DOI: http://dx.doi.org/10.32571/ijct.1381998 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Antibacterial and Antioxidant Molecule Isolated from *Nepeta aristata* Boiss Et Kotschy Ex Boiss plant: 1,5,9-Epideoxyloganic Acid

Yunus BAŞAR[1], Semiha YENİGÜN[2], Lütfi BEHÇET[3], İbrahim DEMİRTAS[1,4], Tevfik OZEN[2]

[1]*Iğdır University, Research Laboratories Application and Research Center (ALUM), Igdir-Türkiye*

[2]*Ondokuz Mayıs University, Faculty of Science, Department of Chemistry, Kurupelit Campus, Samsun-Türkiye*

[3]*Bingöl University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Bingöl-Türkiye*

[4]*Ondokuz Mayıs University, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Samsun-Türkiye*

Received: 27 October 2023; Accepted: 29 December 2023

*****Corresponding author e-mail: tevfikoz@omu.edu.tr

Citation: Başar, Y.; Yenigün, S.; Behçet, L.; Demirtaş, İ.; Ozen, T., *Int. J. Chem. Technol.* **2024**, 8(1), 27-31.

ABSTRACT

1,5,9-Epideoxyloganic acid (ELA) was isolated from the methanol fraction of endemic *Nepeta aristata* crude methanol-chloroform by chromatographic methods (*preparative*-HPLC, silica, and sephadex column). The structure of ELA was determined with ^{1}H NMR and ^{13}C NMR spectrometry. Furthermore, the mass of the molecule was confirmed by mass spectrometry. ELA's antioxidant and antibacterial activities were examined and also compared to standards. ELA exhibited the best antibacterial activity with inhibition zone against *K. pneumoniae* (11.50 mm), *S. aureus* (10.00 mm), and also effective minimum inhibition concentration value against *E. faecalis* (8 µg/mL). The antioxidant properties of ELA were assessed with posphomolybdenium reducing, reducing power, metal chelating, H_2O_2, DPPH$^{\cdot}$, ABTS$^{\cdot+}$, and $O_2^{\cdot-}$ scavenging activities. The posphomolybdenium, $O_2^{\cdot-}$, and H_2O_2 scavenging activities of ELA were found effective at 136.02±3.99, 3.54±0.26 and 9.67±1.26 µg/mL, respectively. As a result, the effective antioxidant and antimicrobial activity values of the ELA molecule may be a source that can be used in pharmacology in the future.

Keywords: *Nepeta aristata*, 1,5,9-epideoxyloganic acid, antibacterial activity, antioxidant activity.

1. INTRODUCTION

The reducing chemicals known as antioxidants have been employed to counteract various free radicals. Atomic groupings or atoms with just one type of electron make up free radicals. Oxygen molecules interacting with certain compounds in medications, air contaminants, and mitochondrial respiratory chain processes can all be found in them. Free radicals harm people and damage DNA, proteins, cell membranes, and other cellular building blocks. They also cause many pathological conditions, including atherosclerosis, myocardial infarction, asthma, diabetes mellitus, anemia, and rheumatoid arthritis.[1,2]

Natural or artificial chemicals that destroy or stop the growth of bacteria are known as antimicrobial agents.[3] These include antibiotics, antiseptics, disinfectants, etc., very wide. Antibiotics are substances created by living things that prevent the proliferation of certain microorganisms or kill them.[4] Plants with antimicrobial properties gain importance as problems arise with the use of antibiotics.[5] Since pathogenic germs can be killed by antibiotics, many researchers are interested in chemicals that can be differentiated from plant species.[6]

The family Lamiaceae includes about 300 taxa. *Nepeta* L, which is distributed in many parts of the world, is the genus with the highest number.[7] It includes 44 taxa, 22 of which are endemic in Turkey.[8] *Nepeta*'s are rich in terpenes, flavonoids, phenolics, and steroids. Moreover, it is known to have various pharmacological activities.[9,10] Iridoid glycosides consisting of cyclopentane and monoterpene skeleton are found in

DOI: http://dx.doi.org/10.32571/ijct.1381998 E-ISSN: 2602-277X

various nepeta species and are known to have bioactive properties.[11] 1,5,9-Epideoxyloganic acid (ELA) is one of the most common compounds in the Lamiaceae family.[12]

Our study examined the antioxidant and antibacterial activities of the ELA molecule isolated from the endemic *N. aristata* Boiss Et Kotschy Ex Boiss plant. In the literature review, detailed studies on the antioxidant activities of the ELA molecule were not found. Therefore, this study is a comprehensive report on biological activity.

2. EXPERIMENTAL

2.1. Plant Materials

In the Bingöl province in the Eastern Anatolia Region of Turkey, *N. aristata* (BIN6195) was gathered south of Ortaköy (Methane rough mountain sides). Prof. Dr. Lütfi Behçet's diagnosis of plants. Plants gathered between July and September were dried in an environment that was conducive to drying.

2.2. Methods

2.2.1. Extraction, Fraction and Isolation

One hundred grams of dried plant materials were ground up and extracted three times using a 1:1 methanol: chloroform solvent solution. The extraction mixture was filtered, and a rotary evaporator removed the solvent at the proper temperature.

In the evaporator, the fractions' solvents were eliminated. Hexane, dichloromethane, ethyl acetate, and methanol were the four fractions produced, respectively when the residual solvent residue was dried in the lyophilizer. The methanol fraction was then used on Sephadex columns and fractionated due to its high activity. Thin-layer chromatography (TLC) was applied to mix fractions with similar composition, yielding nine sub-fractions.

More sensitivity and purity were achieved by separating molecules using *preparative*-HPLC (Thermo ScientificTM DionexTM UltiMateTM 3000). Chromatographic separation was performed with a reverse-phase thermoscientific Hypersil Prep Bds C18 preparative column. The elution gradient was set to methanol and pure water. 100 mg of fraction sample was dissolved with 1:1 w:v methanol and water. It was passed through a 0.22 µm filter, transferred into 5 ml vials, and given to the device. UV scanning was performed for sensitive separation, and the separation process was started according to the appropriate method by setting the appropriate UV value.[13]
CD_3OD was used to dissolve the refined ELA. The Agilent-Premium Compact 14.1 Tesla 600 MHz Frequency NMR equipment was used to identify its structure through two-dimensional COSY, HMBC, and HSQC NMR spectra in addition to 600 MHz ^{1}H- and 150 MHz ^{13}C-NMR.[13]

Mass spectrometry (MS) and liquid chromatography (HPLC) of the chemical were performed using the Agilent 6460 Triple Quad brand. After removing the analytical column, the MS spectrometer was connected to HPLC without the column. Eluent: A (acetonitrile with 5 mM ammonium formate plus 0.1% formic acid), 4 µL of sample injected, 0.400 mL/min injection volume, and solvent flow rate. After 90 seconds, the scanning range was found to be 50–1200 m/z, and the MS spectrum was produced.[14]

2.2.2. Antioxidant Activities

The phosphomolybdenum reduction test was performed according to the molybdenum method. This method was based on reducing Mo^{+6} to Mo^{+5} and generating green phosphate/Mo^{+5} at 695 nm.[15,16] This assay was determined spectrophotometrically based on the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form and the green color formation of the Fe^{2+} at 700 nm.[17,18] The activity was obtained spectrophotometrically based on the measurement principle of the orange complex formed by the ferrous ion (Fe^{2+}) with 1,10-phenanthroline at 510 nm.[19,20] The activity was obtained spectrophotometrically at 517 nm based on DPPH$^{\cdot}$ scavenging.[21] The activity was performed at 734 nm using the results of the oxidation of 2,2-azinobis-(3-ethylbenzothiozine-6-sulfonic acid) ammonium salt with peroxydisulphate.[22,23] The activity was determined spectrophotometrically at 560 nm based on the measurement of the conversion of the yellow color to formazan blue as a result of the reaction of NBT^{+2} with the superoxide radical produced by the PMS-NADH system radical.[24,25] The activity was determined spectrophotometrically at 562 nm based on the formation of Fe^{2+}-ferrozine complex with ferrozine of Fe^{2+}.[26]

2.2.3. Antibacterial Activities

The antimicrobial activity was performed using six bacterial strains (gram-negative bacteria such as *Escherichia coli* ATCC 25922, *Psedomonas aeruginosa* ATCC 15442, and *Klebsiella pneumoniae* ATCC 10031, and gram-positive bacteria such as *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* CCM 99, and *Staphylococcus aureus* ATCC 25213).

100 µL of the bacterial solution, of which 0.5 McFarland value was set, was spread on Mueller Hinton Agar medium, and 6 mm sterile test discs impregnated with 20 µL sample were placed on the agar and then incubated at 37 °C for 16-18 hours. Antibacterial activity was tested by disk diffusion.[27] ELA-prepared solution at a concentration of 1024 µg/mL was used. In methods, tetracycline and amoxicillin antibiotics were

DOI: http://dx.doi.org/10.32571/ijct.1381998 E-ISSN: 2602-277X

used for comparison purposes. The clear inhibition zone was measured in millimeters.

In the MIC method, 100 mL of cationic Mueller Hinton Broth medium (Mueller Hinton Broth medium, 400 µL $MgCl_2$ (2 mg/mL), and 1 mL $CaCl_2$ (2 mg/mL) are added to each well of a sterile 96-well microplate. Antibacterial activity was tested by minimum inhibition concentration.[28] ELA-prepared solution at a concentration of 1024 µg/mL was used. In methods, tetracycline and amoxicillin antibiotics were used for comparison purposes. After cooling, 100 µL of sample (or antibiotic) was added and mixed homogeneously, and serial dilution was made. Then, 5 µL of bacterial solution (0.5 McFarland adjusted bacteria and cationic Mueller Hinton Broth medium) was added to each well at +4°C. It was left for 2 hours, taken at the end of the time, and incubated for 16-18 hours at 37°C. The MIC value was expressed as µg/mL.

2.2.4. Statistical Analysis

To calculate the ± standard deviations for each parameter, triple replication findings from in vitro biological activity tests were used. The SPSS 20.0 software was used to calculate the data. ANOVA was utilized when examining interactions between more than two independent factors and how they affect the dependent variable. For numerous comparisons, the Tukey $HSD^{a,b}$ program was also utilized. The activity results were compared to the statistical significance level of the values, and the result was stated using $p<0.05$ values.

3. RESULTS and DISCUSSION

3.1. Antioxidant Activities

The study of antioxidant activity has drawn more attention recently.[29] Preventing food oxidative deterioration and lowering the risk of oxidative damage at the cellular level requires the use of safe antioxidants.[30,31] It is possible to alter the antioxidant defense mechanisms of living things by diet.[32] The posphomolybdenium reducing (136.02±3.99 µg/mL), metal chelating (109.66±3.02 µg/mL), reducing power (102.00±0.00 µg/mL), $DPPH^{\cdot}$ (11.67±0.38 µg/mL), $ABTS^{\cdot+}$ (84.48±0.95 µg/mL), superoxide anion (3.54±0.26 µg/mL) and H_2O_2 (9.67±1.26 µg/mL) scavenging activities were investigated of ELA and α-tocopherol, EDTA and ascorbic acid standards were used in antioxidant activity tests. Table 1 shows that ELA (Figure 1) has higher than standard antioxidants superoxide anion scavenging (3.54±0.26 µg/mL), H_2O_2 scavenging (9.67±1.26 µg/mL) and posphomolybdenium reducing (136.02±3.99 µg/mL) activities. According to studies, no study has been found in the literature on the antioxidant activity of ELA

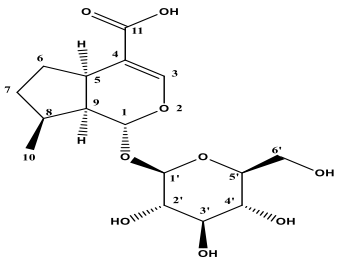

Figure 1. Molecular structure of ELA[13].

Table 1. Antioxidant activities of the ELA.

Antioxidant assays	Activity	ELA*	α-Tocopherol	Ascorbic acid	EDTA
Posphomolybdenium reducing	$A_{0.5}$,	136.02±3.99^a	165.05±0.94^c	145.49±0.20^b	-
Reducing power	µg/mL	102.00±0.00^b	176.63±1.21^c	88.11±0.94^a	-
H_2O_2 scavenging		9.67±1.26^a	56.84±0.96^b	250.52±0.47^c	-
$DPPH^{\cdot}$ scavenging	IC_{50},	11.67±0.38^b	3.44±0.62^a	3.11±0.07^a	-
$ABTS^{\cdot+}$ scavenging	µg/mL	84.48±0.95^b	11.16±0.09^a	11.70±0.01^a	-
$O^{\cdot}_2$ scavenging		3.54±0.26^a	-	78.33±0.49^b	-
Metal chelate		109.66±3.02^b	-	-	30.01±0.38^a

* ELA: 1,5,9-epideoxyloganic acid, EDTA: Etilendiamintetraacetic acid, n:3, $p<0.05$

3.2. Antibacterial Activities

The gram-positive bacteria's cell barrier structure permits hydrophobic compounds to diffuse cells quickly and act in the cell wall and cytoplasm.[33] Contrary to this, the cell barrier of gram-negative bacteria is more complicated. In these bacteria, lipopolysaccharides consisting of lipid A, core polysaccharide, and O antigen provide a "quid" structure.[34,35] Antibacterial activity was examined against gram-negative and gram-positive, and amoxicillin and tetracycline were used as antibiotics. When the antibacterial activity of ELA was examined, it was determined that the MIC value was 8 µg/mL against *E. faecalis*. In contrast, it was determined that the inhibition zones 11.50±0.00 mm and 10.00±0.00 mm against *K. pneumoniae* and *S. aureus* bacteria, respectively. These results show that it has good antibacterial activity when compared with amoxicillin; however, it was lower antibacterial activity than tetracycline (Table 2).

Aničić, et all. [36] investigated the antibacterial activities of ELA and determined the MIC values of 600, 50, 300, 400, and 200 µg/mL against *B. cereus*, *S. aureus*, *E. feacelis*, *P. auregonisa*, and *E. coli* bacteria,

DOI: http://dx.doi.org/10.32571/ijct.1381998 **E-ISSN: 2602-277X**

respectively. According to these results, as indicated in Table 2, it was observed that the MIC values of *B. cereus* and *E. feacelis* bacteria that we obtained in our study were higher.

Table 3. Antibacterial activities of the ELA.

Bacteria	Inhibition zone, mm			Minimum inhibition concentration, µg/mL		
	ELA	Amoxicillin	Tetracycline	ELA	Amoxicillin	Tetracycline
E. coli (ATCC 25922)	-	21.00±5.66	31.00±0.00	256	1024	8
P. aeruginosa (ATCC 15442	-	-	32.00±0.00	>512	1024	<2
K. pneumoniae (ATCC 10031)	11.50±0.00	-	31.00±0.00	>512	1024	8
E. faecalis (ATCC 29212)	-	-	32.00±0.00	8	1024	8
B. cereus (CCM 99)	-	27.00±0.00	37.00±0.00	256	<1	<0.5
S. aureus (ATCC 25213)	10.00±0.00	8.00±0.00	31.00±0.00	>512	1024	8

*ELA: 1,5,9-epideoxyloganic acid

4.CONCLUSION

Plants normally contain little levels of secondary metabolites, yet these compounds have medicinal characteristics. The interest in herbal medicines for the synthesis of natural goods is growing every day due to the highly toxic effects of synthetic pharmaceuticals used for treatment. Though millions of chemical molecules make up a plant, only a few exhibit bioactive characteristics. High concentrations of phenolic, steroids, flavonoids, and terpene chemicals are found in *Nepeta* species. Furthermore, because of their bacteriostatic properties, these species are favored by the general population as remedies for skin conditions like eczema. The antibacterial and antioxidant properties of ELA molecules. As a result of the studies, it was seen that ELA has high antioxidant properties in H_2O_2 and $O_2^{\cdot-}$ scavenging activities. In addition, its antibacterial activity is high against *E. feacelis*, *K. pneumoniae*, and *S. aureus* bacteria. The antioxidant activities of ELA were investigated for the first time and contributed to the literature. Further studies on this molecule, which is effective against antioxidant and antibacterial activities, can be used in the pharmaceutical industry.

Acknowledgments

This study was supported by TUBITAK (119Z442).

Conflict of Interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. Zhou, G.; Zhang, X.; Shah, M.; Che, Q.; Zhang, G.; Gu, Q.; Zhu, T.; Li, D. *Mar. Drug.* **2021,** 19 (2), 82.
2. Wang, L.; Kuang, Z.; Zhang, D.; Gao, Y.; Ying, M.; Wang, T. *Biomed. pharmacother* **2021,** 133, 110978.
3. Çopuroğlu, Ö. Antimicrobial activities of some endemic plant species from Nigde region. Master's Thesis, Niğde University, **2013**.
4. Temiz, A., *General Microbiology Application Tecniques*. Hatiboğlu Publisher: 2010.
5. Emori, T. G.; Gaynes, R. P. *Clin. Microbiol. Rev.* **1993,** 6 (4), 428-442.
6. Essawi, T.; Srour, M. *J. Ethnopharm.* **2000,** 70 (3), 343-349.
7. Hedge, I. C., *P. Roy. Soc. B-Biol. Sci* **2011,** 89, 23-35.
8. Dirmenci, T., *Bot. J. Linn. Soc.* **2005,** 147, 229-233.
9. Eser, F.; Altun, M.; Demirtas, I.; Behcet, L.; Aktas, E. *Nat. Prod. Res.* **2022**, 1-10.
10. Süntar, I.; Nabavi, S. M.; Barreca, D.; Fischer, N.; Efferth, T., *Phytother. Res.* **2018,** 32 (2), 185-198.
11. El-Moaty, H. I. A. *J. Nat. Prod.* **2010**,, 3, 103-111
12. Anicic, N.; Gasix, U.; Lu, F.; Ciric, A.; Ivanov, M.; Jevtic, B.; Dimitrijevic, M.; Andelkovic, B.; Skoric, M.; Nestorovic Zivkovic, J.; Mao, Y.; Liu, J.; Tang, C.; Sokovic, M.; Ye, Y.; Misic, D. *Pharmaceuticals (Basel)* **2021,** 14 (5).
13. Başar, Y.; Yenigün, S.; İpek, Y.; Behçet, L.; Gül, F.; Özen, T.; Demirtaş, İ. *J. Biomol. Struct. Dyn.* **2023**, 42, 1-14.
14. Yenigün, S.; Başar, Y.; İpek, Y.; Behçet, L.; Özen, T.; Demirtaş, İ. *J. Biomol. Struct. Dyn.* **2023**, 42,1-18.
15. Mohamed, R.; Pineda, M.; Aguilar, M. *J. Food Sci.* **2007,** 72 (1), S059-S063.
16. Prieto, P.; Pineda, M.; Aguilar, M. *Anal. Biochem.* **1999,** 269 (2), 337-341.
17. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005,** 53 (6), 1841-1856.
18. Oyaizu, M. *The Jpn. J. Nutr. Diet.* **1986,** *44* (6), 307-315.

DOI: http://dx.doi.org/10.32571/ijct.1381998 **E-ISSN: 2602-277X**

19. Mukhopadhyay, D.; Dasgupta, P.; Roy, D. S.; Palchoudhuri, S.; Chatterjee, I.; Ali, S.; Dastidar, S. G. *Free Radic/ Antioxid.* **2016,** 6 (1).

20. Utami, S.; Adityaningsari, P.; Sosiawan, I.; Endrini, S.; Sachrowardi, Q. R.; Laksono, S. P.; Nafik, S.; Arrahmani, B. C.; Afifah, E.; Widowati, W. *Trad. Med. J* **2017,** 22 (3), 160-165.

21. Blois, M. S. *Nature* **1958,** 181 (4617), 1199-1200.

22. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. *Free Radic. Biol. Med.* **1999,** 26 (9-10), 1231-1237.

23. Dechayont, B.; Ruamdee, P.; Poonnaimuang, S.; Mokmued, K.; Chunthorng-Orn, J. *J. Bot.* **2017** (2), 1-6.

24. Nishikimi, M.; Rao, N. A.; Yagi, K. *Biochem. Biophys. Res. Commun.* **1972,** 46 (2), 849-854.

25. Yang, J.; Guo, J.; Yuan, J. *LWT - Food Sci. Technol.* **2008,** 41 (6), 1060-1066.

26. Dinis, T. C.; Madeira, V. M.; Almeida, L. M. *Arch. Biochem. Biophys.* **1994,** 315 (1), 161-169.

27. Reller, L. B.; Weinstein, M.; Jorgensen, J. H.; Ferraro, M. J. *Clinical Infectious Diseases* **2009,** 49 (11), 1749-1755.

28. Andrews, J. M. *Clin. Infect. Dis.* **2001,** 48 (suppl_1), 5-16.

29. Barba, F. J.; de Souza Sant'Ana, A.; Orlien, V.; Koubaa, M., *Innovative technologies for food preservation: Inactivation of spoilage and pathogenic microorganisms*. Academic Press: **2017**.

30. Gülçin, İ.; Bingöl, Z.; Taslimi, P.; Gören, A. C.; Alwasel, S. H.; Tel, A. Z. *Chem. Biodiv.* **2022,** 19 (3), e202100775.

31. Durmaz, L.; Erturk, A.; Akyüz, M.; Polat Kose, L.; Uc, E. M.; Bingol, Z.; Saglamtas, R.; Alwasel, S.; Gulcin, İ. *Molecules* **2022,** 27 (10), 3091.

32. Barba-Orellana, S.; Barba, F. J.; Quilez, F.; Cuesta, L.; Denoya, G. I.; Vieira, P.; Pinto, C. A.; Saraiva, J. A. Nutrition, public health, and sustainability: an overview of current challenges and future perspectives: *In Agri-Food Industry Strategies for Healthy Diets and Sustainability*, Elsevier: 2020; pp 3-50.

33. Tiwari, B. K.; Valdramidis, V. P.; O'Donnell, C. P.; Muthukumarappan, K.; Bourke, P.; Cullen, P. *J. Agric. Food Chem.* **2009,** 57 (14), 5987-6000.

34. Nikaido, H. *Science* **1994,** 264 (5157), 382-388.

35. Vaara, M. *Microbiol. Rev.* **1992,** 56 (3), 395-411.