed in many different ways, such as the rule of five elimination [6], developing pharmacophore hypotheses [7], using the mimesis method [8], *etc*. Whatever method is used, eventually the designed compounds are synthesized and then tested for their activity. Also, considering recent studies [9, 10], eliminating compounds through the "rule of five" may sometimes miss some valuable compounds (drugs that violate the rule of five). Therefore, if the basic theory, "lock and key model" is working, the compound should be chosen and then should be evaluated/improved for the unfavorable parts of the compound.

In 2019, our study group designed and synthesized a series of thiazole-bearing piperazine derivatives using the mimesis method [11]. Anticancer activity of the synthesized compounds was evaluated against C6 rat glioma and A549 adenocarcinomic human alveolar basal epithelial cell lines. According to the results of the study, C6 cells were more sensitive to active compounds than A549 cells. After that, potential analogs were investigated for

their apoptotic effects against both cell lines. The results showed *N*-(4-(pyridin-4-yl)thiazol-2-yl)-2-(4-(pyrimidin-2-yl)piperazin-1-yl)acetamide (a moderately active compound, **MAC**) has a positive impact on apoptosis, especially on A549 cells. As mentioned in that study, the design of the compounds is based on dasatinib and imatinib. Therefore, **MAC** has been investigated deeply for its mechanism of action. Similar to the effects of dasatinib and imatinib, the caspase-3 enzyme should be stimulated to result in an apoptotic effect; hence, we focused on this enzyme and clarified the molecular mechanics.

Briefly, in this study, we aimed to offer new design strategies that can be useful approaches to designing and developing original compounds using molecular mechanics studies.

2. MATERIALS AND METHODS

2.1. DFT Studies

Theoretical analyses for our MAC were carried out using the Gaussian 09 W package [12] and GaussView 5.0 [13] molecular visualization tools. Using previously published procedures, the DFT study method was applied to MAC [14-16]). It is also critical to analyze charge transfer through intramolecular interactions. To explain this parameter, the energy levels of the examined compound's HOMO and LUMO orbitals must be time-dependent (TD). HOMO-LUMO energy values were used to compute the various chemical activity parameters.

2.2. Molecular Docking Study

Molecular docking studies were performed using an *in-silico* procedure to define the binding modes of active compounds in the allosteric regions of the enzyme X-ray crystal structures of caspase-3 (PDB ID: 1NMS) to understand how to stimulate the caspase-3 activity. The data were retrieved from the Protein Data Bank server (www.pdb.org, accessed 07 December 2021). The Schrödinger Maestro [17] interface was used for the molecular docking study, and the enzyme crystal was processed using the Protein Preparation Wizard protocol of the Schrödinger Suite 2020. Similar to previous studies [18, 19], **MAC** and dasatinib were prepared using the LigPrep module [20] to correctly assign the protonation states as well as the atom types. Bond orders were assigned, and hydrogen atoms were added to the structures. The grid generation was formed using the Glide module [21], and docking runs were performed in extra-precision docking mode (XP) for dasatinib. After obtaining the best pose for the dasatinib-caspase-3 complex, the ligand was removed, and the protein that remained was used as the generated grid map. The docking runs for **MAC** were performed in extra-precision docking mode (XP).

2.3. Molecular Dynamics Study (MDS)

MDS have been considered an important computational tool for evaluating the time-dependent stability of the ligand-receptor complex. In this study, MDS for 100 ns was carried out to ensure the stability of the identified hits from the docking results, and then the interaction types and strengths were analyzed. The method was applied similarly to our previous studies [14, 22-24]. Using the standard force field (OPLS3e) of Schrodinger's Suite with a transferable intermolecular potential with 3 points (TIP3P) water model followed by energy minimization of the complex in the Desmond application [25]. The neutralization of the system was achieved using Na^+ and Cl^- ions, and 150 mM NaCl was added to the dynamics condition. The molecular dynamics simulation was performed following the completion of the system setup. The radius of gyration (Rg), root mean square fluctuation (RMSF), and root mean square deviation (RMSD) values were calculated by the Desmond application.

3. RESULTS AND DISCUSSION

3.1. DFT Studies

The variables that affect the reactivity of various biomolecules based on DFT have piqued the interest of many researchers in recent years. Several critical factors, such as the properties, stability, and composition of **MAC** compounds, have been determined using global reactivity data based on DFT. The best molecular structures produced by

theoretical methods can be used to describe the three-dimensional structure of the investigated substances. When the B3LYP/6-31G (d,p) basis range was used, no imaginary frequency was found after using DFT to optimize the structure of **MAC**. **Figure 1** displays **MAC**'s optimized structures.

Certain highly significant chemical properties, such as optical polarization, chemical softness and strength, molecular electronic transport, and so on, may be computed using the energy difference between HOMO and LUMO [26]. Table 1 shows several reactive characteristics of the MAC molecule. The studied molecule is highly stable, as evidenced by the negative energy levels of the HOMO and LUMO orbitals (**Figure 2**). The most prominent ionization potential (I) and electron affinity (A) values associated with HOMO and LUMO energy, respectively, are found in the MAC structure, which has a low I value and a high A value.

The ability of an atom to attract other atoms' shared electrons, also known as electron density, is known as electronegativity (χ). The more electrons an atom or substituent group attracts, the higher its corresponding electronegativity. It can be shown that MAC has a lower electron affinity (0.1305 eV) and higher electronegativity (0.0519 eV). The MAC has a low η value and a high S value in terms of chemical hardness-softness values that are useful in determining intramolecular charge transfer of molecular structures.

The molecular electrostatic potential (MEP) estimates ligand binding and hydrogen bonds with biomolecules and validates the charge distribution (positive and negative) of 3D molecules [27]. The MEP mapping findings for **MAC** are shown in **Figure 3**.

In the MEP scheme, red represents a partial negative charge brought on by an electron-rich zone, blue represents a partial positive charge brought on by an electron-deficient zone, yellow represents a moderately electron-rich zone, and green represents a neutral zone [28]. The MEP mapping indicates that the oxygen and nitrogen atoms in MAC have the highest negative potential.

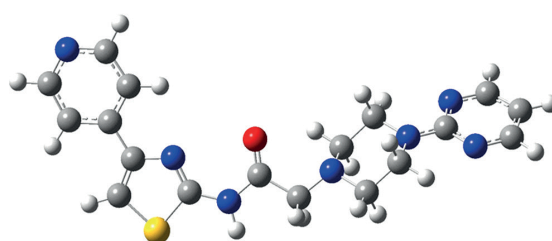

Figure 1. Optimized molecular structures and total energy values of MAC

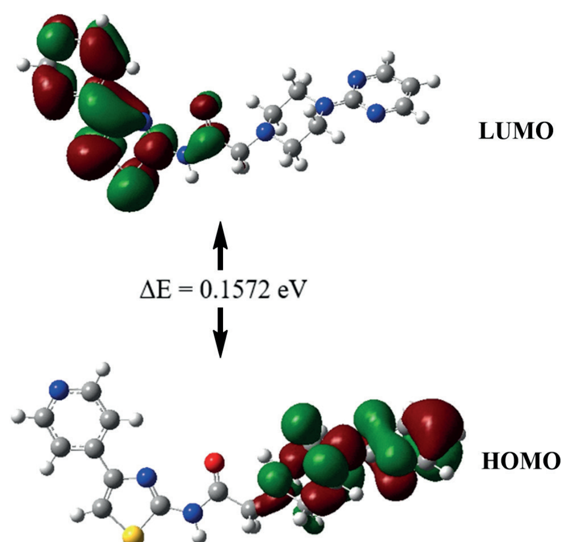

Figure 2. HOMO-LUMO diagrams of the compound MAC

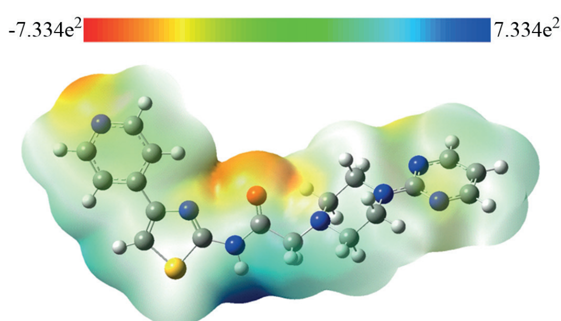

Figure 3. Molecular electrostatic potential (MEP) surfaces presentation of the MAC

3.2. Molecular Docking Study

For the caspase-3 enzyme, Arg64, Ser120, His121, Gly122, Gln161, Cys163, Tyr204, Ser205, Trp206, Arg207, Asn208, Ser209, Ser249, and Phe250 amino

Table 1. Some reactivity parameters of the MAC.

Compounds	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	I (eV)	A (eV)	χ (eV)	η (eV)	S (eV−1)	µ (eV)	ω (eV)
MAC	-0.2091	-0.0519	0.1572	0.2091	0.0519	0.1305	0.0786	6.3613	-0.1305	0.1083

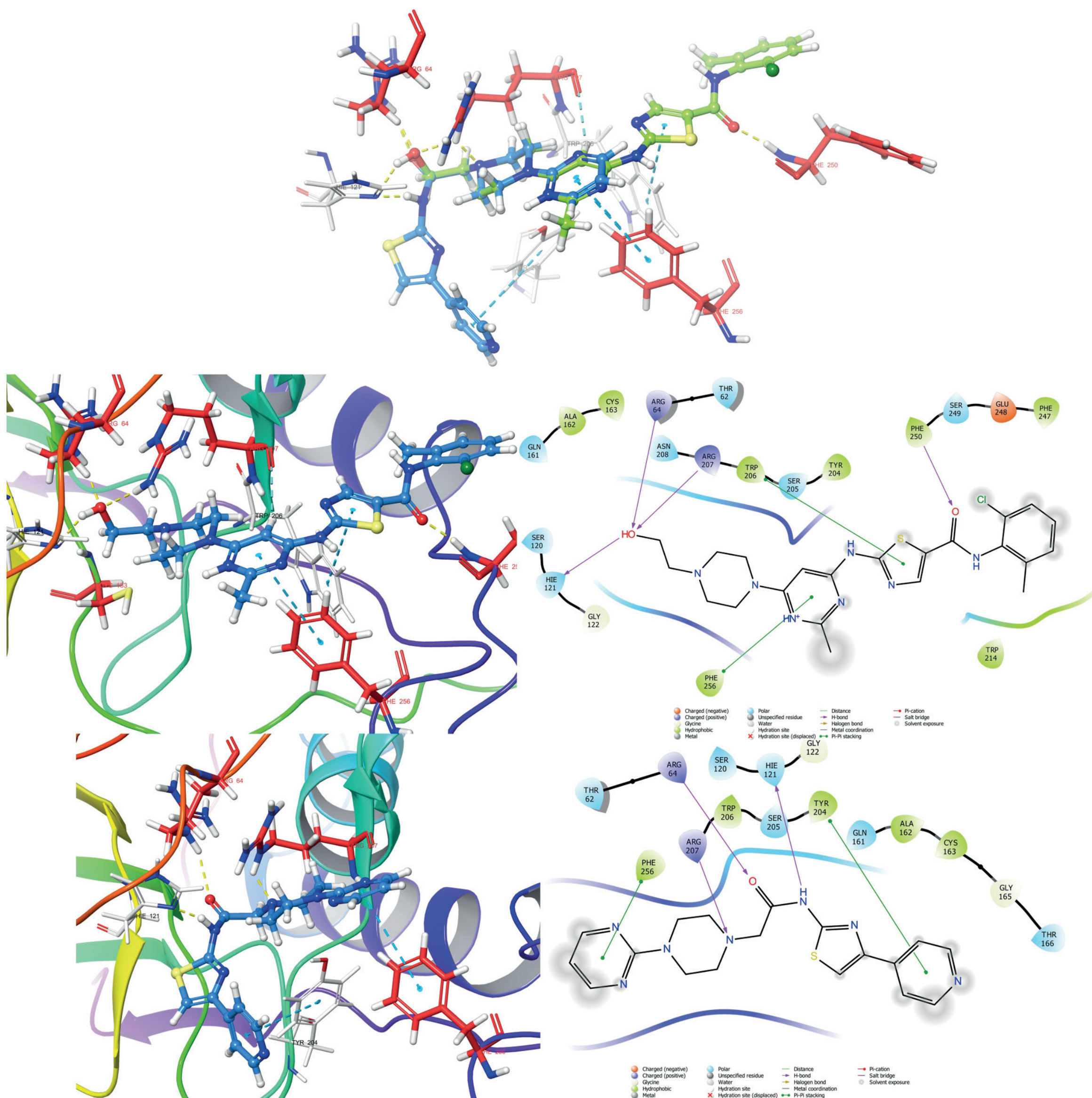

Figure 4. The best docking poses for dasatinib and active compound. **A:** Superimposed of both molecules (green carbons for dasatinib and blue carbons for active compound); **B:** Dasatinib and caspase-3 complex; **C:** MAC and caspase-3 enzyme complex.

acids were identified as binding region amino acids to stimulate the enzyme activity allosterically [29].

According to results (**Figure 4**), dasatinib interacted with Arg64 (H-bond), His121 (H-bond), Trp206 (π-π stacking), Arg207 (H-bond and aromatic H-bonds), Phe250 (H-bond), and Phe256 (π-π stacking) amino acids, while **MAC** interacted with Arg64 (H-bond), His121 (H-bond), Tyr204 (π-π stacking), Arg207 (H-bond), and Phe256 (π-π stacking) amino acids. Both interactions were found to be very similar to

each other. However, the difference between the molecules is dasatinib tailing to the hydrophobic pocket of the enzyme, in which it can interact with Phe250 amino acid. Meanwhile, because the MAC curled into the polar pocket, the H-bond interaction between its acetamide moiety and the Arg64 and Arg207 residues was allowed to occur.

As a result, the following statements can be suggested to the pharmaceutical chemist who will design and synthesize the new caspase-3 stimulants:

The presence of nitrogen-rich structures is valuable for activity, and the presence of aza-cyclic structures and protonable nitrogen significantly increases the activity. Because this structure can form hydrogen bonds with the amino acid Arg207.

The presence of one sp^3 carbon distance between the acetamide structure and the protonable nitrogen structure allows it to form hydrogen bonds with both the arginine loop amino acids (Arg64 and Arg207), and the peripheral loop amino acids (His121).

Interaction with hydrophobic cycle amino acids (Phe256) requires the presence of an aromatic structure linked to a heterocyclic structure with protonable nitrogen. Furthermore, the 3- to 5-carbon-long linkage that can be substituted at the 3rd position of this aromatic structure, as well as this linkage, should be suitable for hydrogen bonding. Finally, the heteroaromatic structure connected to this linkage will significantly affect the intensity of the activity.

3.3. Molecular Dynamics Study (MDS)

After determining the best poses of dasatinib and **MAC**, further analysis was carried out using MDS to check the complex stability and understand the structure-activity relationship (SAR) under environmental changes. The stability diagrams (**Figure 5**) point out that the values of both complexes are in the acceptable range. Two Rg plots indicated that there were no drastic changes during the simulation. RMSD values of **MAC** are under 3 Å, while RMSD values of dasatinib sometimes cross the line of 3 Å, yet it can be concluded that both complexes protect their stability. The RMSF plots displayed that the interactions with loop amino acids (green line in the white area) have a positive impact, such as decreasing the fluctuation intensity, hence, it can be said that both molecules stabilized their own ligand-protein complex. Although the interactions with Phe247-Pro263 loop amino acids differed between the two complexes, these interactions had no direct impact on the complex's stability because the major impact is revealed by Asn52-Gly66 loops and Ser198-Ser213 loops.

After approving the stability of the complex, the interaction types, their continuity, and their interaction strength were evaluated (**Figure 6**).

Dasatinib was interested in Arg64, Ser120, His121, Gln161, Tyr204, Trp206, Arg207, Asn208, Trp214, Phe250, Ser251, Asp253 and Phe256 residues (>0.2 interaction fraction). These interactions were observed as H-bond, water-mediated H-bond, aromatic H-bonds, hydrophobic, and ionic interactions (**Figure 6** and **video1**). The interaction continuity was between dasatinib and Arg207 (H-bond strength: 39% and 49%), Phe250 (H-bond strength: 88%), and Phe256 (π-π stacking strength: 36%; π-cation strength: 30%) amino acids. Interestingly, when connections with Tyr204 and Phe256 were observed, interactions with the Asn52-Gly66 loop region were lost, which points out that even if Arg64 and the other amino acids of this loop region support the stability of the complex, the connections with this loop may not be necessary to observe the activity. Thus, we suggest that it's enough to interact with at least one arginine amino acid to observe caspase-3 activity, although the recognition site is constituted by both arginine amino acids (Arg64 and Arg207) [30].

Meanwhile, **MAC** interacted with Met61, Thr62, Ser63, Arg64, Ser120, His121, Gly122, Glu123, Cys163, Tyr204, Ser205, Trp206, and Arg207 residues. These are mostly water-mediated H-bonds, as well as H-bonds, aromatic H-bonds, and hydrophobic interactions (**Figure 6** and **video2**). The interaction continuity was observed between **MAC**

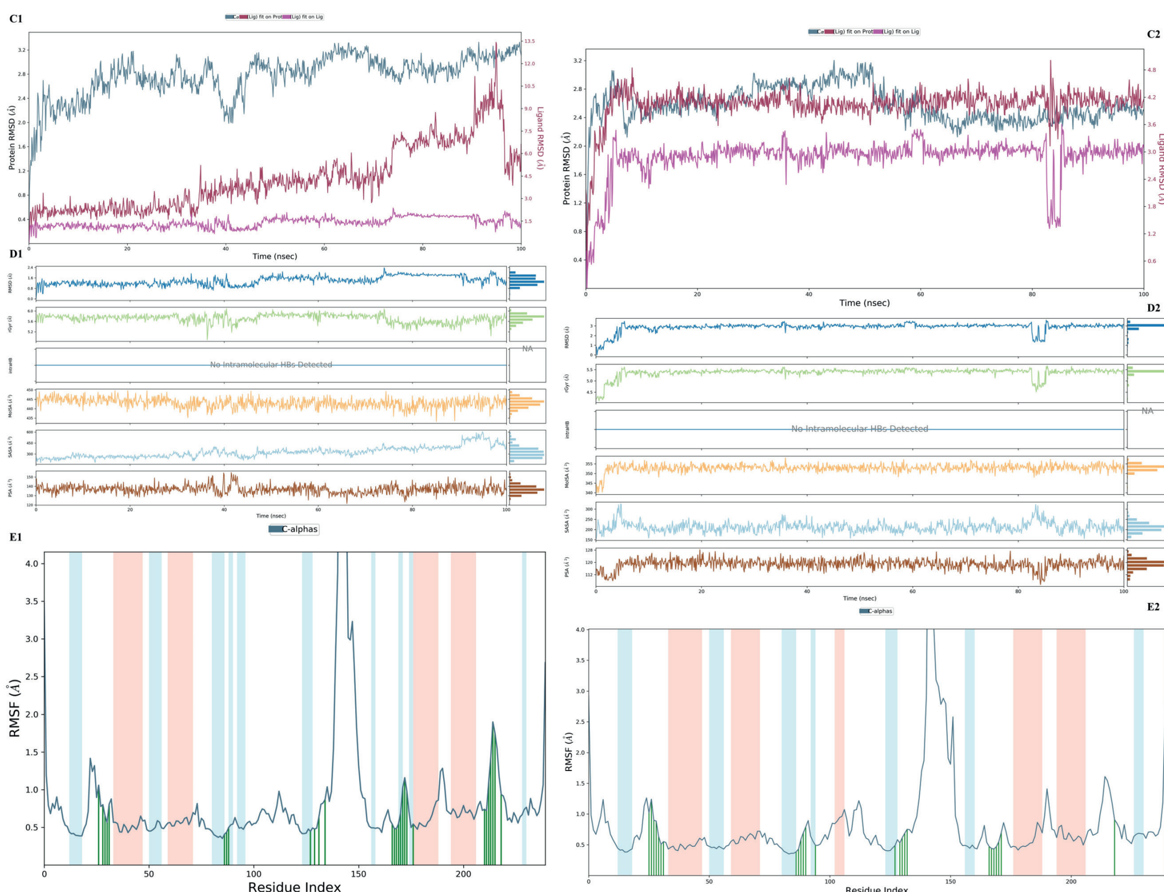

Figure 5. The stability diagrams of the complexes of caspase-3-dasatinib (**C1**, **D1** and **E1**) and MAC (**C2**, **D2** and **E2**); **C**,**D**: Ligand properties (RMSD, Rg, MolSA, SASA, PSA); **E**: RMSF, respectively.

and Gly122 (water-mediated H-bond strength: 57%), Glu123 (water-mediated H-bond strength: 52%), Ser205 (water-mediated H-bond strength: 59%), Trp206 (π-π stacking strength: 34%), and Arg207 (H-bond strength: 91%; water-mediated H-bond strength: 60% and 45%) residues (cutoff: 20%). Interestingly, even if the bond strength of **MAC**-Cys163 amino acids was not enough for the diagram, it is remarkable since it showed parallelism with the interactions of **MAC**-Arg207. Cys163 amino acid is an important amino acid, as the caspase-3 enzyme is a member of the cysteine-aspartic acid protease family. The functional effect of the enzyme starts here. Thus, we suggest that the moiety of the **MAC** [*N*-(thiazole-2-yl)acetamide] that interacts with Cys163 is a pharmacophore group (**video2**).

Generally, in addition to the above suggestions, the following statements can be suggested to the medicinal chemist who will design and synthesize new caspase-3 stimulants:

The *N*-(thiazol-2-yl)acetamide moiety is a pharmacophore structure, thus, this structure should be protected.

4-Pyridinyl substitution on the thiazole ring is remarkable, however, the pyridine ring can be substituted with a nucleophilic group (such as NH_2) to increase the activity. In fact, it also supports forming H-bonds. However, the carbonyl group on the pyridine ring may also be appropriate to interact with histamine and glycine amino acids, two members of the ß-sheet region of the enzyme.

The piperazine moiety is an essential group to observe caspase-3 activity since the proton able nitrogen atom interacts with important arginine amino acids (Arg64, Arg207).

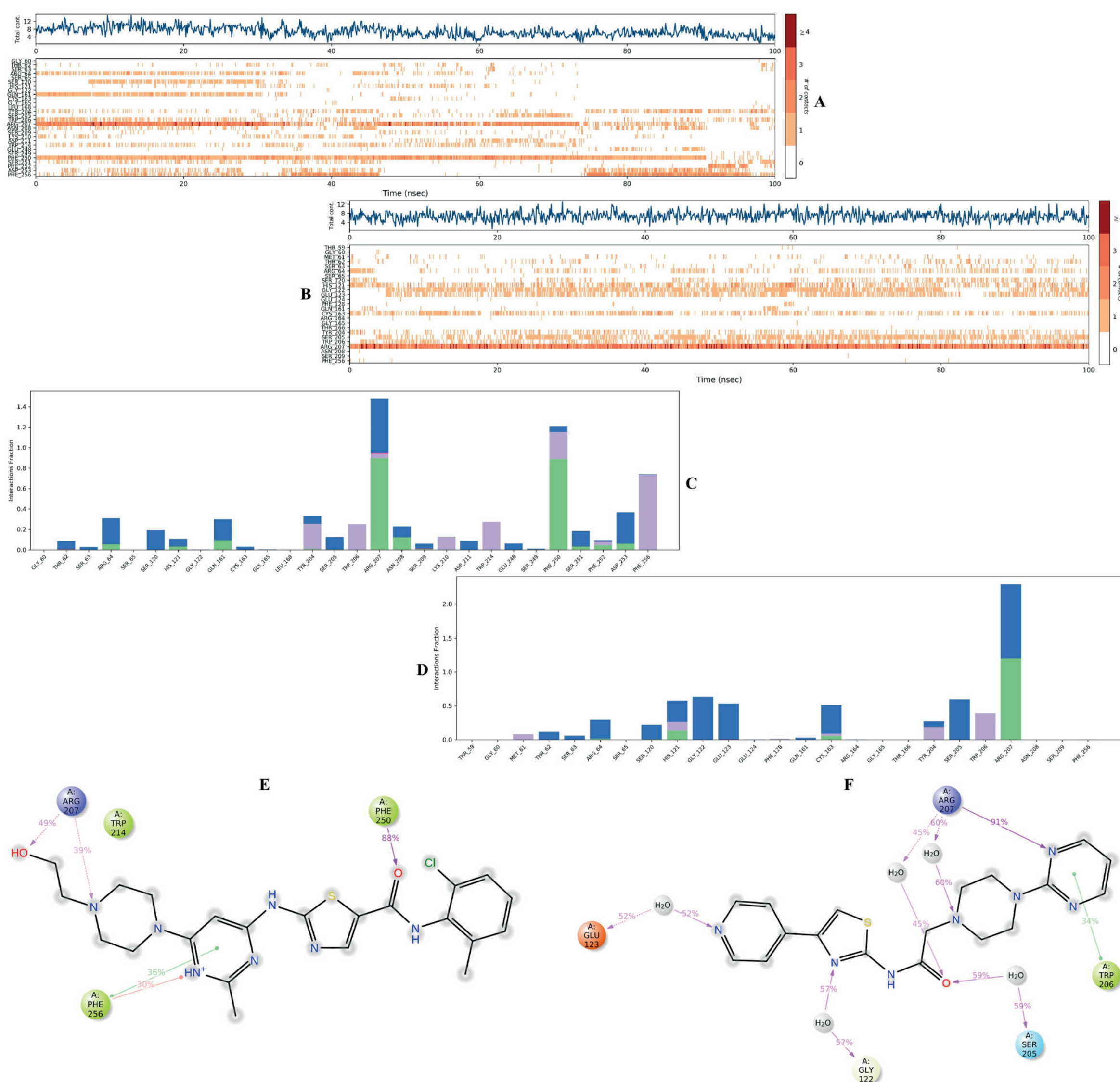

Figure 6. The interaction diagrams of the complexes of caspase-3-dasatinib (left) and MAC (right). **A**,**B**: The plot of total bond number-amino acid fraction during the simulation time; **C**,**D**: Types of interactions with the amino acids and their fraction graphic; **E**,**F**: The bond strength (cutoff=20%), respectively.

4. CONCLUSION

In this study, new design strategies were offered using a moderately active compound to get better activity and increase the apoptotic effect. For this purpose, a thiazole-piperazine derivative that had previously been synthesized and evaluated for its anticancer properties was used. The DFT, docking, and molecular dynamics simulation studies were performed on the caspase-3 enzyme, and the results were evaluated to understand the structure-activity relationship (SAR) and enable the explicate of structural modifications, thereby leading to the suggestion of new design strategies. As a result, the **MAC** has enabled us to develop new molecules based on its molecular core, supported by molecular mechanics.

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Author contribution

Concept: AEE, DN; Design: AEE, DN; Supervision: LY; Materials: AEE, DN; Data Collection and/or Processing: AEE, DN; Analysis and/or Interpretation: AEE, DN; Literature Search: AEE, DN; Writing: AEE, DN; Critical Reviews: LY.

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Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Lagorce D, Sperandio O, Galons H, Miteva MA, Villoutreix BO. FAF-Drugs2: free ADME/tox filtering tool to assist drug discovery and chemical biology projects. BMC Bioinformatics. 2008;9:396. https://doi.org/10.1186/1471-2105-9-396
2. Acar Cevik U, Saglik BN, Osmaniye D, et al. Synthesis and docking study of benzimidazole-triazolothiadiazine hybrids as aromatase inhibitors. Arch Pharm (Weinheim). 2020;353(5):e2000008. https://doi.org/10.1002/ardp.202000008
3. Saglik BN, Sen AM, Evren AE, et al. Synthesis, investigation of biological effects and in silico studies of new benzimidazole derivatives as aromatase inhibitors. Z Naturforsch C J Biosci. 2020;75(9-10):353-362. https://doi.org/10.1515/znc-2020-0104
4. Osmaniye D, Karaca Ş, Kurban B, et al. Design, synthesis, molecular docking and molecular dynamics studies of novel triazolothiadiazine derivatives containing furan or thiophene rings as anticancer agents. Bioorg Chem. 2022;122:105709. https://doi.org/10.1016/j.bioorg.2022.105709
5. Dawbaa S, Evren AE, Cantürk Z, Yurttaş L. Synthesis of new thiazole derivatives and evaluation of their antimicrobial and cytotoxic activities. Phosphorus Sulfur Silicon Relat Elem. 2021;196(12):1093-1102. https://doi.org/10.1080/10426507.2021.1972299
6. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997;23(1-3):3-25. https://doi.org/10.1016/s0169-409x(96)00423-1
7. Wani RR, Chaudhari HK. Docking and 3D QSAR Studies on Substituted Cyclobutylphenyl Quinoline Derivatives as Inhibitors of Bacterial DNA Gyrase. Curr Comput Aided Drug Des. 2018;14(4):322-337. https://doi.org/10.2174/1573409914666180516114700
8. Nuha D, Evren AE, Yılmaz Cankılıç M, Yurttaş L. Design and synthesis of novel 2,4,5-thiazole derivatives as 6-APA mimics and antimicrobial activity evaluation. Phosphorus Sulfur Silicon Relat Elem. 2021;196(10):954-960. https://doi.org/10.1080/10426507.2021.1946537
9. Doak BC, Zheng J, Dobritzsch D, Kihlberg J. How Beyond Rule of 5 Drugs and Clinical Candidates Bind to Their Targets. J Med Chem. 2016;59(6):2312-2327. https://doi.org/10.1021/acs.jmedchem.5b01286
10. Shultz MD. Two Decades under the Influence of the Rule of Five and the Changing Properties of Approved Oral Drugs. J Med Chem. 2019;62(4):1701-1714. https://doi.org/10.1021/acs.jmedchem.8b00686
11. Evren AE, Yurttaş L, Eksellı B, Akalın-Cıftcı G. Novel Tri-substituted Thiazoles Bearing Piperazine Ring: Synthesis and Evaluation of their Anticancer Activity. Lett Drug Des Discov. 2019;16(5):547-555. https://doi.org/10.2174/1570180815666180731122118
12. Frisch M, Trucks G, Schlegel H, et al. Gaussian09, Revision A. Wallingford CT Inc.; 2009. p. 150-166.
13. Dennington R, Keith T, Millam J. GaussView, version 5. 2009.
14. Nuha D, Evren AE, Ciyanci ZS, Temel HE, Akalin Ciftci G, Yurttas L. Synthesis, density functional theory calculation, molecular docking studies, and evaluation of novel 5-nitrothiophene derivatives for anticancer activity. Arch Pharm (Weinheim). 2022;355(9):e2200105. https://doi.org/10.1002/ardp.202200105
15. Nuha D, Evren AE, Kapusiz Ö, et al. Design, synthesis, and antimicrobial activity of novel coumarin derivatives: An in-silico and in-vitro study. J Mol Struct. 2023;1272:134166. https://doi.org/10.1016/j.molstruc.2022.134166
16. Dawbaa S, Nuha D, Evren AE, CankiliÇ MY, YurttaŞ L, Turan G. New Oxadiazole/Triazole Derivatives with Antimicrobial and Antioxidant Properties. J Mol Struct. 2023:135213. https://doi.org/10.1016/j.molstruc.2023.135213
17. Schrödinger Release. 2020-3, Maestro. Schrödinger, LLC, New York, NY, USA; 2020.
18. Yurttaş L, Evren AE, Özkay Y. Synthesis and In Silico Evaluation of Some New 2,4-Disubstituted Thiazole Derivatives. European Journal of Life Sciences. 2022;1(1):8-19. https://doi.org/10.55971/ejls.1089425
19. Evren AE. Bazi yeni kinolin türevlerinin sentezi, antimikrobiyal ve DNA giraz inhibitörü etkilerinin araştırılması [master's thesis]. Eskişehir, Türkiye: Anadolu Üniversitesi; 2019.

20. Schrödinger Release. 2020-3, LigPrep. Schrödinger, LLC, New York, NY, USA; 2020.
21. Schrödinger Release. 2020-3, Glide. Schrödinger, LLC, New York, NY, USA; 2020.
22. Turan Yucel N, Evren AE, Kandemir U, Can OD. Antidepressant-like effect of tofisopam in mice: A behavioural, molecular docking and MD simulation study. J Psychopharmacol. 2022;36(7):819-835. https://doi.org/10.1177/02698811221095528
23. Dawbaa S, Evren AE, Saglik BN, Gundogdu-Karaburun N, Karaburun AC. Biological activity evaluation of novel monoamine oxidase inhibitory compounds targeting Parkinson disease. Future Med Chem. 2022;14(22):1663-1679. https://doi.org/10.4155/fmc-2022-0167
24. Evren AE, Nuha D, Dawbaa S, Saglik BN, Yurttas L. Synthesis of novel thiazolyl hydrazone derivatives as potent dual monoamine oxidase-aromatase inhibitors. Eur J Med Chem. 2022;229:114097. https://doi.org/10.1016/j.ejmech.2021.114097
25. Schrödinger Release. 2020-3, Desmond. Schrödinger, LLC, New York, NY, USA; 2020.
26. Fukui K. Role of frontier orbitals in chemical reactions. Science. 1982;218(4574):747-754. https://doi.org/10.1126/science.218.4574.747
27. Politzer P, Murray JS. The fundamental nature and role of the electrostatic potential in atoms and molecules. Theoretical Chemistry Accounts: Theory, Computation, and Modeling (Theoretica Chimica Acta). 2002;108(3):134-142. https://doi.org/10.1007/s00214-002-0363-9
28. Luque FJ, Orozco M, Bhadane PK, Gadre SR. SCRF calculation of the effect of water on the topology of the molecular electrostatic potential. The Journal of Physical Chemistry. 2002;97(37):9380-9384. https://doi.org/10.1021/j100139a021
29. Cade C, Swartz P, MacKenzie SH, Clark AC. Modifying caspase-3 activity by altering allosteric networks. Biochemistry. 2014;53(48):7582-7595. https://doi.org/10.1021/bi500874k
30. Luo M, Lu Z, Sun H, et al. Nuclear entry of active caspase-3 is facilitated by its p3-recognition-based specific cleavage activity. Cell Res. 2010;20(2):211-222. https://doi.org/10.1038/cr.2010.9

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Review Article

Latest research about active pharmaceutical ingredient loaded Poly Lactic Acid-co-Glycolic Acid (PLGA) based drug delivery system in Türkiye

Berna Kaval[1,2], Fatma Dilara Şen[1], Kemal Kaya Batmaz[1], Meliha Ekinci[3], A. Alper Öztürk[✉4]

[1]Anadolu University, Graduate School of Health Sciences Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskişehir, Türkiye.

[2]Muğla Sıtkı Koçman University, Köyceğiz Vocational School of Health Services, Department of Pharmacy Services, Muğla, Türkiye.

[3]Ege University, Faculty of Pharmacy, Department of Radiopharmacy, İzmir, Türkiye.

[4]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskişehir, Türkiye.

✉ A. Alper Öztürk
aaozturk@anadolu.edu.tr

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ABSTRACT

Some of the most well-engineered and produced biomaterials are polyesters based on polyglycolic acid (PGA), polylactic acid (PLA), and their copolymers, polylactic acid co-glycolic acid (PLGA). In controlled release systems, PLGA is the most extensively used and popular polymer. Because of its biodegradability, biocompatibility, and favorable release kinetics, but also because of the reliability of protein delivery issues, this synthetic polymer has been found to be very successful. PLGA is approved in various human drug delivery systems by EMA and FDA. In this review, first, PLGA and historical development, usage, physico-chemical structure, drug release properties, degradation specifications, solubility, crystallinity, thermal stability, release properties, types of PLGA will be mentioned. In the last stage of the review, studies conducted in Türkiye are included. In conclusion, we believe that this review is a resource for researchers doing research with PLGA.

Keywords: PLGA, Drug Delivery Systems, Drug Carrier, Nanotechnology

1. INTRODUCTION

Polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers, polylactic acid co-glycolic acid (PLGA) based polyesters are some of the approved, optimized, well-designed and manufactured biomaterials. Lactic acid contains an asymmetric R-carbon, usually defined in classical stereochemical terms as the D or L shape and sometimes as the R and S form [1].

Because of its methyl side group, PLA is a hydrophobic polymer and is more resistant to hydrolysis than PGA due to methyl group steric hindrance. Semi-crystalline poly (L-lactide) (about 37 percent crystallinity) while poly (DL-lactide) is amorphous due to structural irregularities. PLA has brittleness and low thermal stability. PGA does not contain methyl side groups and has a crystalline structure of 45-55 percent. As a result, a lot of organic solvent is insoluble. Glycolide copolymerisation is used for PLGA L-lactide or DL-lactide. The differentiation of the glycolide/lactide ratio of the PLGA crystallinity can be controlled [2].

Because lactic acid and glycolic acid, two biodegradable metabolite monomers, are hydrolyzed by the body, PLGA is one of the most often utilized

biodegradable polymers for the development of nanomedicines. During the Krebs cycle, the body simply metabolizes these monomers and removes them as carbon dioxide and water. This results in a minimal toxicity to the system. PLGA is approved in various human drug delivery systems by EMA and FDA. Polymers are available commercially with a variety of molecular weights and compositions of copolymers. Depending on the ratio of molecular weight copolymer, the time of degradation might range from several months to many years [3].

In this study, firstly an introduction about PLGA is made and the history of PLGA is mentioned. Then brief information about usage, physico-chemical structure, drug release, degradation, solubility, cristallinity, thermal stability, release properties and types of PLGA are presented. Finally, pharmaceutical Research & Development work done with PLGA in Türkiye have been included.

2. HISTORICAL DEVELOPMENT

First used as a matrix in 1967 was copolymer PLA/PGA. This copolymer has a surgical thread under the trade name Vicryl® [4]. To date, PLGA is the most popular polymer in controlled release systems and is widely used. This synthetic polymer has been proven to be particularly effective because to its biodegradability, biocompatibility, and favorable release kinetics, as well as the reliability of protein delivery difficulties. PGA and PLA together with copolymer PLGA are biodegradable synthetic polymers used in the 1960s as surgical sutures. These polymers were successfully developed as surgical sutures, which led to an increase in their application as polymeric biomaterials. The copolymer has since been developed for usage in controlled release systems and is regarded as the most efficient and extensively researched polymer for controlled delivery systems of biodegradable polymers. PLGA has been utilized to release a variety of small-molecule drugs, peptides, and proteins, such as steroid hormones, insulin, anti-inflammatory drugs, cytokines, growth hormones, chemotherapeutics, antibiotics, and narcotic antagonists, as well as hormones that control fertility [5].

3. USAGE

One of the most important uses of PLGA is that it is a polymer used in nanoparticle production technology. PLGA has different weights and copolymer technologies available on the market. When the literature is examined, one of the important parameters in the preparation of PLGA nanoparticles is the water solubility of the drug to be loaded into the nanoparticle system. While preparing PLGA nanoparticles of drugs with low water solubility, double emulsion solvent evaporation technique is generally used, while nanoprecipitation method is used for drugs with low water solubility. In the literature, it is seen that many active pharmaceutical ingredients are encapsulated into PLGA nanoparticles. Examples of these active ingredients are: paclitaxel, nitrocamptothecin, cisplatin, haloperidol, estradiol [6-8,12]. Another carrier system in which PLGA is frequently used is scaffold, fiber, microbubble [9-13].

Biological targeting ligands such as cytokines, hormones, chemotherapeutic drugs and other agents can be conjugated to PLGA nanoparticles or microspheres to target tumors. Early diagnosis and imaging of cancer can be done with PLGA nanoparticles used in the field of radiopharmacy [14]. One of the most extensively researched polymers for vaccines is PLGA. Systems prepared with PLGA as a controlled release system can deliver antigens or adjuvants to the target site [15].

4. PHYSICO-CHEMICAL STRUCTURE

PLGA is able to be shaped into virtually any size or form and can hold practically any quantity of molecules. The inherent viscosity of PLGA polymers that are commercially accessible on the market is often correlated with their molecular weights. The PLGA polymer's crystallinity directly influences its mechanical strength, swelling characteristics, hydrolysis propensity, and ultimately the rate of biodegradation [16].

The mechanical strength of the polymer and its capacity to be designed as a tool for drug delivery are influenced by physical parameters like molecular weight and polydispersity index. Such characteristics

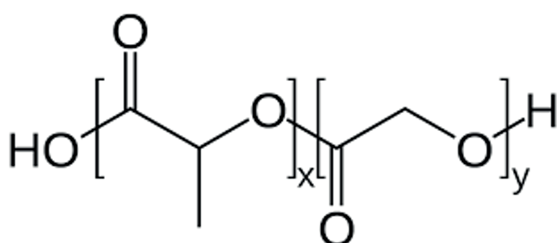

Figure 1. Chemical structures of PLGA

can control how quickly polymers hydrolyze and degrade biologically [16]. These qualities can also be used to design a drug delivery system and regulate how quickly the system degrades and hydrolyzes [17]. The chemical structure of PLGA is presented in Figure 1.

5. DRUG RELEASE PROPERTIES

When the PLGA polymer undergoes biodegradation or hydrolysis, the ester bonds in the polymer chain are broken and split into oligomers and then monomers. The two main release mechanisms associated with drug release from PLGA-based delivery systems are diffusion and degradation/ erosion. It is reported that release rate of the drug is initially controlled by diffusion followed by the last phase of release by degredation/erosion. Direct correlation between the release rate and nanoparticle size can be seen. The fact that large microspheres degrade faster than small microspheres was shown in the literature. This is presumably because of the expanded collection of acidic items in huge polymeric microspheres. Hydrolysis occuring in PLGA systems starts immediately after being in contact with water; hydrolysis produces acids which catalyze hydrolysis. This autocatalytic process leads to a faster degradation at the center of PLGA matrix rather than from the surface [17-20].

6. DEGREDATION SPECIFICATIONS

Understanding the variables that affect PLGA's degradation is crucial for improving its desirable qualities and designing a drug delivery system that considers each of these variables will increase its effectiveness and efficiency. The most crucial element in determining the hydrophilicity and rate of degradation of a delivery matrix is its polymer composition. Numerous studies have shown how to conduct an organized analysis of polymer composition and degradation [21]. These findings indicate that the weight loss of polymers is accelerated by an increase in the amount of glycolic acid in oligomers. Degradation rate is indirectly impacted by copolymer composition, which also influences crucial attributes like glass transition temperature and crystallinity. There are conflicting reports right now on how crystallinity affects the rate of degradation [22].

The copolymer's rate of degradation is influenced by its molecular weight. Higher molecular weight polymers typically showed slower rates of degradation. The length of the polymer chain and molecular weight are directly correlated. Longer polymer chains in higher molecular weight polymers require longer to degrade than small polymer chains to do so [17].

Large system degradation has been demonstrated to be significantly influenced by the surface-to-volume ratio. Higher surface area ratio causes the matrix to degrade more quickly [23].

Both alkaline and highly acidic environments increase speed polymer degradation, as shown by *in vitro* PLGA biodegradation and hydrolysis.

7. SOLUBILITY

Solubility in common organic solvents has a substantial impact on how easily polymers can be processed, applied, and characterized. Less than 50% of the glycolyl units in many common organic solvents, including halogenated hydrocarbons (chloroform and dichloromethane), acetone, ethyl acetate, dioxane, and tetrahydrofuran (glycolic acid units). Only a few organic solvents, like hexafluoroiso-propanol, can be used to characterize and process PLGA with high glycolyl unit content (50 percent and greater) because it is insoluble in most of them [7]. By hydrolysis, PLGA biodegrades the ester associations in water. Due to the presence of methyl side groups in PLA, it is more hydrophobic than PGA; as a result, lactide copolymers high in PLGA are less hydrophilic, absorb less water, and degrade more slowly [17].

8. CRYSTALLINITY

Cristallinity affects the rate of degradation and mechanical characteristics of PLGA. The lactide to glycolide ratio and the stereoisomeric composition of the lactide units in the copolymer affect the poly(lactidecoglycolide) crystallinity. Amorphous poly(DL-lactide-co-glycolide) has 0–75 percent glycolyl units. With 25-75 percent glycolyl units, poly(L-lactide-co-glycolide) is also amorphous [7].

The molecular weight of the polymer and its melting point are both directly correlated. According to reports, the PLGA copolymers' Tg (glass transition temperature) is higher than the physiological temperature of 37°C, making them glassy by nature and providing them a very robust chain structure. Depends on the polymer having a specific mechanical strength in order to be produced as a drug delivery system. Tg of PLGAs decreases with a reduction in the molecular weight and lactide concentration of the copolymer composition [16].

9. THERMAL STABILITY

In the absence of moisture, PLGA copolymers are thermoplastic materials with adequate thermal stability. As a result, these materials can be melted down to produce sutures, orthopedic fixation devices, and drug delivery systems. Poly(lactide-co-glycolide) degrades to lactide and glycolide under nitrogen or vacuum after being heated for a long time over 200°C. Thermal degradation occurs at lower temperatures as a function of time, temperature, and is accelerated by impurities, residual monomers, and humidity [7].

Table 1. Glass transition temperatures of PLA and PLGA [24]

Polymers	Tg (°C)
PLA 100	45.3
PLGA (90:10) (Lactide:Glycolide)	48.7
PLGA (75:25) (Lactide:Glycolide)	36.5
PLGA (65:35) (Lactide:Glycolide)	42.8
PLGA (50:50) (Lactide:Glycolide)	35.7

10. RELEASE PROPERTIES

A drug delivery system based on PLGA may release drug molecules in only three different ways:

-Transport by water-filled pores,

-Transport by polymer,

-Transport by dissolution of the encapsulating polymer (which does not involve the transfer of drugs).

Because the encapsulated product is often a biopharmaceutical, such as a peptide or protein, which is too large and too hydrophilic for transportation through the polymer process, transportation through water-filled pores is the most popular mode of release [25].

11. TYPES

Monomer ratios and PLGA forms are generally categorized as PLGA 50:50 and PLGA 25:75. A copolymer composed of 50% lactide and 50% glycolide is known as PLGA 50:50. As a result, PLGA 25:75 (lactide: glycolide) and PLGA 80:20 (lactide: glycolide) both have amorphous structures. PLGA is hydrolyzed by ester linkages when there is water present. They are hydrolyzed into monomeric acids and eliminated through the creb cycle as CO_2 and water in the urine. The biodegradation of PLGA is influenced by its molecular weight, lactide: glycolide ratios, glass transition temperature (Tg), and degree of crystallinity. High lactide concentration PLGA is less hydrophilic, less water absorbing, and hydrolyzes more slowly. Properties of PLGA, such as molecular weight, lactide: glycolide ratio (rate of the active substance extracted from the carrier system: 50:50 > 65:35 > 75:25 > 80:20), and factors affecting the release of the active substance, such as particle size and surface properties of the prepared carrier systems [17].

Table 2. The pharmaceutical Research & Development studies with PLGA in Türkiye

Drug	**PLGA type**	**Type of drug delivery system**	**Method of Preparation**	**Ref**
-	PLGA (50:50) (Lactide:Glycolide) [2A (14 kDa), 3A (46 kDa), 4A (58.8 kDa) Medisorb®]	Nanoparticles	Spontaneous Emulsification Diffusion	[11]
-	PLGA (50:50) (Lactide:Glycolide) with molecular weights of 30,000–60,000 Da (High) and 24,000–38,000 Da (Low)	Nanomicelles	Oil/Water Emulsification-Solvent Evaporation	[13]
1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate	Resomer® RG 503	Nanoparticles	Nanoprecipitation	[14]
5-fluorouracil	PLGA (74:26) (Lactide:Glycolide) PLGA (73:27) (Lactide:Glycolide)	Nanoparticles	Nanoprecipitation-Solvent Displacement	[26]
20-S-Camptothecin	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	Nanoprecipitation	[27]
Antisense oligonucleotides (ODNs) (5’ CTT CAT CTT CAG CTA GTC GG)	Resomer® RG 503 Resomer® RG 503 H	Nanospheres	Emulsification-Diffusion	[28]
Atorvastatin, Alpha-lipoic acid	PLGA (50:50) (Lactide:Glycolide)	Microspheres	Spray-Drying	[29]
Betamethasone-17-valerate	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	Emulsion–Diffusion–Evaporation	[30]
Bovine Serum Albumin	Resomer® RG 502 Resomer® RG 503	Nanoparticles	Double Emulsion-Solvent Evaporation	[31]
Carvedilol	Resomer® RG 502 Resomer® RG 504	Nanoparticles	Nanoprecipitation	[20]
Clarithromycin	Resomer® RG 502 H Resomer® RG 503 H Resomer® RG 504 H	Nanoparticles	Nanoprecipitation	[32]
Curcumin (diferuloylmethane)	Resomer® RG 503	Hybrid Nanoparticles	Emulsion Sonication	[33]
Dexamethasone	Resomer® RG 502 H	Nanoparticles	Emulsification-Solvent Evaporation	[34]
Dexketoprofen trometamol	Resomer® RG 504 H	Nanoparticles	Double Emulsion-Solvent Evaporation	[35]
Dexpanthenol	Resomer® RG 756 S	Nanofiber Mats	Electrospinning	[36]

Table 2. Continued

Drug	PLGA type	Type of drug delivery system	Method of Preparation	Ref
Diclofenac sodium	PLGA (50:50) (Lactide:Glycolide) (34000 Da) PLGA (50:50) (Lactide:Glycolide) (88000 Da)	Microspheres	Solvent Evaporation	[37]
Diclofenac sodium	Resomer® RG 503 H	Nanoparticles	Nanoprecipitation	[38]
Docetaxel	Resomer® RG 502 Resomer® RG 502 H Resomer® RG 503 Resomer® RG 503 H	Nanoparticles	Emulsification-Solvent Evaporation	[39]
Doxorubicin	PLGA (50:50) (Lactide:Glycolide)	Hybrid Nanoparticles	Oil/Water Emulsion-Solvent Evaporation	[40]
Eletriptan hydrobromide	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	Sonication Evaporation	[41]
Epidermal Growth Factor	Resomer® RG 503 H	Microspheres	Double Emulsion-Solvent Evaporation	[42]
Etodolac	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	Nanoprecipitation	[43]
Flurbiprofen sodium	PLGA (50:50) (Lactide:Glycolide) (69 kDa), PLGA (50:50) (Lactide:Glycolide) (63 kDa), PLGA (75:25) (Lactide:Glycolide) (130 kDa), PLGA (75:25) (Lactide:Glycolide) (92 kDa), PLGA (85:15) (Lactide:Glycolide) (149 kDa)	Microspheres	Solvent Evaporation	[44]
Flurbiprofen, Folic Acid	Resomer® RG 504 H	Nanoparticles	Nanoprecipitation	[45]
Gemcitabine hydrochloride	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	Modified Double Emulsion-Solvent Evaporation	[46]
Gemcitabine hydrochloride	PLGA (50:50) (Lactide:Glycolide) PLGA 65:35 (Lactide:Glycolide)	Lipid Polymer Hybrid Nanoparticles	Emulsion-Solvent Evaporation	[47]

Table 2. Continued

Drug	PLGA type	Type of drug delivery system	Method of Preparation	Ref
Heparin	Resomer® RG502 Resomer® RG503 Resomer® RG504	Microparticles	Spray-Drying	[48]
Ibuprofen	Resomer® RG 503 H	Nanoparticles	Nanoprecipitation	[49]
Imatinib mesylate	PLGA (50:50) (Lactide:Glycolide) PLGA (75:25) (Lactide:Glycolide) PLGA (85:15) (Lactide:Glycolide)	Microspheres	Water/Oil/Water Double Emulsion-Solvent Evaporation	[50]
Interleukin-2	Resomer® RG 502	Microparticles	Double Emulsion-Solvent Extraction	[51]
Ketoprofen lysine	Resomer® RG 504 H	Nanoparticles	Spray-Drying	[52]
Lamivudine	Resomer® RG 504 H	Nanoparticles	Double Emulsion-Solvent Evaporation	[53]
Meloxicam	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	In Situ Coating Technique-The Surface Adsorption	[54]
Meloxicam	PLGA (50:50) (Lactide:Glicolyde)	Nanoparticles	Using Salting-Out-Emulsion-Evaporation	[55]
Meloxicam	PLGA (50:50) (Lactide:Glycolide)	Nanoparticles	Ultrasonication-Solvent Evaporation	[56]
Metformin HCl	Resomer® RG 502	Nanoparticles	Ultrasonication-Solvent Evaporation	[57]
Nifedipine	Resomer® RG 756 S	Nanoparticles	Nanoprecipitation-Emulsion-Solvent Evaporation	[58]
Nimesulide	PLGA (50:50) (Lactide:Glicolyde)	Nanoparticles	Ultrasonication-Solvent Evaporation	[59]
Ondansetron	Resomer® RG 502 Resomer® RG 503	Microspheres	Emulsification-Spray-Drying	[60]
Paclitaxel	Resomer® RG 502 H	Nanoparticles	Oil/Water Emulsification-Solvent Evaporation	[61]
Paclitaxel	Resomer® RG 503	Nanoparticles	Nanoprecipitation	[62]
Paclitaxel, Flurbiprofen	PLGA	Nanoparticles	Nanoprecipitation	[63]
Phenyl Butyl Nitrone (α-phenyl-N-tert-butyl nitrone)	Resomer® RG 502 H	Nanoparticles	Homogenization-Solvent Evaporation	[64]
Plasmid DNA (pDNA)	Resomer® RG 502 Resomer® RG 503 Resomer® RG 503 H	Microspheres	Water/Oil/Water Double Emulsion-Solvent Evaporation	[65]

Table 2. Continued

Drug	PLGA type	Type of drug delivery system	Method of Preparation	Ref
Recombinant Human Interleukin-2	Resomer® RG 502 Resomer® RG 502H Resomer® RG 504 Resomer® RG 752H Resomer® RG 756S	Microparticles	Double Emulsion-Solvent Evaporation	[66]
Recombinant Human Interleukin-2	Resomer® RG 502 Resomer® RG 502 H Resomer® RG 504 Resomer® RG 752 H Resomer® RG 756 S	Microparticles	Double Emulsion- Solvent Evaporation	[67]
Salmon calcitonin	Resomer® RG 502 H	Nanoparticles	Nanoprecipitation	[68]
Small interfering RNA (siRNA)	PLGA (50:50) (Lactide:Glycolide) [2A (14 kDa) and 3A (46 kDa) Medisorb®]	Nanoparticles	Emulsification Diffusion	[69]
Sodium fusidate	PLGA (50:50) (Lactide:Glycolide)	Microspheres	Double Emulsion Solvent Evaporation	[70]
Terbinafine hydrochloride	Resomer® RG 502 H	Nanoparticles	Emulsification-Solvent Evaporation	[71]
Vancomycin	PLGA (75:25) (Lactide:Glycolide)	Microspheres	Oil/Water Emulsion-Solvent Evaporation	[72]
Vancomycin	PLGA (90:10) (Lactide:Glycolide) PLGA (70:30) (Lactide:Glycolide)	Microcapsules	Water/Oil/Water Double Emulsion-Solvent Evaporation	[73]
Vancomycin	PLGA (90:10) (Lactide:Glycolide) PLGA (70:30) (Lactide:Glycolide)	Polymer disks	Solvent Evaporation	[74]
Vascular Endothelial Growth Factor	PLGA (50:50) (Lactide:Glycolide)	Microspheres	Water/Oil/Water Emulsification	[75]
Vascular Endothelial Growth Factor	PLGA (50:50) (Lactide:Glycolide)	Microspheres	Water/Oil/Water Emulsification	[76]
Vincristine, ε-Viniferine	PLGA	Nanoparticles	Nanoprecipitation	[77]
Diclofenac sodium	Resomer® RG 502 H	Nanoparticles	Double Emulsion Solvent Evaporation	[78]
Flurbiprofen	Resomer® RG 502 H Resomer® RG 503 H Resomer® RG 503 H	Nanoparticles	Nanoprecipitation	[79]
Ketoprofen lysine	Resomer® RG 504H	Nanoparticles	Spray drying technique	[80]
Cefaclor Monohydrate	Resomer® RG 504H	Nanoparticles	Nanoprecipitation	[81]
Lamivudine	Resomer® RG 502 H	Nanoparticles	Double Emulsion Solvent Evaporation	[82]

12. CONCLUSION

Drug delivery systems and polymers used in drug delivery systems are a very important issue today and studies are continuing [81,83-87]. Different drug delivery systems prepared with polymers are more advantageous and more promising than traditional drug delivery systems [81,85-93]. Table 2 shows pharmaceutical R&D studies with PLGA in Turkey. PLGA is widely using as a polymer for long time and it is approved in various human drug delivery systems by the FDA and the EMA. This review shows that there are latest studies on medicine industry about beneficial usage of PLGA and also traditional usage of PLGA in Türkiye. In addition because of its good biodegradable properties and able to controlled release PLGA is a very important polymer technology. We can make inference that PLGA is continue to be used for human health and medicine industry.

Author contribution

Concept: AAÖ; Design: AAÖ; Supervision: AAÖ; Data Collection and/or Processing: BK, FDŞ, KKB, ME, AAÖ; Analysis and/or Interpretation: BK, FDŞ, KKB, ME, AAÖ; Literature Search: BK, FDŞ, KKB, ME, AAÖ; Writing: BK, FDŞ, KKB, ME, AAÖ; Critical Reviews: AAÖ.

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Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chem Rev. 1999;99(11):3181-3198. https://doi.org/10.1021/cr940351u
2. Özakar E. Etkin Madde Yüklü PLGA Nanopartiküllerinin Hazırlanması ve *in vitro* Değerlendirilmesi [master's thesis]. Erzurum: Atatürk Üniversitesi Sağlık Bilimleri Enstitüsü; 2014.
3. Tabatabaei Mirakabad FS, Nejati-Koshki K, Akbarzadeh A, et al. PLGA-based nanoparticles as cancer drug delivery systems. Asian Pac J Cancer Prev. 2014;15(2):517-535. https://doi.org/10.7314/apjcp.2014.15.2.517
4. Erden N, Çelebi N. Biyolojik olarak parçalanabilen polimer sistemlerin peptit ve proteinlere uygulanması: Sürekli etki sağlayan laktid/glikolid mikroküreleri. FABAD J Pharm Sci. 1993;18:161-171.
5. Hines DJ, Kaplan DL. Poly(lactic-co-glycolic) acid-controlled-release systems: experimental and modeling insights. Crit Rev Ther Drug Carrier Syst. 2013;30(3):257-276. https://doi.org/10.1615/critrevtherdrugcarriersyst.2013006475
6. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012;161(2):505-522. https://doi.org/10.1016/j.jconrel.2012.01.043
7. Avgoustakis K. Polylactic-Co-Glycolic Acid (PLGA). In: Wnek GE, Bowlin GL, editors. Encyclopedia of Biomaterials and Biomedical Engineering. CRC Press; 2008. p. 2291-2301.
8. Herrero-Vanrell R, Molina-Martinez IT. PLA and PLGA microparticles for intravitreal drug delivery: an overview. J Drug Deliv Sci Technol. 2007;17(1):11-17. https://doi.org/10.1016/S1773-2247(07)50002-X
9. Sarkar S, Lee GY, Wong JY, Desai TA. Development and characterization of a porous micro-patterned scaffold for vascular tissue engineering applications. Biomaterials. 2006;27(27):4775-4782. https://doi.org/10.1016/j.biomaterials.2006.04.038
10. Hwang CM, Khademhosseini A, Park Y, Sun K, Lee SH. Microfluidic chip-based fabrication of PLGA microfiber scaffolds for tissue engineering. Langmuir. 2008;24(13):6845-6851. https://doi.org/10.1021/la800253b
11. Katas H, Cevher E, Alpar HO. Preparation of polyethyleneimine incorporated poly (d, l-lactide-co-glycolide) nanoparticles by spontaneous emulsion diffusion method for small interfering RNA delivery. Int J Pharm. 2009;369(1-2):144-154. https://doi.org/10.1016/j.ijpharm.2008.10.012
12. Cui W, Bei J, Wang S, et al. Preparation and evaluation of poly(-lactide-coglycolide) (PLGA) microbubbles as a contrast agent for myocardial contrast echocardiography. J Biomed Mater Res B Appl Biomater. 2005;73(1):171-178. https://doi.org/10.1002/jbm.b.30189

13. Eskandari Z, Kazdal F, Bahadori F, Ebrahimi N. Quality-by-design model in optimization of PEG-PLGA nano micelles for targeted cancer therapy. J Drug Deliv Sci Technol. 2018;48:393-402. https://doi.org/10.1016/j.jddst.2018.10.009

14. Sahin A, Esendagli G, Yerlikaya F, Caban-Toktas S, Yoyen-Ermis D, Horzum U, Aktas Y, Khan M, Couvreur P, Capan Y. A small variation in average particle size of PLGA nanoparticles prepared by nanoprecipitation leads to considerable change in nanoparticles' characteristics and efficacy of intracellular delivery. Artif Cells Nanomed Biotechnol. 2017;45(8):1657-1664. https://doi.org/10.1080/21691401.2016.1276924

15. Holy CE, Dang SM, Davies JE, Shoichet MS. In vitro degradation of a novel poly(lactide-co-glycolide) 75/25 foam. Biomaterials. 1999;20(13):1177-1185. https://doi.org/10.1016/s0142-9612(98)00256-7

16. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000;21(23):2475-90. https://doi.org/10.1016/s0142-9612(00)00115-0

17. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;3(3):1377-1397. https://doi.org/10.3390/polym3031377

18. Amann LC, Gandal MJ, Lin R, Liang Y, Siegel SJ. In vitro-in vivo correlations of scalable PLGA-risperidone implants for the treatment of schizophrenia. Pharm Res. 2010;27(8):1730-1737. https://doi.org/10.1007/s11095-010-0152-4

19. Crotts G, Park TG. Protein delivery from poly(lactic-co-glycolic acid) biodegradable microspheres: release kinetics and stability issues. J Microencapsul. 1998;15(6):699-713. https://doi.org/10.3109/02652049809008253

20. Öztürk AA, Martin-Banderas L, Cayero-Otero MD, Yenilmez E, Yazan Y. New approach to hypertension treatment: carvediol-loaded PLGA nanoparticles, preparation, in vitro characterization and gastrointestinal stability. Lat Am J Pharm. 2018;37(9):1730-1741.

21. Lu L, Peter SJ, Lyman MD, et al. In vitro and in vivo degradation of porous poly(DL-lactic-co-glycolic acid) foams. Biomaterials. 2000;21(18):1837-1845. https://doi.org/10.1016/s0142-9612(00)00047-8

22. Schliecker G, Schmidt C, Fuchs S, Wombacher R, Kissel T. Hydrolytic degradation of poly(lactide-co-glycolide) films: effect of oligomers on degradation rate and crystallinity. Int J Pharm. 2003;266(1-2):39-49. https://doi.org/10.1016/s0378-5173(03)00379-x

23. Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials. 1995;16(4):305-311. https://doi.org/10.1016/0142-9612(95)93258-f

24. In Pyo Park P, Jonnalagadda S. Predictors of glass transition in the biodegradable poly-lactide and poly-lactide-co-glycolide polymers. J Appl Polym Sci. 2006;100(3):1983-1987. https://doi.org/10.1002/app.22135

25. Fredenberg S, Wahlgren M, Reslow M, Axelsson A. The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems-a review. Int J Pharm. 2011;415(1-2):34-52. https://doi.org/10.1016/j.ijpharm.2011.05.049

26. Bozkir A, Saka OM. Formulation and investigation of 5-FU nanoparticles with factorial design-based studies. Farmaco. 2005;60(10):840-846. https://doi.org/10.1016/j.farmac.2005.06.016

27. Cirpanli Y. Kamptotesin İçeren Polimerik ve Oligosakkarit Bazlı Nanopartiküler Formülasyonların Geliştirilmesi ve in vitro-in vivo Değerlendirilmesi [dissertation]. Ankara: Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü; 2009.

28. Kilic AC, Capan Y, Vural I, et al. Preparation and characterization of PLGA nanospheres for the targeted delivery of NR2B-specific antisense oligonucleotides to the NMDA receptors in the brain. J Microencapsul. 2005;22(6):633-641. https://doi.org/10.1080/02652040500162766

29. Eroğlu H, Haidar MK, Nemutlu E, et al. Dual release behavior of atorvastatin and alpha-lipoic acid from PLGA microspheres for the combination therapy in peripheral nerve injury. J Drug Deliv Sci Technol. 2017;39:455-466. https://doi.org/10.1016/j.jddst.2017.04.028

30. Özcan I, Azizoğlu E, Senyiğit T, Özyazici M, Özer Ö. Comparison of PLGA and lecithin/chitosan nanoparticles for dermal targeting of betamethasone valerate. J Drug Target. 2013;21(6):542-550. https://doi.org/10.3109/1061186X.2013.769106

31. Sinani G, Sessevmez M, Koray Gök M, et al. Nasal vaccination with poly(β-amino ester)-poly(d,l-lactide-co-glycolide) hybrid nanoparticles. Int J Pharm. 2017;529(1-2):1-14. https://doi.org/10.1016/j.ijpharm.2017.06.053

32. Öztürk AA, Yenilmez E, Özarda MG. Clarithromycin-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles for oral administration: Effect of polymer molecular weight and surface modification with chitosan on formulation, nanoparticle characterization and antibacterial effects. Polymers (Basel). 2019;11(10):1632. https://doi.org/10.3390/polym11101632

33. Orunoğlu M, Kaffashi A, Pehlivan SB, et al. Effects of curcumin-loaded PLGA nanoparticles on the RG2 rat glioma model. Materials Science and Engineering: C. 2017;78:32-38.

34. Rençber S, Aydın Köse F, Karavana SY. Dexamethasone loaded PLGA nanoparticles for potential local treatment of oral precancerous lesions. Pharm Dev Technol. 2020;25(2):149-158. https://doi.org/10.1080/10837450.2019.1673407

35. Öztürk AA, Banderas LM, Otero MDC, Yenilmez E, Şenel B, Yazan Y. Dexketoprofen trometamol-loaded poly-lactic-co-glycolic acid (PLGA) nanoparticles: Preparation, in vitro characterization and cyctotoxity. Tropical J Pharm Res. 2019;18(1):1-11. https://doi.org/10.4314/tjpr.v18i1.1

36. Tanrıverdi ST, Suat B, Azizoğlu E, Köse FA. In-vitro evaluation of dexpanthenol-loaded nanofiber mats for wound healing. Tropical J Pharm Res. 2018;17(3):387-394. https://doi.org/10.4314/tjpr.v17i3.1

37. Tunçay M. Diklofenak Sodyum İçeren Mikrokürelerin Biyolojik Parçalanabilen Polimerler ile Formülasyonun Geliştirilmesi ve in vitro, in vivo Değerlendirilmesi [master's thesis]. Ankara: Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü; 1998.

38. Cetin M, Atila A, Kadioglu Y. Formulation and in vitro characterization of Eudragit® L100 and Eudragit® L100-PLGA nanoparticles containing diclofenac sodium. AAPS PharmSciTech. 2010;11(3):1250-1256. https://doi.org/10.1208/s12249-010-9489-6

39. Demir D. Dosetaksel İçeren Nanopartiküllerin Formülasyonu ve in vitro Değerlendirilmesi [master's thesis]. Ankara: Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü; 2013.

40. Tonbul H. Doksorubisin ve elakridar içeren PLGA/silika hibrit nanopartiküllerin formülasyonu ve meme kanserindeki etkinliğinin değerlendirilmesi [dissertation]. Ankara: Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü; 2019.

41. Esim O, Savaser A, Kurbanoglu S, Ozkan CK, Ozkan SA, Ozkan Y. Development of assay for determination of eletriptan hydrobromide in loaded PLGA nanoparticles. J Pharm Biomed Anal. 2017;142:74-83. https://doi.org/10.1016/j.jpba.2017.05.002

42. Orhan Z, Cevher E, Gül M, et al. Preparation and In vivo evaluation of gelatine implants containing EGF-PLGA microspheres in tendon healing. ACTA Pharm Sci. 2007;49(3):219-229.

43. Çırpanlı Y, Robineau C, Çapan Y, Çalış S. Etodolac loaded poly (lactide-co-glycolide) nanoparticles: formulation and in vitro characterization. Hacettepe Univ J Faculty Pharm. 2009;29(2):105-114.

44. Samati Y, Yüksel N, Tarimci N. Preparation and characterization of poly(D,L-lactic-co-glycolic Acid) microspheres containing flurbiprofen sodium. Drug Deliv. 2006;13(2):105- 11. https://doi.org/10.1080/10717540500313331

45. Şenel B, Öztürk AA. New approaches to tumor therapy with siRNA-decorated and chitosan-modified PLGA nanoparticles. Drug Dev Ind Pharm. 2019;45(11):1835-1848. https://doi.org/10.1080/03639045.2019.1665061

46. Yalcin TE, Ilbasmis-Tamer S, Takka S. Effect of organic solvents on gemcitabine loaded PLGA nanoparticles. FABAD J Pharm Sci. 2017;42(1):15.

47. Yalcin TE, Ilbasmis-Tamer S, Takka S. Development and characterization of gemcitabine hydrochloride loaded lipid polymer hybrid nanoparticles (LPHNs) using central composite design. Int J Pharm. 2018;548(1):255-262. https://doi.org/10.1016/j.ijpharm.2018.06.063

48. Yildiz A, John E, Özsoy Y, et al. Inhaled extended-release microparticles of heparin elicit improved pulmonary pharmacodynamics against antigen-mediated airway hyper-reactivity and inflammation. J Control Release. 2012;162(2):456-463. https://doi.org/10.1016/j.jconrel.2012.07.008

49. Sahin A, Spiroux F, Guedon I, et al. Using PVA and TPGS as combined emulsifier in nanoprecipitation method improves characteristics and anticancer activity of ibuprofen loaded PLGA nanoparticles. Pharmazie. 2017;72(9):525-528. https://doi.org/10.1691/ph.2017.7015

50. Karal-Yilmaz O, Ozkan A, Akgun E, et al. Controlled release of imatinib mesylate from PLGA microspheres inhibit craniopharyngioma mediated angiogenesis. J Mater Sci Mater Med. 2013;24(1):147-153. https://doi.org/10.1007/s10856-012-4784-2

51. Devrim B, Bozkır A. Preparation and in vitro evaluation of surface-modified poly (lactide-co-glycolide) microparticles as biodegradable drug carriers for pulmonary peptide and protein delivery. J Microencapsul. 2014;31(4):355-362. https://doi.org/10.3109/02652048.2013.858791

52. Elmaskaya A, Ozturk AA, Buyukkoroglu G, Yenilmez E. Spray-dried ketoprofen lysine-incorporated PLGA nanoparticles; formulation, characterization, evaluation and cytotoxic profile. Indian J Pharm Sci. 2019;81(4):640-650. https://doi.org/10.36468/pharmaceutical-sciences.555

53. Öztürk AA, Kırımlıoğlu GY. Preparation and in vitro of characterization lamivudine loaded nanoparticles prepared by acid or ester terminated PLGA for effective oral antiretroviral therapy. J Res Pharm. 2019;23(5):897-913. https://doi.org/10.35333/jrp.2019.37

54. Sengel-Turk CT, Hascicek C, Dogan AL, Esendagli G, Guc D, Gonul N. Surface modification and evaluation of PLGA nanoparticles: the effects on cellular uptake and cell proliferation on the HT-29 cell line. J Drug Deliv Sci Technol. 2014;24(2):166-172. https://doi.org/10.1016/S1773-2247(14)50027-5

55. Şengel-Türk CT, Hasçiçek C, Dogan AL, Esendagli G, Guc D, Gönül N. Preparation and in vitro evaluation of meloxicam-loaded PLGA nanoparticles on HT-29 human colon adenocarcinoma cells. Drug Dev Ind Pharm. 2012;38(9):1107-1116. https://doi.org/10.3109/03639045.2011.641562

56. Sengel C, Hasçiçek C, Gönül N. Design of vitamin E d-α-tocopheryl polyethylene glycol 1000 succinate-emulsified poly (D,L-lactide-co-glycolide) nanoparticles: Influence of duration of ultrasonication energy. J Young Pharm. 2011;3(3):171-175. https://doi.org/10.4103/0975-1483.83754

57. Gundogdu N, Cetin M. Chitosan-poly (lactide-co-glycolide) (CS-PLGA) nanoparticles containing metformin HCl: preparation and in vitro evaluation. Pak J Pharm Sci. 2014;27(6):1923-1929.

58. Orhan Z, Cevher E, Yildiz A, Ahiskali R, Sensoy D, Mülazimoğlu L. Biodegradable microspherical implants containing teicoplanin for the treatment of methicillin-resistant Staphylococcus aureus osteomyelitis. Arch Orthop Trauma Surg. 2010;130(1):135-142. https://doi.org/10.1007/s00402-009-0886-9

59. Turk CT, Oz UC, Serim TM, Hascicek C. Formulation and optimization of nonionic surfactants emulsified nimesulide-loaded PLGA-based nanoparticles by design of experiments. AAPS PharmSciTech. 2014;15(1):161-176. https://doi.org/10.1208/s12249-013-0048-9

60. Gungor S, Okyar A, Erturk-Toker S, Baktir G, Ozsoy Y. Ondansetron-loaded biodegradable microspheres as a nasal sustained delivery system: in vitro/in vivo studies. Pharm Dev Technol. 2010;15(3):258-265. https://doi.org/10.3109/10837450903148257

61. Aygül G, Yerlikaya F, Caban S, Vural İ, Çapan Y. Formulation and in vitro evaluation of paclitaxel loaded nanoparticles. HUJPHARM. 2013;33(1):25-40.

62. Tonbul H, Sahin A, Tavukcuoglu E, Esendagli G, Capan Y. Combination drug delivery with actively-targeted PLGA nanoparticles to overcome multidrug resistance in breast cancer. J Drug Deliv Sci Technol. 2019;54:101380. https://doi.org/10.1016/j.jddst.2019.101380

63. Sahin A, Caban-Toktas S, Tonbul H, Yerlikaya F, Aktas Y, Capan Y. Development of paclitaxel and flurbiprofen co-loaded PLGA nanoparticles: Understanding critical formulation and process parameters using Plackett–Burman design. Istanbul J Pharm. 2019;49(3):161-166. https://doi.org/10.26650/IstanbulJPharm.2019.19036

64. Ozturk K, Fernández Megía E, Novoa Carballal R, et al. Comparative in vitro studies on PBN loaded nanoparticles prepared by biodegradable chitosan, PLGA polymers and their PEGylated block copolymers. J Drug Deliv Sci Technol. 2014;24(2):148-152. https://doi.org/10.1016/S1773-2247(14)50024-X

65. Capan Y, Woo BH, Gebrekidan S, Ahmed S, DeLuca PP. Influence of formulation parameters on the characteristics of poly(D, L-lactide-co-glycolide) microspheres containing poly(L-lysine) complexed plasmid DNA. J Control Release. 1999;60(2-3):279-286. https://doi.org/10.1016/s0168-3659(99)00076-0

66. Devrim B, Bozkir A, Canefe K. Preparation and evaluation of PLGA microparticles as carrier for the pulmonary delivery of rhIL-2 : I. Effects of some formulation parameters on microparticle characteristics. J Microencapsul. 2011;28(6):582-594. https://doi.org/10.3109/02652048.2011.599438

67. Devrim B, Bozkır A, Canefe K. Preparation and evaluation of poly(lactic-co-glycolic acid) microparticles as a carrier for pulmonary delivery of recombinant human interleukin-2: II. In vitro studies on aerodynamic properties of dry powder inhaler formulations. Drug Dev Ind Pharm. 2011;37(11):1376-1386. https://doi.org/10.3109/03639045.2011.576680

68. Cetin M, Aktas MS, Vural I, Ozturk M. Salmon calcitonin-loaded Eudragit® and Eudragit®-PLGA nanoparticles: in vitro and in vivo evaluation. J Microencapsul. 2012;29(2):156-166. https://doi.org/10.3109/02652048.2011.635426

69. Katas H, Chen S, Osamuyimen AA, Cevher E, Oya Alpar H. Effect of preparative variables on small interfering RNA loaded Poly(D,L-lactide-co-glycolide)-chitosan submicron particles prepared by emulsification diffusion method. J Microencapsul. 2008;25(8):541-548. https://doi.org/10.1080/02652040802075567

70. Cevher E, Orhan Z, Sensoy D, et al. Sodium fusidate-poly(D,L-lactide-co-glycolide) microspheres: preparation, characterisation and in vivo evaluation of their effectiveness in the treatment of chronic osteomyelitis. J Microencapsul. 2007;24(6):577-595. https://doi.org/10.1080/02652040701472584

71. Rençber S, Tanrıverdi ST. Terbinafine hydrochloride loaded PLGA nanoparticles for topical administration. Proceedings of the 3rd World Congress on Recent Advances in Nanotechnology (RAN'18). 2018;112:1-6. https://doi.org/10.11159/nddte18.112

72. Sayin B, Calis S. Influence of accelerated storage conditions on the stability of vancomycin-loaded poly (d, l-lactide-co-glycolide) microspheres. FABAD J Pharm Sci. 2004;29:111-116.

73. Ozalp Y, Ozdemir N, Kocagöz S, Hasirci V. Controlled release of vancomycin from biodegradable microcapsules. J Microencapsul. 2001;18(1):89-110. https://doi.org/10.1080/026520401750038638

74. Ozalp Y, Ozdemir N, Hasirci V. Vancomycin release from poly(D,L-lactide) and poly(lactide-co-glycolide) disks. J Microencapsul. 2002;19(1):83-94. https://doi.org/10.1080/02652040110065404

75. Karagoz H, Ulkur E, Kerimoglu O, et al. Vascular endothelial growth factor-loaded poly(lactic-co-glycolic acid) microspheres-induced lateral axonal sprouting into the vein graft bridging two healthy nerves: nerve graft prefabrication using controlled release system. Microsurgery. 2012;32(8):635-641. https://doi.org/10.1002/micr.22016

76. Sipahigil O, Alarçin E, Türkoğlu M, et al. Characterization, cell proliferation and cytotoxicity evaluation of vascular endothelial growth factor loaded poly (lactic-co-glycolic acid) microspheres. Nobel Med. 2012;8(1):77-82.

77. Öğünç Y, Demirel M, Yakar A, İncesu Z. Vincristine and ε-viniferine-loaded PLGA-b-PEG nanoparticles: pharmaceutical characteristics, cellular uptake and cytotoxicity. J Microencapsul. 2017;34(1):38-46. https://doi.org/10.1080/02652048.2017.1282549

78. Öztürk AA, Namlı İ, Güleç K, Kıyan HT. Diclofenac sodium loaded PLGA nanoparticles for inflammatory diseases with high anti-inflammatory properties at low dose: Formulation, characterization and in vivo HET-CAM analysis. Microvascular Research. 2020;130:103991. https://doi.org/10.1016/j.mvr.2020.103991

79. Öztürk AA, Yenilmez E, Şenel B, Kıyan HT, Güven UM. Effect of different molecular weight PLGA on flurbiprofen nanoparticles: formulation, characterization, cytotoxicity, and in vivo anti-inflammatory effect by using HET-CAM assay. Drug Development and Industrial Pharmacy. 2020;46(4):682-695. https://doi.org/10.1080/03639045.2020.1755304

80. Elmaskaya A, Ozturk AA, Buyukkoroglu G, Yenilmez E. Spray-dried ketoprofen lysine-incorporated PLGA nanoparticles; formulation, characterization, evaluation and cytotoxic profile. Indian Journal of Pharmaceutical Sciences, 2019;81(4):640-650. 10.36468/pharmaceutical-sciences.555

81. Öztürk AA, Namlı İ, Aygül A. Cefaclor Monohydrate-Loaded Colon-Targeted Nanoparticles for Use in COVID-19 Dependent Coinfections and Intestinal Symptoms: Formulation, Characterization, Release Kinetics, and Antimicrobial Activity. ASSAY and Drug Development Technologies. 2021;19(3):156-175. https://doi.org/10.1089/adt.2020.1014

82. Ekinci M, Öztürk AA, Santos-Oliveira R, İlem-Özdemir D. The use of Lamivudine-loaded PLGA nanoparticles in the diagnosis of lung cancer: Preparation, characterization, radiolabeling with 99mTc and cell binding. Journal of Drug Delivery Science and Technology. 2022;69:103139. https://doi.org/10.1016/j.jddst.2022.103139

83. Öztürk AA. Deksketoprofen trometamol yüklü kontrollü salım gösteren nanoboyutta ilaç taşıyıcı sistem geliştirilmesi ve in Vitro/in vivo değerlendirilmesi [dissertation]. Anadolu Üniversitesi, Sağlık Bilimleri Enstitüsü, Eskişehir, Türkiye; 2019.

84. Öztürk AA, Yenilmez E. Pharmacological and pharmaceutical technological overview for seborrheic dermatitis: A review about topical application and new approaches. ACTA Pharmaceutica Sciencia. 2018;56(4):57-80. https://doi.org/10.23893/1307-2080.APS.05626

85. Güven UM, Öztürk AA, Yenilmez E. Evaluation of carvedilol-loaded Eudragit® nanoparticles. J Res Pharm. 2020;24(1):71-81. https://doi.org/10.35333/jrp.2020.116

86. Öztürk AA, Arpagaus C. Nano Spray-Dried Drugs for Oral Administration: A Review. ASSAY and Drug Development Technologies. 2021;19(7):412-441. https://doi.org/10.1089/adt.2021.053

87. Öztürk AA, Yenilmez E, Arslan R, Şenel B, Yazan Y. Dexketoprofen trometamol loaded solid lipid nanoparticles (SLNs): Formulation, in vitro and in vivo evaluation. J Res Pharm. 2020;24(1):82-99. https://doi.org/10.35333/jrp.2020.114

88. Öztürk AA, Namlı İ, Güleç K, Görgülü Ş. Design of lamivudine loaded nanoparticles for oral application by nano spray drying method: a new approach to use an antiretroviral drug for lung cancer treatment. Combinatorial Chemistry & High Throughput Screening. 2020:23(10):1064-1079. https://doi.org/10.2174/1386207323666200325155020

89. Öztürk AA, Güven U. Cefaclor monohydrate loaded microemulsion formulation for topical application: Characterization with new developed UPLC method and stability study. J Res Pharm. 2019;23(3):426-440. https://doi.org/10.12991/jrp.2019.150

90. Öztürk AA, Gündüz AB, Ozisik O. Supervised machine learning algorithms for evaluation of solid lipid nanoparticles and particle size. Combinatorial Chemistry & High Throughput Screening. 2018;21(9):693-699. https://doi.org/10.2174/1386207322666181218160704

91. Öztürk AA, Güven U, Yenilmez E, Şenel B. Effects of Different Derivatives of Eudragit Polymer on Entrapment Efficiency, In Vitro Dissolution, Release Kinetics and Cell Viability Results on Extended Release Flurbiprofen Loaded Nanomedicines. Lat Am J Pharm. 2018;37(10):1981-1992.

92. Öztürk AA, Martin-Banderas L, Cayero-Otero MD, Yenilmez E, Yazan Y. New approach to hypertension treatment: carvediol-loaded PLGA nanoparticles, preparation, in vitro characterization and gastrointestinal stability. Lat Am J Pharm. 2018;37(9):1730-1741.

93. Öztürk AA, Çınar Nİ, Yenilmez E. Development of nano-sized ketoprofen lysine incorporated Eudragit (R) S100 nanomedicine by double emulsion solvent evaporation and in vitro characterization. J Pharm Pharmacogn Res. 2019;7(1):47-58.