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Geranium tuberosum Metanol Ekstraktının Enzim İnhibitör Özellikleri ve Antimikrobiyal Aktivitesi

Züleyha ALMAZ[1*]

Öne Çıkanlar:

- *Geranium tuberosum* 'un kök ve yaprak kısımlarının metanol ekstraktları hazırlanmıştır.
- Her ekstrakt için antimikrobiyal aktivite ve enzim inhibitör deneyleri yapılmıştır.
- Genel olarak her iki ekstraktta güçlü aktivitelere sahiptir.

Anahtar Kelimeler:

- *Geranium tuberosum*
- Asetilkolinesteraz
- Bütirilkolinesteraz
- Ksantin Oksidaz
- Antimikrobiyal

ÖZET:

Geranium tuberosum kökleri Türkiye'de bazı kırsal bölgelerde taze olarak tüketilmektedir. Bu çalışma, türün geleneksel kullanımını bilimsel olarak ta aydınlatmak için yapılmıştır. *Geranium tuberosum*'un kök ve yaprak kısımlarının enzim inhibisyon etkisi ve antimikrobiyal aktivitesi, metanolik ekstraktlar kullanılarak araştırıldı. Yaprak ekstresi, kök ekstresine göre daha yüksek asetilkolinesteraz ve butirilkolinesteraz aktivitesine sahipken, kök ekstresi ksantin oksidaz enzimi üzerinde daha güçlü inhibitör etki gösterdi. Ayrıca ekstraktların antifungal ve antibakteriyel aktiviteleri içi boş agar tekniği kullanılarak araştırıldı. Her iki ekstraktın da farklı bakteri suşları üzerinde yüksek antimikrobiyal aktivite gösterdiği gözlendi. Bu sonuçlar *Geranium tuberosum*'un doğal bir antibakteriyel olduğunu ve enzim inhibe etme potansiyeline sahip olduğunu doğruladı.

Enzyme Inhibitory Properties and Antimicrobial Activity of *Geranium tuberosum* Methanol Extract

Highlights:

- Methanol extracts of the root and leaf of *Geranium tuberosum* were prepared.
- Antimicrobial activity and enzyme inhibitory experiments were performed for each extract.
- Overall, both extracts had potent activities.

Keywords:

- *Geranium tuberosum*
- Acetylcholinesterase
- Butyrylcholinesterase
- Xanthine Oxidase
- Antimicrobial

ABSTRACT:

Geranium tuberosum roots are consumed fresh in some areas of the countryside of Turkey. This study was conducted to scientifically validate the traditional use of the species. The enzyme inhibition effect and antimicrobial activity of the root and leaf parts of *Geranium tuberosum* were investigated using a methanol extract. The leaf extract had higher acetylcholinesterase and butyrylcholinesterase activity than the root extract, while the root extract showed a stronger inhibitory effect on xanthine oxidase enzyme.. Also, the antifungal and antibacterial activities of the extracts were investigated using the hollow agar technique. It was observed that both extracts gave high antimicrobial activity on different bacterial strains. These results confirmed that *Geranium tuberosum* is a natural antibacterial, and has enzyme inhibitory potential.

[1] Züleyha ALMAZ (**Orcid ID:** 0000-0002-4532-4311), Muş Alparslan University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Muş, Türkiye

***Sorumlu Yazar/*Corresponding Author**: Züleyha ALMAZ, e-mail: z.turkoglu@alparslan.edu.tr

INTRODUCTION

The use of herbal medicines for therapeutic purposes on various patients has a very old history all over the world and the interest in natural treatments is increasing significantly. In addition to its use as natural medicine, edible wild plants associated with poverty and famine and seen as a last resort in these cases have now become popular in developed countries. Scientific studies on traditional species are needed in order not to lose the existing traditional knowledge about wild medicinal plants, which is transmitted only orally, to contribute to the conservation of biological diversity, and to prove the importance of these edible plants (Graça et al., 2020; Newman & Cragg, 2016). The genus *Geranium*, which is medically important and the leaves and tubers of some species can be consumed as food, includes about 400 species that are distributed in temperate regions in most of the world (İlçim et al., 2008; Şöhretoğlu et al., 2008). This number is 35 in the flora of Turkey (Şöhretoğlu et al., 2012). A significant number of *Geranium* species are used as tonic, diuretic, hemorrhoid, diabetes and antidiarrheal in folk medicine which is reported a decade ago (Sabuncuoğlu & Şöhretoğlu, 2012). Some of these features have been highlighted in various scientific studies over the past two decades. It is possible to find many studies examining the various biological characteristics of a big amount of this genus in the literature. For example, ringworm, diarrhea, hypotensive agent, central depression, cancer, cardiovascular, skin, gastrointestinal ulcers, antibacterial, and antiviral activities have been reported (Kosuge et al., 1985; Ivancheva & Stantcheva, 2000; Chalchat et al., 2002; Williamson, 2002; González-Tejero et al., 2008; Ngezahayo et al., 2015; Oh et al., 2015;).

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a decline in learning abilities that interferes with personal activities in older people and has no clinical treatment to halt its progression (Güleç et al., 2022). AD, one of the most common causes of death in the elderly population in developed countries, gradually destroys memory and thinking skills (Anil et al., 2022). Since the most dramatic abnormalities in this disease result from loss of cholinergic conduction, they are mainly associated with changes in cholinesterase (ChE) metabolism. While acetylcholinesterases (AChE) hydrolyze acetylcholine to a large extent, butyrylcholinesterases (BChE) play a complementary role (Almaz et al., 2021). Anti-AD drugs prescribed as ChE inhibitors developed so far act by increasing acetylcholine levels by inhibiting these enzymes (Sever et al., 2021; Yaşar et al., 2021). However, since these drugs have many side effects, there is a need to develop anti-ChE drugs of natural origin (Mahmudov et al., 2022). Galantamine was the first compound to be a potent AChE inhibitor isolated from a plant source, but inhibitors such as tacrine, donepezil, and physostigmine are used synthetically (Khan et al., 2018).

Hyperuricemia associated with gout, which has a worldwide distribution, is due to the overproduction or under-excretion of uric acid. This disease, which can be prevented by lowering uric acid plasma levels, can be treated with uricosuric drugs that increase urinary excretion of uric acid or xanthine oxidase (XO) inhibitors, which block the terminal step in uric acid biosynthesis (Nguyen et al., 2004). XO catalyzes the oxidation of xanthine and hypoxanthine to uric acid. Allopurinol, an XO inhibitor used clinically in the treatment of gout, has many side effects such as nephropathy, hepatitis, and allergic reactions (Osada et al., 1993; Ishibuchi et al., 2001).

The use of herbal medicines, which is one of the elements of complementary and alternative medicine, is enhancing swiftly all around the countries. For this reason, scientific evaluation and verification of the properties of these plants, which are also used in traditional medicine, is one of the main goals. Some biological activity studies of *Geranium tuberosum* species have been informed

(Şöhretoğlu et al., 2008; Şöhretoğlu et al., 2009). The current study was planned to evaluate the antimicrobial and enzyme inhibitory activities of *G. tuberosum*, on underexplored species.

MATERIALS AND METHODS

Chemicals

Acetylcholinesterase (AChE): lyophilisate from *Electrophorus electricus* (electric eel), Butyrylcholinesterase (BChE): lyophilisate from equine serum, Xanthine oxidase (XO): lyophilisate from bovine milk were provided from Sigma-Aldrich (St. Louise, MO), and stored at −80°C. Acetylthiocholine iodide (ASCh, substrate for the AChE inhibition assay), S-butyrylthiocholine iodide (BSCh, substrate for the BChE inhibition assay), Xanthine (substrate for the XO inhibition assay), DTNB (5,5′dithiobis-2-nitrobenzoic acid, Ellman's reagent) were obtained from Sigma-Aldrich (St. Louis, MO), Merck and Acros Organics.

Preparation of Plant Samples

G. tuberosum samples were gathered from Elazığ province during the vegetation period in 2021 (38° 57' 47.0916" N; 38° 38' 7.5588" E) (Figure 1). Classification of the collected plant samples with respect to the flora of Turkey was made by Murat Kürşat (Bitlis, Turkey).The coded (Z. Almaz: 2300) plant sample was turned into herbarium material. The roots and leaves of the plant were removed and left to dry in shade. *G. tuberosum* leaf and root were prepared in 350 mL of methanol by the Soxhlet extraction method in the central laboratory of the Muş Alparslan University. Methanol was eliminated by evaporation. The samples were tagged as methanolic leaf (*Gt*L(MeOH)), methanolic root (*Gt*R(MeOH)).

Figure 1. Turkey / Elazig / Agin images by *G. tuberosum*

Determination of Enzyme Inhibition Study

The enzyme activity of XO, in which xanthine used as a substrate, was measured spectrophotometrically using a modification (Tan et al., 2022) of the protocol used by Noro by screening uric acid formation from xanthine at 294 nm (Noro et al., 1983). The inhibition of this enzyme was found by measuring the amount of uric acid, and firstly, various concentrations of the extracts were incubated

at 37°C for 10 minutes with buffer (50mM) and enzyme (0.2U). Then, the reaction was begun by adding 1 mM substrate prepared daily. The measurement of the absorption of the reaction mixture was carried out via the Agilent Cary 60 UV–Vis Spectrophotometer. The standard compound allopurinol was used for XO enzyme inhibition and the IC_{50} value was determined by the reduction in the amount of uric acid formed. Inhibitory activities of extracts against cholinesterase enzymes were measured by comparison with the standart compound galantamine with an modification of Ellman's technique (Ellman et al., 1961) as previously expressed (Almaz et al., 2021; Köse & Gulcin, 2017). First, 1 mg of each extract was weighed and dissolved in 1 ml of DMSO, and then diluted in distilled water at different concentrations. To determine the inhibitory activity of the enzymes, 5 serial dilutions were carried out as described in the previous study (Almaz, 2023). Absorbance measurement was made within 5 minutes at 412 nm using a Thermo Fisher Scientific Multiskan Go Finland. Percent activity-[I] was plotted to decide the inhibition potential of the extracts on cholinesterases and XO. IC_{50}values were determined by the plotted graphs.

Antimicrobial Activity Assay

The antifungal and antibacterial activities of the extracts were found by using the hollow agar method (Sagdic et al., 2003). *G. tuberosum* extracts were separately tested against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 70063), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (NCTC 5348) bacteria and *Saccharomyces cerevisiae* (ATCC 834), *Candida albicans* (ATCC 10231) fungi. The bacterial and fungal strains investigated in this work were provided by Yusuf ALAN in Bitlis Eren University. For this purpose, the tested microbial strains were cultured overnight in Nutrient Broth medium at 37 °C and in Sabourand 2% Glucose Broth at 27 °C, respectively. Then, these media were rushed into petri dishes. 9 mm diameter wells were opened in agar after the medium had solidified. Extracts prepared at concentrations of 40 µg/mL, 80 µg/mL, 100 µg/mL dissolved in DMSO were filled into the wells and the petri dishes were incubated for 24 hours at 37 °C and 48 hours at 27 °C for bacterial and fungal strains, respectively (Aras et al., 2018). The diameters average inhibition zones formed were measured after repeating the assay for three times. The inhibition zone values were compared with 5 different standard antibiotic discs used as controls (erythromycin (15), gentamicin (10), amikacin (30), ampicillin (10), and fluconazole) to compare the antimicrobial activities of the extracts.

Statistical Analyses

The antimicrobial activity results determined by the zone diameters formed were compared among themselves with standard antibiotics (Erythromycin, Ampicillin, Gentamicin, Amikacin, and Fluconazole) by using Tukey's Multiple Comparison and t-test after One-Way ANOVA. Those with $p > 0.05$ were not considered statistically significant and $p < 0.05$ were considered statistically significant. The statistical significance levels are designated with the symbol "*" as follows:
$p > 0.05$; ns, $0.01 > p < 0.05$; *, $0.001 > p < 0.01$; **, $0.0001 > p < 0.001$; ****.

RESULTS AND DISCUSSION

Discovering and identifying inhibitors of natural catalysts required for all biochemical processes is the first phase of drug discovery research. A healthy human body has normal levels of enzymes, but when enzymes are overexpressed, abnormal biological processes can occur. Due to the negative side effects of synthetic drugs, the importance of medicinal plants is increasing day by day. Plant-derived inhibitors are being studied because of the significant enzyme inhibition potential of compounds isolated from plants (Bayrak et al., 2020; Öztürk et al., 2022). The activity of enzymes related to Alzheimer's

disease (AChE) and (BChE) and Gout disease (XO) of *G. tuberosum* methanolic extracts were studied and the results are given in Table 1.

Table 1. Anticholinesterase and Xanthine oxidase activity of *G. tuberosum* extracts

G. tuberosum	**AChE**		**BChE**		**XO**	
Extracts	**IC_{50} value**	**R^2**	**IC_{50} value**	**R^2**	**IC_{50} value**	**R^2**
***Gt*L(MeOH)**	4.03	0.904	7.81	0.928	7.46	0.911
***Gt*R(MeOH)**	4.72	0.947	8.44	0.918	4.38	0.904
Galantamine	0.198	0.978	1.734	0.967		
Allopurinol					1.102	0.978

*Galantamine was used as positive control for AChE and BChE enzymes and allopurinol was used as positive control for XO enzyme and determined as µg/mL levels. The enzyme inhibitory activity of the extracts was tested at a concentration of 1 mg/mL

Anticholinesterase activities of *G. tuberosum* extracts were calculated by their inhibitory capacity on AChE and BChE enzymes as mentioned. According to the data of the study, it was found that the extracts showed strong AChE and BChE inhibitory effects. It was found that the methanol leaf extract had the highest IC_{50} values for AChE and BChE, and these values were calculated as 4.03 (r^2: 0.904) and 7.81 (r^2: 0.928), respectively. When the IC_{50} values for AChE and BChE of the standard molecule galantamine were calculated as 0.198 (r^2: 0.978) and 1.734 (r^2: 0.967), it was observed that extracts exhibited moderate anticholinesterase activity. In the study with *Geranium pyrenaicum* Burm. f., it was found that it showed high anti-AChE (4.49 mg GALAE/g) activity with ethyl acetate and methanol extract, and strong anti-BChE (12.26 mg GALAE/g) activity with ethyl acetate extract. ChE inhibitory activity was expressed as galantamine equivalents (mgGALAE/g extract) (Świątek et al., 2021).

In addition, the extracts were compared with allopurinol, a standard XO inhibitor widely used in the treatment of gout, and it was found to have moderate inhibitory activity. It was determined that root extract 4.38 (r^2: 0.904) showed more effective inhibition against XO enzyme than leaf extract 7.46 (r^2: 0.911). In the study examining the XO inhibitory effect of polyphenolic compounds extracted from *Geranium sibiricum*, they reported that the IC_{50} values were lower than the standard allopurinol and in the range of 0.87 to 2.53 µM (Wu et al., 2010). It was determined that xanthine oxidase inhibition of ellagic acid obtained from *Geranium wilfordii* Maxim was higher than that of allopurinol (Liu, Mei, Xiao, & Liu, 2020).

The effects of the antibiotics used for control purposes on microorganisms are given in Table 2. The antimicrobial activities of *Gt*L(MeOH) and *Gt*R(MeOH) extracts of *G. tuberosum* against *E. coli, K. pneumoniae, P. aeruginosa, E. faecalis, L. monocytogenes, S.aureus, C. albicans,* and *S. cerevisiae* microorganisms were determined in mm (Table 3).

Table 2. Antimicrobial effects of antibiotic discs on test microorganisms

***MIC.**		**Erythromycin (15 µg)**	**Ampicillin Sulbactam (10 µg)**	**Gentamicin (10 µg)**	**Amikacin (30 µg)**	**Fluconazole (25 µg)**
GR + bacteria	**B1**	30.33±0.57	30.33±0.57	15.00±1.00	20.67±0.57	0.00±0.00
	B2	10.67±1.52	12.67±0.58	10.67±0.58	13.33±0.57	0.00±0.00
	B3	7.50±0.50	14.00±0.00	8.00±0.00	7.50±0.50	0.00±0.00
GR – bacteria	**B4**	0.00±0.00	0.00±0.00	13.33±0.58	15.33±0.58	0.00±0.00
	B5	0.00±0.00	0.00±0.00	15.33±0.57	22.00±1.00	0.00±0.00
	B6	10.33±0.58	0.00±0.00	15.00±1.00	15.67±0.58	0.00±0.00
Fungus	**F7**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	30.33±0.58
	F8	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.33±0.57

* B1: *S. aureus*; B2: *E. faecalis*; B3: *L. monocytogenes*; B4: *E. coli*; B5: *P. aeruginosa*; B6: *K. pneumoniae*; F7: *C. albicans*; F8: *S. cereviciae,* * Diameter of standard discs 5mm

Among the extracts, it was found that the methanol extract indicated high antimicrobial activity against gram-negative bacteria, *E.coli (*30.33±0.57), *P. aeruginosa (*27.67±1.52), and *K. pneumoniae* (30.67±0.57). However, this extract did not show antibacterial activity against *E. faecalis* and *L. monocytogenes*. It was determined that the *Gt*L(MeOH) extract exhibited better antibacterial activity against *S. aureus* (25.33±1.52) and *Gt*R(MeOH) extract against *E. faecalis* (23.33±0.58), which are Gram-positive bacteria. It was determined that *Gt*R(MeOH) extract indicated the best activity against *E. faecalis* (23.33±0.58) and it was determined that this extract showed high antibacterial activity against this bacterium than all antibiotics $p<0.001$ (highly important).

No activity against *L. monocytogenes* and fungi was observed in any of the two extracts. In addition, it was observed that the activities of the extracts increased depending on the increasing concentration in general. It was determined that *Gt*L(MeOH) extract showed high antibacterial activity against *K. pneumoniae* bacteria than all antibiotics ($p<0.001$).

Table 3. Effect of *G. tuberosum* extracts on test microorganisms

*MIC.		*Gt*L(MeOH)			*Gt*R(MeOH)		
		40 µg/mL	80 µg/mL	100 µg/mL	40 µg/mL	80 µg/mL	100 µg/mL
GR (+) bacteria	B1	18.00±0.00^{eeede}	20.33±0.57^{eeeae}	25.33±1.52^{ddede}	15.33±0.58^{eeaee}	21.00±1.00^{eeeae}	25.00±1.00^{eeede}
	B2	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}	14.67±0.58^{ebeae}	20.33±0.58^{eeeee}	23.33±0.58^{eeeee}
	B3	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}	0.00±0.00^{eeeea}
GR (-) bacteria	B4	19.33±1.15^{eeeee}	29.00±2.00^{eeeee}	30.33±0.57^{eeeee}	0.00±0.00^{aaeea}	15.00±1.00^{eeaae}	21.00±0.00^{eeeee}
	B5	15.33±0.57^{eeaee}	25.33±1.53^{eeece}	27.67±1.52^{eeeee}	14.33±0.58^{eeaee}	20.33±1.52^{eeeae}	24.33±0.57^{eeebe}
	B6	17.00±1.00^{eebae}	26.67±1.15^{eeeee}	30.67±0.57^{eeeee}	0.00±0.00^{eaeea}	14.67±0.57^{ceaae}	25.00±1.00^{eeeee}
Fungus	F7	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}
	F8	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}	0.00±0.00^{aaaae}

* MIC: Microorganisms; GR+ (GR positive), GR- (GR negative), B1: *S. aureus*; B2: *E. faecalis*; B3: *L. monocytogenes*; B4: *E. coli*; B5: *P. aeruginosa*; B6: *K. pneumoniae*; F7: *C. albicans*; F8: *S. cereviciae*; ns: a; *: b; **: c; ***: d ; ****: e

In a study in which the antimicrobial activity of *Geranium thunbergii* was investigated using the minimum inhibitory concentration (MIC) test and the paper disc method, it was reported that the ethyl acetate fraction showed higher antimicrobial activity than the others with the inhibition zone diameters ranging from 13.33 to 15.67 mm. *S. aureus* bacterial strain (15.67±1.04) showed the highest antimicrobial activity, which is similar to the current study (Kwon et al., 2017). It was reported that the leaf extract of *G. macrorrhizum* was not susceptible to pathogenic *C. albicans* (Radulović et al., 2012). A group of researchers examined the antimicrobial effect of *G. wallichianum* and found that the ethyl acetate extract showed a strong effect against *K. pneumonia* bacteria with a value of 25 µg/ml, but showed less antimicrobial activity against fungal strains such as *C. albicans* (Mir et al., 2022).

CONCLUSION

The genus Geranium includes species with scientifically documented biological activities, well known for their traditional uses worldwide. In addition, it develops information about antimicrobial and enzyme-inhibitory effects. However, in order to benefit from these traditional plants medicinally, it is necessary to evaluate the properties of different extracts and support them with in vitro and in vivo studies. Wild plants can be considered a source of with functional properties, not only for their pharmacological properties but also for applications in the food industry. Therefore, more reliable information is needed for their safety and effectiveness. The data from the present study suggest that in the future this plant may serve as fundamental scientific research data for its possible use as a natural source of antibacterial, anticholinesterase, and xanthine oxidase inhibitors.

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Conflict of Interest

The author declared that there is no conflict of interest.

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