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Antimicrobial Activities and Enzyme Inhibition Effects of *Nepeta* Species

Semiha YENIGUN[1], Yunus BASAR[2], Yaşar IPEK[3], Mesut GOK[4], Lutfi BEHCET[5], Ibrahim DEMİRTAS[2,6], Tevfik OZEN[1*]

[1] Department of Chemistry, Faculty of Science, Ondokuz Mayıs University, 55139, Samsun, Türkiye
[2] Research Laboratories Application and Research Center (ALUM), University of Igdir, 76000, Igdir, Türkiye
[3] Department of Biochemistry, Faculty of Science, University of Cankiri Karatekin, 18100, Cankiri, Türkiye
[4] Science and Technology Application and Research Center, University of Siirt, 56210, Siirt, Türkiye
[5] Department of Molecular Biology and Genetics, Faculty of Science and Art, University of Bingol, Bingol, Türkiye
[6] Department of Pharmacy Vocational Sciences, Faculty of Pharmacy, Ondokuz Mayıs University, Samsun, Türkiye

*Corresponding author: tevfikoz@omu.edu.tr

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Abstract

Various *Nepeta* species, widely used among the public, have valuable phytochemical contents and clinical and biological activities. For this reason, our study examined the enzyme inhibition and antibacterial properties of methanol: chloroform (1:1) extracts of six *Nepeta* species. *N. aristata* showed a higher inhibitory effect than the standard drug on seven of the eight enzymes studied. *N. baytopii* had a high inhibition effect on urease and lipase. It was determined that *N. italica* inhibited other enzymes except for urease, CA, and lipase. In addition, BChE is also the only effective plant. *N. nuda* subsp. *albiflora* has a high effect on inhibiting urease, AChE, and lipase. *N. stenantha* and *N. trachonitica* also showed inhibition effects on urease, AChE, and tyrosinase. In the disc diffusion method of antibacterial activity, extracts against *B. cereus* had antibacterial activity. The antimicrobial activity of *N. aristata* extract was effective against *P. aerugonisa* and *K. pneumoniae*. Additionally, when looking at the minimum inhibition concentration method of antibacterial activity, *Nepeta* extracts were effective against most bacteria. This research determined *Nepeta* extracts are effective natural products with antioxidant and enzyme inhibition activities.

Key Words: *Nepeta* species, bioactivity, enzyme inhibition activity, antibacterial activity

1. Introduction

The *Nepeta* genus, which has the largest place in the Lamiaceae family, contains three hundred species and is used by humans for medicinal purposes in many countries, including Turkey (Sharma et al., 2021). There are 40 different taxa in Turkey, and 19 of them are endemic. Most of *Nepeta* species in Turkey are used to treat many diseases such as stomach, bacteriostatic, diuretic, skin diseases, infusion, and nauseation (Baytop, 1999). Enzymes are among the biological macromolecules that are regarded as potential therapeutic targets. As of late, almost half of all pharmaceuticals utilized in clinical settings are enzyme inhibitors (Copeland, 2005). The inhibitions of certain

Nepeta species' extracts against various enzymes were investigated for this reason. *N. baytopii* (Zengin et al., 2021), *N. italica* (Acquaviva et al., 2023), *N. cadmea* and *N. nuda* subsp. *glandulifera* (Sarikurkcu et al., 2019), and so on are a few examples. Antimicrobial agents are substances, either manufactured or natural, that eliminate or stop germs' reproduction (Moreno et al., 2000). Numerous *Nepeta* species, including *N. trachonitica* (Köksal et al., 2017), *N. distans* (Alkahtani et al., 2022), etc. are among these plants.

A limited number of studies have been conducted on the antibacterial and enzyme inhibition activities of *N. baytopii* (Zengin et al., 2021), *N. italica* (Acquaviva et al., 2023), *N. stenantha* (Kazemi et al., 2016), and *N. trachonitica* (Köksal et al., 2017). Therefore, in this study, the enzyme inhibition effects and antibacterial activities of methanol:chloroform extracts of the previously unstudied *N. aristata*, and *N. nuda* subsp. *albiflora*, and the few studied.

N. baytopii, N. italica, N. stenantha, and *N. trachonitica* were investigated. Enzyme inhibitions of the extracts were determined using carbonic anhydrase (CA), urease, acetylcholinesterase (AChE), lipase, butyrylcholinesterase (BChE), α-amylase, tyrosinase and α-glucosidase enzymes. In addition, the antibacterial activities of these extracts were examined against six different bacteria by disk diffusion and minimum inhibition concentration methods. The enzyme inhibition effects and antibacterial activities of *Nepeta* plants, which have not been studied much, were evaluated by comparing them with each other, standard substances and drugs.

2. Material and Methods

2.1. Chemicals

Urease from *Jack bean*, AChE from *Electrophorus electrius*, α-amylase from *Porcine pancreas*, BChE from *Horse serum*, lipase from *Porcine pancreas*, α-glucosidase from *Saccharomyces cerevisiae*, tyrosinase from mushroom, galantamine, CA from *Bovine erythrocytes*, 5,5′-dithiobis(2-nitrobenzoic acid), NaOCl, sodium nitroprusside, phenol, NaOH, urea, orlistat, starch, iodide, acetazolamide, *p*-nitrophenyl acetate, *p*-nitrophenyl octanoate, thiourea, amoxicillin, 3,4-dihydroxy-L-phenylalanine, tetracycline, $MgCl_2.6H_2O$ and $CaCl_2.2H_2O$, NaCl, Trisma, HCl, K_2HPO_4, KH_2PO_4, Na_2HPO_4 from Sigma-Aldrich; acarbose from TCI; Muller Hinton Agar (MHA), and Muller Hinton II Broth (MHB) from Himedia; kojic acid from Gelentham.

2.2. Extraction and Plant Materials

While five *Nepeta* L. species were gathered from the Bingöl province in Turkey's Eastern Anatolia Region, one was collected from the Ağrı province. These plant species were described in the 7th volume of the book "Flora of Turkey and the Eastern Aegean Islands" (Hedge & Lamond, 1982). The identification of plants Prof. Dr. It was made by Lütfi Behçet and kept in the Herbarium of Bingöl University, Department of Biology (Yenigün et al., 2023).

The aerial parts of *Nepeta* species were cleaned and dried in a calm and dark laboratory environment. Dried plant samples (624-1120 g) were ground to powder with a laboratory grinder. The high efficiency of the chloroform/methanol solvent mixture is widely used to extract of wide range of plants. The solvent mixture is used because it is the most suitable solvent mixture required for obtaining secondary metabolites in plants and for the fractionation process (Başar et al., 2023; Yenigun et al., 2024; Yenigün et al., 2023). Then, methanol: chloroform (1:1, 9-15 L) solvent mixture was added and kept closed. This process was repeated three times at one-week intervals. A rotary evaporator operating at +40 °C was used to extract the solvent mixture after the mixture had been filtered through Whatman no. 1 filter paper. In order to prepare the acquired

crude extracts for additional tests and analyses, they were dried using a lyophilizer device until they were transformed into a powder and then kept in a refrigerator at -20°C (Yenigün et al., 2023).

2.3. Enzyme Inhibition Activities

The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against urease (Zhang et al., 2006) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 10 µL of samples of different concentrations or thiourea as standard into each well, 25 µL of 1 U urease (in 100 mM pH 8.2 sodium-phosphate buffer) and 50 µL of 100 mM urea solution were mixed until homogeneous and incubated for 15 minutes at 30°C. It was kept waiting for minutes. 45 µL of phenol reagent [8% (w/v) phenol and 0.1% (w/v) sodium nitroprusside] and 70 µL of alkaline reagent [2.5% (w/v) NaOH and 4.7% NaOCl] solutions were added to the mixture in each well. The samples were kept at 30°C for 50 minutes. The absorbance values of each well were measured at 630 nm and IC_{50} (µg/mL) values were determined.

The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against AChE and BChE (Ellman et al., 1961) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 20 µL of samples of different concentrations or galantamine, 20 µL of 0.03 U AChE or BChE (in 100 mM pH 8.0 phosphate buffer), 20 µL of 3.3 mM DTNB, and 140 µL of 100 mM pH 8.0 phosphate buffer were added to each well and mixed until homogeneous. and left at room temperature for 15 minutes. 10 µL of 1 mM ATCh (acetylthiocholine iodide) or B solution was added to the mixture in each well. The absorbance values of each well were measured at 412 nm and IC_{50} (µg/mL) values were determined.

The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against CA (Chanda et al., 2019) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 60 µL of samples of different concentrations or acetazolamide and 90 µL of 115 U CA solution (in 0.05 M pH 7.4 Tris-SO4 buffer) were mixed into each well until homogeneous and left at room temperature for 15 minutes. 60 µL of 10 mM 4-nitrophenyl acetate solution was added to the mixture in each well and left at room temperature for 15 min. The absorbance values of each well were measured at 400 nm and IC_{50} (µg/mL) values were determined.

The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against α-amylase (Yang et al., 2012) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 82 µL of samples of different concentrations or acarbose and 10 µL of 1 U α-amylase (in 20 mM pH 6.9 potassium phosphate buffer) solution were mixed into each well until homogeneous and kept at 37°C for 10 min. 8 µL of 1% starch solution was added to the mixture in each well and kept at 37°C for 12 minutes. 50 µL 10% HCl and 15 µL iodine-KI (2.5 mM iodine+6.5 mM KI) solutions were added. The samples were kept in boiling water for 10 minutes and the absorbance values of each well were measured at 620 nm and IC_{50} (µg/mL) values were determined.

The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against lipase (Trentin et al., 2020) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 20 µL of samples at different concentrations or orlistat, 200 µL of 100 mM

pH 8.2 Tris-HCl buffer and 20 µL of 1 mg/mL lipase (in 100 mM pH 8.2 Tris-HCl buffer) solution were added to each well and mixed until homogeneous. 20 µL of 5.1 mM p-nitrophenyl octanoate (100 mM pH 8.2 in Tris-HCl buffer) solution was added to the mixture in each well and kept at 37°C for 30 min. The absorbance values of each well were measured at 410 nm, and IC_{50} (µg/mL) values were determined. The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against α-glucosidase (Mayur et al., 2010) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 10 µL of samples of different concentrations or acarbose and 25 µL of 0.2 U α-glucosidase (in 20 mM pH 6.9 potassium phosphate buffer) solution were mixed into each well until homogeneous. 25 µL of 0.5 mM p-NPG and 20 µL of 20 mM pH 6.9 potassium phosphate buffer were added to the mixture in each well. The samples were kept at 37°C for 12 minutes. 100 µL of 0.2 M Na2CO3 solution was added to the mixture in each well and mixed until homogeneous. The absorbance values of each well were measured at 410 nm, and IC_{50} (µg/mL) values were determined.

The possible inhibition activities of the *Nepeta* extracts (1024–0.5 µg/mL) against tyrosinase (Addar et al., 2019) inhibitory activity were measured by previously described methods with slight modifications (Başar et al., 2023). In a 96-well plate, 10 µL of samples of different concentrations or kojic acid were into each well, 20 µL of 150 U tyrosinase (in 0.1 M pH 6.8 potassium phosphate buffer) and 20 µL of 0.1 M pH 6.8 potassium phosphate buffer were mixed until homogeneous and incubated at 37°C. It was kept for 10 minutes. 20 µL of 5 mM L-DOPA solution was added to the mixture in each 96-well. The absorbance values of each well were measured at 475 nm, and IC_{50} (µg/mL) values were determined.

2.4. Antibacterial Activity

Three gram-positive (*Bacillus cereus* CCM99, *Staphylococcus aureus* ATCC 25213, *Enterococcus faecalis* ATCC 29212) and three gram-negative (*Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 25922, *Psedomonas aeruginosa* ATCC 15442) bacteria were tested by the *Nepeta* extracts for antibacterial activity. In the Biochemistry Research Laboratory (Ondokuz Mayis University, Department of Chemistry), both newly developed and pre-existing microorganisms were cultivated.

2.4.1. Disc diffusion method (DDM)

The agar DDM was used with an MHA (Mueller Hinton Agar) medium and incorporated into the MHA for *Nepeta* extracts diffusion (Reller et al., 2009). The turbidity of the newly cultured bacteria was adjusted to 0.5 McFarland standards (10^8 CFU/mL). 0.5 McFarland gram-negative and positive bacteria were spread on MHA. After impregnating 6 mm sterile discs with 40 µL of extracts or antibiotics (amoxicillin and tetracycline), they incubated at 37 °C for 16-18 hours. The antimicrobial activity of the apparent transparent zone of inhibition diameter was measured around the wells and compared with antibiotic drugs to evaluate the sensitivity of the strains.

2.4.2. Minimum inhibition concentration (MIC) method

The antimicrobial activities of *Nepeta* extracts using the MIC method were performed for each bacterium (Andrews, 2001). The activity was performed using 96-well microplates in a cationic MHB medium containing $MgCl_2.6H_2O$ and $CaCl_2.2H_2O$. Before applying dilution, 100 µL of cationic MHB and 100 µL of extracts (or antibiotics) were combined evenly in the well. After adding 5 µL of bacterial solution (including cationic MHB and a McFarland value of 0.5) to each well, they were incubated for 16–18 hours at 37 °C after being held at +4 °C for two hours. The MIC was expressed as µg/mL. Solvents were utilized as the

negative control, and amoxicillin and tetracycline as the positive controls.

2.5. Statistical analysis

SPSS 20.0, an IBM statistical package for social studies, was used to examine the data. Multiple comparisons were conducted using One-Way ANOVA-Tukey HSD^{a,b} by the collected data. Statistical significance was determined by comparing the values' statistical importance to that of the activity analysis result group. A significant value of $p<0.05$ was agreed upon.

3. Results and Discussion

3.1. Enzyme Inhibitory Activities

The *Nepeta* species extracts were evaluated for inhibition activities, the results of which were depicted in Table 1. Thiourea, acetazolamide, kojic acid, galantamine, orlistat, and acarbose were used to compare the inhibitory enzyme potential of *Nepeta* extracts. In the present work, methanol-chloroform extracts of *N. trachonitica* presented considerable urease inhibition capacity with the IC_{50} of 1.51±0.28 µg/mL (Table 1). Fareed et al. (2013) found that the urease inhibition activities of chloroform (C), ethyl acetate (EA), methanol (M), water (W), and n-hexane (H) extracts of *N. praetervisa* were determined as 45.00, 68.00, 10.00, 25.00 and 30.00%, respectively. At the different concentrations of extracts and acetazolamide, the CA inhibition activity of *N. aristata* extract was determined to be the most effective (Table 1).

Table 1. Enzyme inhibition results of methanol-chloroform extracts of six *Nepeta* species

Sample	Inhibition, IC_{50} (µg/mL)							
	Urease	AChE	BChE	CA	α-Amylase	α-Glucosidase	Lipase	Tyrosinase
N. aristata	1.65±0.34^{a}	33.96±1.20^{d}	-	5.29±0.51^{b}	3.48±0.51^{a}	11.89±0.42^{a}	4.84±0.21^{a}	6.23±0.25^{b}
N. baytopii	4.95±0.00^{c}	-	-	12.50±0.40^{c}	101.39±0.45^{f}	41.30±0.43^{e}	8.15±0.28^{bc}	29.96±0.00^{f}
N. italica	-	25.94±1.11^{c}	9.35±0.88^{a}	19.35±0.27^{d}	27.71±0.38^{b}	14.59±0.00^{b}	16.48±0.51^{d}	13.20±0.86^{c}
N. nuda	5.29±0.00^{d}	4.06±0.19^{a}	-	148.46±0.94^{f}	67.17±0.69^{e}	158.58±0.52^{f}	4.24±0.33^{a}	69.21±0.14^{g}
N. stenantha	2.79±0.30^{b}	13.92±0.33^{b}	-	12.63±0.04^{c}	63.70±0.75^{d}	33.25±0.81^{d}	41.83±0.10^{e}	22.57±0.53^{d}
N. trachonitica	1.51±0.28^{a}	3.18±0.09^{a}	-	33.85±0.17^{e}	39.30±0.46^{c}	21.02±0.89^{c}	10.16±0.38^{c}	2.05±0.02^{a}
Standards	9.97±0.00^{e} (Thiourea)	38.47±0.00^{e} (Galantamine)	22.20±0.00^{b} (Galantamine)	2.35±0.06^{a} (Acetazolamide)	25.93±0.16^{b} (Acarbose)	13.50±0.04^{ab} (Acarbose)	6.30±1.01^{ab} (Orlistat)	25.83±1.58^{e} (Kojic Acid)

Data are means of three repetitions ± standard deviation (SD), Different superscripts (a–e) in the same column indicate significant differences between the tested extracts ($p<0.05$, as determined by ANOVA).
Abbreviations: AChE: Acetylcholinesterase; BChE: butyrylcholinesterase; CA: Carbonic anhydrase.

In this work, the AChE inhibitory effect of *N. trachonitica* was determined in the highest inhibition and exhibited in Table 1. Zengin et al. (2021) determined the AChE inhibitions of H, EA, M, and water/methanol (W/M) extracts of *N. baytopii* as 3.97±0.32, 4.57±0.06, 3.65±0.11 and 2.68±0.07 mg galantamine equivalent (GALAE)/g. The previous study conducted with *N. baytopii* determined that AChE inhibition of methanol and water/methanol extracts was low. However, in our study, no inhibition was observed by the methanol-chloroform extract of the same plant. The reason for observing this result may be due to the phenolic compounds, fatty acids, or volatile compounds contained in this extract. Acquaviva et al. (2023) obtained the AChE inhibitions of H, dichloromethane (DCM), EA, ethanol (E), ethanol-water (E-W), and water (W) extracts of *N. italica* as 3.02±0.47, 2.93±0.01, 2.69±0.17, 2.88±0.03, 2.80±0.02 and 0.04±0.01 mg GALAE/g. The previous study conducted with *N. italica* determined that AChE inhibition of different extracts was low. Nonetheless, in our study, low inhibition was also observed by the methanol-chloroform extract of the same

plant. This may be due to the compounds contained in this extract. Sarikurkcu et al. (2019) found that AChE inhibition of *N. nuda* and *N. cadmea* methanol extracts were determined as 1.26±0.01 and 1.35±0.02 mg GALAE/g extract. In the previous study with *N. nuda*, it was found that the AChE inhibition of the methanol extract was low. However, our study noted that the methanol-chloroform extract of a different species of *N. nuda* had high inhibition. This may be because both plants are high in and their species are different, or the components they contain may be different due to the different extracts.

BChE inhibition potential activities were not observed in all *Nepeta* extracts. In the *N. italica* extract, the IC_{50} value of the inhibition effect of BChE is higher than in galantamine, but the BChE inhibition effect is in no other extract (Table 1). Zengin et al. (2021) determined the BChE inhibitions of H, EA, and M extracts of *N. baytopii* as 6.93±1.14, 10.85±0.73, and 2.98±0.46 mg GALAE/g. The previous study with *N. baytopii* determined low BChE inhibition of methanol and water/methanol extracts. However, the identical plant's methanol-chloroform extract showed no inhibition in our investigation. The reason for observing this result may be due to the phenolic compounds, fatty acids, or volatile compounds contained in this extract. Acquaviva et al. (2023) obtained the BChE inhibitions of H, DCM, EA, E, and E-W extracts of *N. italica* as 1.88±0.20, 2.40±0.37, 1.79±0.40, 4.01±0.28, and 1.24±0.07 mg GALAE/g. The previous study conducted with *N. italica* determined that BChE inhibition of different extracts was low. However, in our study, high inhibition was observed by the methanol-chloroform extract of the same plant. This may be due to the compounds contained in this extract. Akdeniz et al. (2020) found that BChE inhibition of root, stem, leaf, flower, and the mixture of ethanol extracts of *N. heliotropifolia* was between 14.58±0.87 to 54.79±0.77%. Also, *N. congesta* was determined as between 4.50±0.81 and 48.35±0.77%.

The extract of *Nepeta* species exhibited a remarkable α-amylase inhibition activity and found the highest inhibition in *N. aristata* (Table 1). Zengin et al. (2021) determined the α-amylase inhibitions of H, EA, M, W/M, and W extracts of *N. baytopii* as 0.66±0.01, 0.84±0.02, 0.67±0.02, 0.50±0.01, and 0.10±0.01 mmol acarbose equivalent (ACAE)/g. The previous study conducted with *N. baytopii* determined that α-amylase inhibition of methanol and water/methanol extracts was moderate. However, in our study, low inhibition was observed by the methanol-chloroform extract of the same plant. The reason for observing this result may be due to the phenolic compounds, fatty acids, or volatile compounds contained in this extract. Acquaviva et al. (2023) obtained the α-amylase inhibitions of H, DCM, EA, E, E-W, and W extracts of *N. italica* as 0.37±0.02, 0.58±0.02, 0.51±0.01, 0.33±0.01, 0.25±0.01 and 0.05±0.01 mmol ACAE/g. The previous study conducted with *N. italica* determined that the α-amylase inhibition of different extracts was good. However, in our investigation, the same plant's methanol-chloroform extract also showed comparable inhibition when used with the usual medication. This may be due to the compounds contained in this extract. Malik, Roy [12] found that α-amylase inhibition of ethanol, methanol, and water extracts of *N. cataria* were 29.37±1.45, 52.03±0.71, and 16.59±1.79%, respectively. In reference, Sarikurkcu et al. (2019) showed that α-amylase inhibition of *N. nuda* and *N. cadmea* methanol extracts were 0.36±0.01 and 0.24±0.01 mg ACAE/g extract. In the previous study with *N. nuda*, it was found that the α-amylase inhibition of the methanol extract was high. However, our study noted that the methanol-chloroform extract of a different species of *N. nuda* had low inhibition. This may be because both plant species are different, or

the components they contain may be different due to the different extracts.

The α-glucosidase inhibition potency of *N. aristata* was determined to be the most effective at 11.89±0.42 µg/mL (Table 1). Zengin et al. (2021) determined the α-glucosidase inhibitions of H, EA, M, W/M, and W extracts of *N. baytopii* as 7.87±0.02, 7.76±0.01, 8.15±0.08, 0.61±0.04, and 1.06±0.09 mmol ACAE/g. In the previous study conducted with *N. baytopii*, it was obtained that α-glucosidase inhibition of methanol extract was high. However, the methanol-chloroform extract of the same plant showed considerable inhibition in our investigation. The reason for observing this result may be due to the phenolic compounds, fatty acids, or volatile compounds contained in this extract. Acquaviva et al. (2023) obtained the α-glucosidase inhibitions of H, DCM, EA, E, E-W, and W extracts of *N. italica* as 4.91±0.01, 0.14±0.01, 0.53±0.07, 5.38±0.01, 5.60±0.01 and 0.94±0.04 mmol ACAE/g. The previous study conducted with *N. italica* determined a low α-glucosidase inhibition of different extracts. However, the methanol-chloroform extract of the same plant also showed significant inhibition in our investigation. This may be due to the compounds contained in this extract. Sarikurkcu et al. (2019) found that α-glucosidase inhibition of *N. nuda* and *N. cadmea* methanol extracts were determined as 3.67±0.02 and 2.02±0.01 mg ACAE/g extract. In the previous study with *N. nuda*, it was found that the α-glucosidase inhibition of the methanol extract was high. However, our study noted that the methanol: chloroform extract of a different species of *N. nuda* had low inhibition. This may be because both plant species are different, or their components may differ due to the different extracts.

In our study, the inhibition effect of lipase was observed to be the most effective in the *N. nuda* extract (Table 1). Roh and Jung (2012) found that lipase inhibition of ethanol extract of *N. japonica* was determined as 37.3±2.5%.

In our work, *N. trachonitica* extract exhibited an effective tyrosinase inhibition activity with the IC_{50} of 2.05±0.02 µg/mL (Table 1). Zengin et al. (2021) determined the tyrosinase inhibitions of H, EA, M, W/M, and W extracts of *N. baytopii* as 77.84±1.83, 78.60±1.58, 96.06±0.70, 95.31±1.77, and 6.15±1.02 mg kojic acid equivalent (KAE)/g. The previous study conducted with *N. baytopii* determined that tyrosinase inhibition of methanol extract was high. Nevertheless, our investigation found that the methanol-chloroform extracts of six *Nepeta* species inhibited the medicine in a similar way to the conventional treatment. The reason for observing this result may be due to the phenolic compounds, fatty acids, or volatile compounds contained in this extract. Acquaviva et al. (2023) obtained the tyrosinase inhibitions of H, DCM, EA, E, E-W, and W extracts of *N. italica* as 72.12±2.44, 49.91±1.32, 56.29±7.29, 64.61±0.94, 59.52±1.31 and 15.04±0.22 mg KAE/g. The previous study conducted with *N. italica* determined that tyrosinase inhibition of different extracts was high. Yet in our investigation, the methanol-chloroform extract of six *Nepeta* species also showed significant inhibition. This may be due to the compounds contained in this extract. Akdeniz et al. (2020) found tyrosinase inhibition capacity of ethanol extracts of *N. heliotropifolia* root, stem, and leaf between 51.78±0.82 to 22.04±1.12%, and also *N. congesta* extracts exhibited between 26.25±1.51 and 16.27±1.14%.

3.2. Antibacterial Activities

The antibacterial activity of six *Nepeta* species extracts against three-gram negative bacteria and three-gram positive bacteria was investigated, and different *Nepeta* extracts varied in antibacterial potential. The antimicrobial activities of six *Nepeta* species extracts were illustrated as

the mm and µg/mL values in Table 2. In this work, *N. aristata* extract showed effective activity against *P. aeruginosa* and *K. pneumoniae* bacteria while not showing antibacterial activity on *E. coli*, *E. faecalis*, and *S. aureus,* according to DDM. However, other *Nepeta* extracts did not show antibacterial activity on *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. faecalis*, and *S. aureus*. In addition, *Nepeta* extracts had a strong against *B. cereus* bacteria. In a previous research, Köksal et al. (2017) showed that the antimicrobial activities against *E. coli*, and *P. aeruginosa* in DDM, measured by absorbing 90 µL of *N. trachonitica* ethanol extract at a concentration of 20 mg/mL onto the disc, was 12.00±1.24, and 9.00±0.00 mm, respectively. A previous study showed that *N. trachonitica* has antibacterial properties on the bacteria we used. However, our study determined that it had no antibacterial effect on solid media. The reason for this is that the extract solvent is different because the secondary components in the plant allow different components to pass into the extract in different solvents. In reference, Ahmad et al. (2020) showed that antimicrobial activities in DDM of extracts of *N. deflersiana* prepared in different polarities were determined the ethanol extract against *P. aeruginosa* as 14.00±0.47 mm, the ethanol, acetone, and ethyl acetate extract against *K. pneumoniae* as 11.00±0.65, 13.00±0.82, and 13.00±0.70 mm, respectively, and the ethyl acetate extract against *E. coli* as 16.00±0.22 mm. A previous study showed that solvent extracts of *N. deflersiana* of different polarities had antibacterial properties on the bacteria we used in our study. However, we found that the plants we used in our study did not have an antibacterial effect on solid media. The reason for this is the difference in the components in the extract when a single solvent is used and the components in the extract when a solvent mixture is used.

Table 2. Antimicrobial activity results of methanol-chloroform extracts of six *Nepeta* species

Antibacterial properties	Samples	Gram-negative bacteria			Gram-positive bacteria		
		E. coli	*P. aeruginosa*	*K. pneumoniae*	*E. faecalis*	*B. cereus*	*S. aureus*
DDM, mm	*N. aristata*	-	28.00±2.83	14.00±2.83	-	17.00±0.00	-
	N. baytopii	-	-	-	-	14.00±1.41	-
	N. italica	-	-	-	-	12.00±4.24	-
	N. nuda	-	-	-	-	10.00±1.41	-
	N. stenantha	-	-	-	-	10.00±0.50	-
	N. trachonitica	-	-	-	-	28.00±0.00	-
	Amoxicillin	21.00±5.66	-	-	-	27.00±0.00	8.00±0.00
	Tetracycline	31.00±0.00	32.00±0.00	31.00±0.00	32.00±0.00	37.00±0.00	31.00±0.00
MIC, µg/mL	*N. aristata*	256	128	256	64	512	256
	N. baytopii	128	256	128	128	1024	512
	N. italica	512	512	1024	512	256	1024
	N. nuda	256	128	256	128	1024	512
	N. stenantha	64	128	256	128	512	256
	N. trachonitica	128	128	256	64	1024	512
	Amoxicillin	1024	1024	1024	1024	<1	1024
	Tetracycline	8	<2	8	8	<0.5	8

Abbreviations: DDM: Disc diffusion method; **MIC:** Minimum inhibition concentration

The following six *Nepeta* extracts exhibited effective antimicrobial activity against all six bacteria used, which are *N. aristata* (64 to 512 µg/mL), *N. baytopii* (128 to 1024 µg/mL); *N. italica* (256 to 1024 µg/mL), *N. stenantha* (64 to 512 µg/mL), *N. nuda* (128 to 1024 µg/mL) (64 to 1024 µg/mL). These results prove bacteria were resistant to methanol: chloroform extracts were used. All six *Nepeta* extracts for which the MIC test was applied had antimicrobial properties, and these results were consistent with previous reports. In reference, Ahmad et al. (2020) showed that antimicrobial activities in MIC of *N. deflersiana* prepared in ethanol extract were determined as 250 µg/mL

against *P. aeruginosa*, and *K. pneumoniae*.

A previous study showed that solvent extracts of *N. deflersiana* of different polarities had antibacterial properties on the bacteria we used in our study. However, we determined that the plants we used in our study had a higher antibacterial effect against *P. aeruginosa* bacteria and antibacterial activity against *K. pneumoniae* bacteria, similar to the *N. deflersiana*. This is because of the difference in the components in the extract when a single solvent is used and the components in the extract when a solvent mixture is used.

4. Conclusion

Since the enzyme inhibition and antibacterial activities of *N. aristata*, *N. stenantha*, and the enzyme inhibition of *N. trachonitica* have not been investigated, this study was the first of its kind. *N. aristata*; urease (1.65±0.34 µg/mL), AChE (33.96±1.20 µg/mL), α-amylase (3.48±0.51 µg/mL), α-glucosidase (11.89±0.42 µg/mL), lipase (4.84±0.21 µg/mL), tyrosinase (6.23±0.25 µg/mL), *N. baytopii*; urease (4.95±0.00 µg/mL), *N. italica*; AChE (25.94±1.11 µg/mL), BChE (9.35±0.88 µg/mL), tyrosinase (13.20±0.86 µg/mL), *N. nuda* ; urease (5.29±0.00 µg/mL), AChE (4.06±0.19 µg/mL), lipase (4.24±0.33 19 µg/mL), *N. stenantha*; urease (2.79±0.30 µg/mL), AChE (13.92±0.33 µg/mL), tyrosinase (22.57±0.53 µg/mL) and *N. trachonitica*; urease (1.51±0.28 µg/mL), AChE (3.18±0.09 µg/mL), tyrosinase (2.05±0.02 µg/mL) showed the highest enzyme inhibition effect than standards. *N. aristata* showed the highest effect in enzyme inhibition. Additionally, antibacterial activity in DDM showed an effect against *N. aristata* as *P. aeruginosa*, *K. pneumoniae*, *B. cereus* bacteria, while other plants showed an effect only against *B. cereus* bacteria. The MIC method determined that almost all plants were effective against all bacteria. For this reason, it was predicted that plants would lead to further studies.

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Author Contribution

Semiha Yenigun, Yunus Basar, Yasar Ipek, Mesut Gok: Writing–Review, Visualization & Editing. Semiha Yenigun: Antibacterial and Enzyme Inhibitor Activities, Writing–Review. Tevfik Ozen: Biologic Studies, Writing–Review, Supervision. Lutfi Behcet: Sourcing plants. Ibrahim Demirtas: Writing–Review, Supervision.

Conflicts of Interest

The authors declare no conflict of interest.

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