CHAPTER 8

DETERMINATION OF THE BIOLOGICAL ACTIVITY OF *Lavandula stoechas* L.

Hasan DURUKAN[1]
Ahmet DEMİRBAŞ[2]
Nuraniye ERUYGUR[3]

[1] Sivas Cumhuriyet University, Sivas Technical Sciences Vocational School, Department of Plant and Animal Production, Sivas, Türkiye,
e-mail: hasandurukan@cumhuriyet.edu.tr, ORCID ID: 0000-0002-2255-7016
[2] Sivas Cumhuriyet University, Sivas Technical Sciences Vocational School, Department of Plant and Animal Production, Sivas, Türkiye,
e-mail: ademirbas@cumhuriyet.edu.tr, ORCID ID: 0000-0003-2523- 7322
[3] Selçuk University, Faculty of Pharmacy, Department of Pharmacognosy, Konya, Türkiye, e-mail: nuraniye.eruygur@selcuk.edu.tr,
ORCID ID: 0000-0002-4674-7009

INTRODUCTION

Lavandula stoechas is an aromatic plant belonging to the *Lamiaceae* family (Dob et al., 2006; Kaplan et al., 2019; Bilenler and Gökbulut, 2019). It is known that *Lavandula stoechas* plant, which is in the form of a bush, does not shed its leaves (Carrasco et al., 2015). *Lavandula stoechas* plant prefers non-acidic soils, especially in open forests and dry hills (Mokhtarzadeh and Khawar, 2022). *Lavandula stoechas* is used in many areas such as cosmetics and food (Öztürk et al., 2005). *Lavandula stoechas* plant also has antimicrobial properties (Bilenler and Gökbulut, 2019). In traditional medicine, *Lavandula stoechas* is used as an analgesic, antiseptic (Celep et al., 2018), expectorant, antispasmodic, carminative (Giray et al., 2008; Zuzarte et al., 2013) in the treatment of ear, nose and throat diseases (Mokhtarzadeh and Khawar, 2022). *Lavandula stoechas* plant, whose dried flowers and leaves are used, is preferred in the treatment of insomnia and high blood pressure disorders (Leblebici et al., 2012).

Chemicals that prevent possible harmful effects by interacting with free radicals are called antioxidants (Tüzün et al., 2020). Many reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) are natural byproducts of body metabolism (Amarowicz et al., 2004). Herbal products have antioxidant properties thanks to their content such as flavonoids, cinnamic acid derivatives and phenolic compounds (Kolaç et al., 2017). Phenolic compounds, the second most abundant metabolite in plants, play an important role in the growth of plants (Noreen et al., 2017).

It is known that Alzheimer's disease is expressed as a type of disease that can be defined by the progressive degeneration of memory and cognitive function (Eruygur et al., 2019). Acetylcholinesterase (AChE), one of the cholinesterases, plays a role in the hydrolysis of acetylcholine to choline and acetic acid (Eruygur and Uçar, 2018). Diabetes mellitus is a disease that causes serious complications (Eruygur and Uçar, 2018). In the treatment of diabetes, inhibitors that reduce carbohydrate digestion such as α-amylase and α-glucosidase inhibitors are used (Aksoy et al., 2021). Tyrosinase; it is an enzyme that plays an important role in the synthesis of melanin in mammalian cells and in browning in plants and microorganisms. Since melanin, which is produced and accumulated in high amounts in human skin, causes hyperpigmentation, it is important to control excessive melanin production by using tyrosinase inhibitor agents (Eruygur and Uçar, 2018).

The study is important in terms of determining the chemical composition, antioxidant activity, TPC, TFC and inhibitory activities of some enzymes of 80% ethanol extract of *Lavandula stoechas* plant.

1. MATERIALS AND METHODS

Lavandula stoechas plant used in the study was collected from Sivas natural flora in 2021. The data obtained in the study were made in Sivas Cumhuriyet University Advanced Technology Research Center laboratories.

Preparation of the extract

After the plant sample was ground in the herb grinding mill, 10 g of the leaf sample was weighed and placed in 50 mL of 80% ethanol solvent and left to agitate in a shaker for one day. After shaking, the solution was filtered through coarse filter paper. To obtain the extract from the filtrate, the filtrate was dried at 40^{o}C with an evaporator under low pressure, and this process was repeated three times.

Determination of chemical components

The chemical components of the extract were analyzed by GC-MS (Gas Chromatography/Mass Spectrometry) (Sacchetti et al., 2005).

Determination of antioxidant activity

Determination of antioxidant activity in *Lavandula stoechas* plant was made in 80% ethanol extract. In determining the free radical scavenging activity of the extract, using 2,2-diphenyl-1picrylhydrazyl (DPPH) with slight modification according to the method of Blois (1958), 2,2'-azinobi (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used with slight modification by Re et al. (1999) method.

Determination of total phenol content (TPC) and total flavonoid content (TFC)

While determining the total phenol content (TPC), the Folin-Ciocalteu spectrophotometric method was used (Gámez-Meza et al., 1999). A gallic acid was used as the standard when determining the total phenol content, and the phenol content was calculated as milligram gallic acid

equivalents per gram of dry extract. Total flavonoid content (TFC) was determined according to the aluminum chloride colorimetric method of Molan and Mahdy (2014). Total flavonoid content is expressed as milligram of catechin equivalents per gram dry weight of the extract.

Determination of enzyme inhibitory activities

Determination of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition was performed according to the method of Ellman (1961) (Ergül et al., 2019; Güçlü et al., 2022). In the study, inhibition of α-glucosidase Kumar et al. (2012), inhibition of α-amylase Kumar et al. (2013) according to the method reported In the study, acarbose was used as a positive control in both α-glucosidase and α-amylase inhibition methods. Tyrosinase inhibitory activity was determined by slightly modifying the 96-well plate spectrophotometric method described by Jeong et al. (2009).

2. RESULTS

2.1. Chemical Components

In the study, the chemical components determined by GC-MS (Gas Chromatography / Mass Spectrometry) analysis method in 80% ethanol extract of *Lavandula stoechas* are given in Table 1.

Table 1: Chemical components of 80% ethanol extract of *Lavandula stoechas*

No	R.T.	(%) Area	Chemical components
1	15.275	0.99	Butylethylacetaldehyde
2	18.691	1.21	1,4:3,6-Dianhydro-alpha-d-glucopyranose
3	19.017	4.19	4-Vinylphenol
4	22.604	3.23	2-Methoxy-4-vinylphenol
5	28.269	8.65	1-Dodecanol
6	28.876	1.19	Adamantane
7	29.385	2.50	2,4-Di-tert-butylphenol
8	32.629	3.17	N2-dimethylguanine
9	33.476	23.07	2-Propenoic acid, dodecyl ester
10	35.141	1.95	(-)-Loliolide
11	36.223	1.13	Neophytadiene
12	36.349	5.84	Hexahydrofarnesyl acetone
13	38.843	1.95	Ethyl palmitate
14	46.042	2.57	Steviol Methyl Ester

In the study, the highest amount of chemical components determined from 80% ethanol extract of *Lavandula stoechas* was "2- Propenoic acid, dodecyl ester" with 23.07%, followed by "1-Dodecanol" with 8.65% and "Hexahydrofarnesyl acetone" with 5.84% (Table 1).

2.2. Antioxidant Activity

DPPH and ABTS radical scavenging activity

DPPH and ABTS radical scavenging activity determined in 80% ethanol extract of *Lavandula stoechas* plant is given in Figure 1.

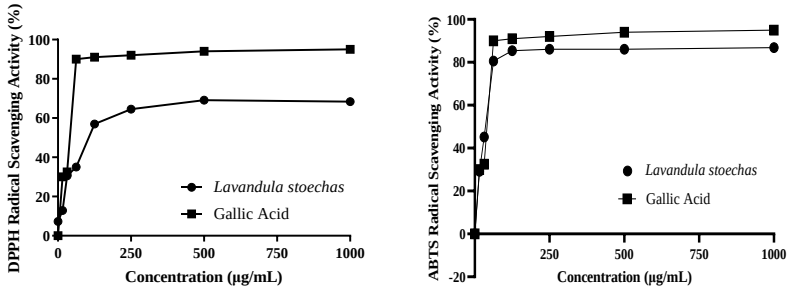

Figure 1: DPPH and ABTS radical scavenging activity of *Lavandula stoechas*

When the data obtained from the DPPH and ABTS radical scavenging activity test of *Lavandula stoechas* 80% ethanol extract were compared with the results of the gallic acid standard, it was determined that the antioxidant activity value was moderate compared to the DPPH test, but good in the ABTS test (Figure 1).

Total phenol (TPC) and total flavonoid contents (TFC)

TPC and TFC determined in 80% ethanol extract of *Lavandula stoechas* plant are given in Figure 2.

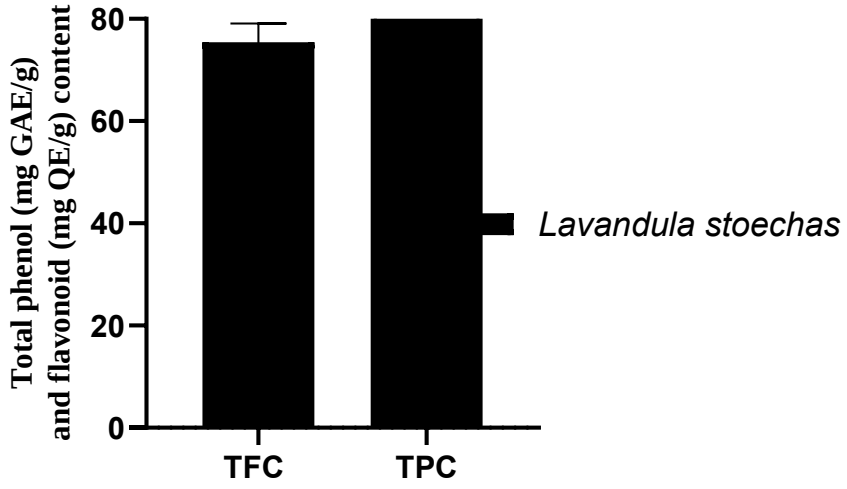

Figure 2: Total Phenol and Total Flavonoid Contents in 80% Ethanol Extract of *Lavandula stoechas* Plant

When the study was evaluated in terms of total phenol and total flavonoid contents, it was concluded that 80% ethanol extract of *Lavandula stoechas* plant had high levels of TFC and TPC (Figure 2.).

Enzyme inhibition activity

Comparison of the inhibition activities of some enzymes of 80% ethanol extract of *Lavandula stoechas* plant with reference drugs is given in Figure 3.

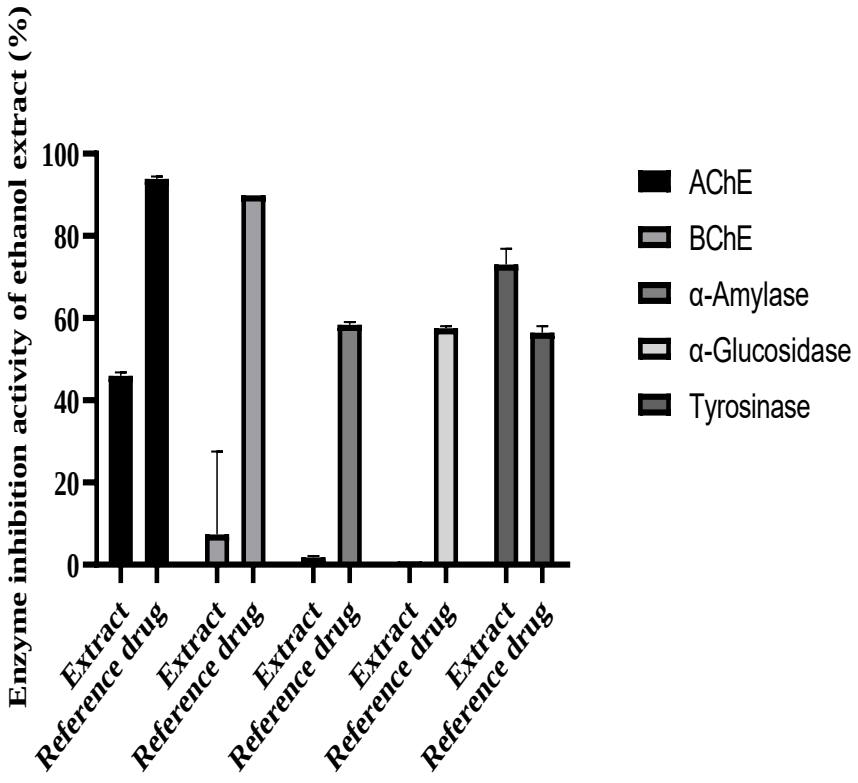

Figure 3: Comparison of the Inhibition Activities of AChE, BChE, α-Amylase, α-Glucosidase and Tyrosinase Enzymes of 80% Ethanol Extract of *Lavandula stoechas* Plant With Reference Drugs

AChE and BChE enzymes are effective on Alzheimer's, α-amylase and α-glucosidase enzymes are effective on diabetes and tyrosinase enzyme is effective on skin spots. Galanthamine, which was used as a reference drug in the study, was used to compare the inhibition activities of AChE and BChE enzymes. Acarbose, which was used as a reference drug in the study, was used to compare the inhibition activities of α-amylase and α-glucosidase enzymes. Kojic acid, which was used as a reference drug in the study, was used to compare the inhibition activity of

tyrosinase enzyme. When the enzyme inhibition activity values of *Lavandula stoechas* plant on these diseases were examined, it was determined that the tyrosinase enzyme showed higher activity than the standard drug.

CONCLUSIONS

As a result of the study, 80% ethanol extract of *Lavandula stoechas* plant; chemical composition, antioxidant activity, TPC and TFC, and inhibitory activities of some enzymes were determined. The chemical components of *Lavandula stoechas* plant were determined as "2-propenoic acid, dodecyl ester", "1-dodecanol" and "hexahydrofarnesyl acetone" in the highest amount, respectively. It was determined that 80% ethanol extract of *Lavandula stoechas* plant showed moderate antioxidant activity in DPPH test and good level of antioxidant activity in ABTS test when compared with gallic acid. In the study, it was determined that the TPC and TFC of the plant were high. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes associated with Alzheimer's disease, α-glucosidase and α-amylase enzymes associated with diabetes were found to be at low levels compared to the reference drugs, while tyrosinase enzyme, which is effective on skin spots, was found to be at a higher level than the reference drug.

REFERENCES

Aksoy, L., Suyundikov, M., & Düz, M. (2021). α-amylase, α-glucosidase, tyrosinase, acetylcholine esterase enzyme inhibition properties and essential oil composition of *Thermopsis turcica* Kit Tan, Vural&Küçüködük. Anadolu Journal of Agricultural Sciences, 36(3): 357-364.

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B., & Weil, J.A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry, 84: 551-562.

Bilenler, T., & Gökbulut, İ. (2019). Determination of sensitivity of hospital pathogens against commercial essential oil. Gümüşhane University Journal of Science and Technology, 9(3): 716-723. (In Turkish).

Blois, M.S. (1958). Antioxidant determinations by the use of stable free radical. Nature, 181: 1199-1200.

Carrasco, A., Ortiz-Ruiz, V., Martinez-Gutierrez, R., Tomas, V., & Tudela, J. (2015). *Lavandula stoechas* essential oil from Spain: Aromatic profile determined by gas chromatography–mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. Industrial Crops and Products, 73: 16-27.

Celep, E., Akyüz, S., İnan, Y., & Yesilada, E. (2018). Assessment of potential bioavailability of major phenolic compounds in *Lavandula stoechas* L. ssp. *stoechas*. Industrial Crops & Products, 118: 111-117.

Dob, T., Dahmane, D., Agli, M., & Chelghoum, C. (2006). Essential oil composition of *Lavandula stoechas* from Algeria. Pharmaceutical Biology, 44(1): 60-64.

Ellman, G.L., Courtney, K.D., Andres, V., & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2): 88-95.

Ergül, M., Ergül, M., Eruygur, N., Ataş, M., & Uçar, E. (2019). *In vitro* evaluation of the chemical composition and various biological activities of *Ficus carica* leaf extracts. Turkish J. Pharm. Sci., 16(4), 401-409.

Eruygur, N., Ucar, E., Akpulat, H.A., Shahsavari, K., Safavi, S.M., & Kahrizi, D. (2019). In vitro antioxidant assessment, screening of enzyme inhibitory

activities of methanol and water extracts and gene expression in *Hypericum lydium*. Molecular Biology Reports, 46: 2121-2129.

Eruygur, N., & Uçar, E. 2018. Cholinesterase, α-Glucosidase, α-Amylase, and Tyrosinase Inhibitory Effects and Antioxidant Activity of *Veronica officinalis* Extracts. Türkiye Tarımsal Araştırmalar Dergisi, 5(3): 253-259.

Gámez-Meza, N., Noriega-Rodríguez, J.A., Medina-Juárez, L.A., Ortega-García, J., Cázarez-Casanova, R., & Angulo-Guerrero, O. (1999). Antioxidant activity in soybean oil of extracts from thompson grape bagasse. JAOCS, 76: 1445-1447.

Giray, E.S., Kırıcı, S., Kaya, D.A., Türk, M., Sönmez, Ö., & İnan, M. (2008). Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of *Lavandula stoechas*. Talanta, 74: 930-935.

Güçlü, G., Ergül, M., Uçar, E., Eruygur, N., Ataş, M., & Akpulat, H.A. (2022). Anticancer, antioxidant, antimicrobial and enzyme ınhibitory activities of *Inula aucheriana*. KSÜ Tarım ve Doğa Dergisi, 25(5): 946-954.

Jeong, S.H., Ryu, Y.B., Curtis-Long, M.J., Ryu, H.W., Baek, Y.S., Kang, J.E., Lee, W.S., & Park, K.H. (2009). Tyrosinase inhibitory polyphenols from roots of *Morus lhou*. J. Agric. Food Chem., 57: 1195-1203.

Kaplan, E.N., Çakı, M.N., & Paşa, S. (2019). *Lavandula stoechas* (karabaş otu) bitki özütünden kozmetik ürün eldesi. UMÜFED Uluslararası Batı Karadeniz Mühendislik ve Fen Bilimleri Dergisi, 1(1): 24-37.

Kolaç, T., Gürbüz, P., & Yetiş, G. (2017). Phenolic content and antioxidant characteristics of natural products. Journal of Inonu University Health Services Vocational School, 5(1): 26-42. (In Turkish).

Kumar, D., Gupta, N., Ghosh, R., Gaonkar, R.H., & Pal, B.C. (2013). α-Glucosidase and α-amylase inhibitory constituent of *Carex baccans*: Bio-assay guided isolation and quantification by validated RP-HPLC-DAD. Journal of Functional Foods, 5(1): 211-218.

Kumar, D., Kumar, H., Vedasiromoni, J.R., & Pal, B.C. (2012). Bio-assay guided isolation of α-glucosidase inhibitory constituents from *Hibiscus mutabilis* leaves. Phytochemical Analysis, 23(5): 421-425.

Leblebici, S., Bahtiyar, S.D., & Özyurt, M.S. (2012). Determination of the amount of heavy metal in some medicinal plants sold in herbalist in Kütahya. Journal of Science and Technology of Dumlupınar University, 29: 1-6.

Mokhtarzadeh, S., & Khawar, K.M. (2022). Optimization of tissue culture studies in *L. stoechas L. subsp. L. stoechas*. Turkish Journal of Agricultural and Natural Science, 9(2): 330-339. (In Turkish).

Molan, A.L., & Mahdy, A.S. (2014). Iraqi medicinal plants: Total flavonoid contents, free-radical scavenging and bacterial beta-glucuronidase inhibition activities. IOSR Journal of Dental and Medical Sciences, 13(5): 72-77.

Noreen, H., Semmar, N., Farman, M., & McCullagh, J.S.O. (2017). Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. Asian Pacific Journal of Tropical Medicine, 10(8): 792-801.

Öztürk, B., Konyalıoğlu, S., Kantarcı, G., & Çetinkol, D. (2005). Essentıal oil composition, antibacterial antifungal and antioxidant capacity of wıld Lavandula stoechas L. subsp. stoechas from İzmir. ANADOLU Journal of Aegean Agricultural Research Institute, 15(1): 61-72.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant acivity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 26(9/10): 1231-1237.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, 91(4): 621-632.

Tüzün, S., Baş, İ., Karakavuk, E., Sanyürek, N.K., & Benzer, F. (2020). Comparison of antioxidant activities detected by different methods in various molasses. Turkish Journal of Agricultural and Natural Science, 7(2): 323-330.

Zuzarte, M., Gonçalves, M.J., Cavaleiro, C., Cruz, M.T., Benzarti, A., Marongiu, B., Maxia, A., Piras, A., & Salgueiro, L. (2013). Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. Industrial Crops and Products, 44: 97-103.