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In Vitro of *Melaleuca viridiflora* Sol. ex Gaertn Plant Investigation of Antimicrobial, Anticancer and Cytotoxic Activities

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ABSTRACT

In this study; it was aimed to investigate the anticancer and cytotoxic activities on DU-145 prostate cancer and MCF-7 breast cancer cell lines and WI-38 human fibroblast cell line of essential oil (Nioli) obtained from *Melaleuca viridiflora* Sol. ex Gaertn. plant and its antimicrobial effects on various bacteria and yeast cells. Content analyzes of Nioli essential oil were made by Gas Chromatography-Mass Spectrometry (GK-KS). The antimicrobial effects of the essential oil were determined using Disk Diffusion and Minimum Inhibition Concentration (MIC) tests. Its cytotoxic effects were determined by the XTT (2,3-bis-(2-methoxy-4-) nitro-5)-sulfophenyl)-2H-tetrazolium-5-carboxanilide) test; were investigated at different concentrations in breast cancer (MCF-7), prostate cancer (DU-145) and healthy human fibroblast (WI-38) cell lines. In the disc diffusion method of essential oil; It was determined that it formed an inhibition zone against *Klebsiella pneumoniae* bacteria at 200, 100, 50, 25 and 12.5 µg/mL concentrations, and against *Candida albicans* yeast at 200 and 100 µg/mL concentrations. Nioli essential oil showed the highest antibacterial effect against *Bacillus cereus*. While it was moderately effective against *Pseudomonas aeruginosa* and *Candida albicans*, it reached MIC values effective against other bacteria. It was observed that the anticancer activity of Nioli essential oil was more effective in DU-145 prostate cancer cells compared to MCF-7 breast cancer cells, and it had no toxicity in WI-38 healthy human fibroblast cells.

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Introduction

Medicinal and aromatic plants have been used in the prevention of diseases and in the treatment due to their healing effects since the existence of humanity (Bayram et al., 2010; Sevindik et al., 2017). Accordingly, plant essential oils have been used in many scientific and commercial fields for many years. Food industry, cosmetics, medicine, aromatherapy and phytotherapy are the leading areas among these fields (Hammer et al., 1998; Mohammed et al., 2022). The fact that essential oils have a wide range of uses has attracted the attention of many scientists. As a result of many researches, their chemical structures and biological activities were evaluated and these properties were put into practice (Mouhssen, 2004; Uysal et al., 2021; Akgül et al., 2022). Essential oils contain aromatic compounds with volatile and unique odor, obtained from medicinal and aromatic plants, in liquid form at room temperature (Oussalah et al., 2007; Kına et al., 2021; Unal et al., 2022). Essential oils can be found in the organs of plants such as flowers, fruits, bark, leaves, roots, resins and wood. Plant essential oils contain terpenic substances (about 90%), terpenic hydrocarbons and their oxygenated derivatives, as well as organic acids, alcohols, phenols and ketones (Çelik and Çelik, 2007; Tanker et al., 1990).

Melaleuca genus is a plant species belonging to the Myrtaceae family, growing naturally in the Australian continent and Asia, generally spreading in tropical regions, and consisting of evergreen trees and shrubs in winter. It is a fruit-like capsule up to 6 m in length, with a bare body and spongy leaves, with alternating leaves (Nevin et al., 2016).

The essential oil produced by distillation of the leaf parts of plants belonging to the *M. viridiflora* species of the Myrtaceae family is called Nioli. This oil, which is obtained from the plant and has volatile properties, contains a large amount of monoterpenes and sesquiterpenes (Bombarda et al., 2001). The antibacterial and antifungal activities of the essential oil obtained from plants belonging to the *Melaleuca* genus have been proven.

The data suggest that 1,8-cineol, terpinen-4-ol, and methyl eugenol, in particular, play key roles in mediating the antimicrobial activity of this oil (Sharifi-Rad et al., 2017). Through its high content of eucalyptol; it is included in the content of pharmaceutical products due to its therapeutic effect in diseases such as cough, cold, rheumatism, neuralgia and is used for upper respiratory tract disorders in aromatherapy (Elliot and Jones, 1993). In external use, due to its fungicidal and bactericidal effect, it is used in the treatment of cystitis, in vaginal washing, and its solutions at a rate of 5-15 % in the treatment of acne (Nevin et al., 2016). It is known that niolin suppresses the hormone called α-melanocyte, which activates oxidative stress in B16 melanoma cells (Chao et al., 2017).

Microorganisms can gain resistance to antibiotics over time, and as a result of this resistance, the importance of studies on the development of new generation drugs in the fight against infections is increasing (Sevindik, 2019). It has been observed that microorganisms do not form the resistance mechanism against synthetic antibiotics against plants. This situation inevitably increases the importance of herbal medicines or plant extracts (Çelik and Çelik, 2007; Toroğlu and Çenet, 2006).

In cancer treatment, various treatments are applied according to the location of the tumor in the body, the physiological condition of the patient and the structure of the tumor. Chemotherapy and radiotherapy are among the most commonly used methods in routine (Kiltie et al., 2005).

Cancer is one of the diseases with the highest mortality rate in the world. This has given great importance to cancer treatment research. Of late years, there has been an growing trend in the preference of medicinal and aromatic plants in cancer treatment due to their high activity, low toxicity and many natural anticancer agents in their content (Daştan et al., 2016).

In this study, the anticancer activities of Nioli (*M. viridiflora*) essential oil on DU-145 prostate cancer and MCF-7 breast cancer cells, cytotoxic activities on WI-38 human fibroblast cell line and antimicrobial effects on various bacteria and yeast cells were investigated.

Materials and Methods

Essential Oil Sample

Nioli essential oil was supplied by Art de Huile (Turkey).

GC-MS (Gas Chromatopraphy-Mess Spectrometry)

GC-MS analyzes for the determination of the components in the Nioli essential oil sample were commissioned by Art de Huile, which supplied the oil. The average values of the essential oil composition are given in (Table 1). These components are alpha pinene, alpha terpineol, beta pinene, cineol 1.8, gamma terpinene, limonene, viridiflorol. When the GC-MS results were examined, it was determined that the content of Nioli essential oil had the highest rate of cineol 1.8 (52,63%) and the lowest rate of gamma terpinene (1.80%). Cineol 1.8 was determined as the major essential oil component.

Antimicrobial Activity Tests

Disc diffusion (Kirby-Bauer) and microdilution broth methods were used to determine the antimicrobial activity of Nioli essential oil.

Disc Diffusion Method

In the dis diffusion test, *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Candida albicans* (ATCC 10231) strains were used. Dilutions of Nioli essential oil (100-1.56 µg/mL) were absorbed into blank discs with 20 µL. Mueller-Hinton Agar (MERCK) and Sabouraud Dextrose Agar (Neogen) agars were inoculated with 0.5 McFarland turbidity bacterial and yeast solutions using sterile swab sticks. Essential oil impregnated discs were placed. Antibiotic discs were used for positive control (OXOID). Bacterial plaques were incubated at 37 ± 0,1°C for 24 hours, and yeast plaques at 25 ± 0,1°C for 48 hours. At the end of the expected time, the diameters of the zones observed in the medium were measured in mm (CLSI M02, 2018).

Minimum Inhibition Concentration (MIC)

For the experiment, 96-well microtiter plates with U-type wells were used. MHB was used for bacteria and SDB was used for yeast. 10 µL essential oil (200 µg/mL) was applied to the first well, followed by serial dilutions. Bacteria and yeasts adjusted to McFarland 0.5 turbidity were diluted to 5×10^5 cfu/mL for bacteria and $0.5\text{-}2.5 \times 10^3$ cfu/mL for yeasts and 50 µl was added onto the wells (CLSI M07-A9, 2012; CLSI M27-A2, 2012). Plates were incubated for 24 hours at 37°C. In order to better observe the growth at the end of the incubation, 50 µL of 2,3,5-Triphenyltetrazolium chloride (TTC) (Meck, Germany) solution of 2 mg/mL was applied to all. Incubation process complete at 37°C for 2 hours. The first well in which a decrease in the color of Formazan due to the presence of live microorganisms in the wells was observed was accepted as the MIC. MIC results according to reference sources; It was evaluated as effective (MIC <100 µg/mL), Moderate (100 <MIC ≤ 625 µg/mL), Weak (MIC >625 µg/mL) (Kuete, 2010; Awouafack et al., 2013).

Table 1. Components of Nioli essential oil

Name of Component	Reference	GC-MS (%)
Alpha pinene	6.0-15.0	8.28
Alpha terpineol	5.0-10.0	7.63
Beta pinene	1.0-5.0	2.11
Cineol 1.8	45.0-65.0	52,63
Gamma terpinene	1.0-4.0	1.80
Limonene	3.0-12.0	7.73
Viridiflorol	2.0-10.0	5.08

Cytotoxic Activity

In our study, MCF-7, DU-145, WI-38 cell lines were used for cytotoxic activity determination. Essential oils were prepared at different concentrations (200-1.56 µg/mL) and used. Cells were incubated in 5% CO_2 at 37°C. 1% penicillin (100 U/mL) and streptomycin (100 µg/mL) and 10% fetal bovine serum (FBS) were added to all wells.

Cytotoxicity was interpreted according to the XTT method. Cells were added to the wells in the appropriate medium, test compounds were applied in varying proportions and incubated in CO_2 medium at 37°C for 24 hours. When the time was up, 100 µL of XTT solution was added to all wells and incubated for an additional 2 hours and optical density values were determined at 475 nm (Adukwu et al., 2016).

Results and Discussion

Globally, cancer is the cause of the majority of diseases and deaths caused by them, which can affect almost every organ in the human body. World Health Organization data show that; Deaths due to cancer in the world are 1 in 6 of all other deaths. According to statistics, worldwide cancer cases will rise to 29.4 million per year by 2040 (Jain et al., 2021). While the most common type of cancer in women is breast cancer, it is stated as prostate cancer in men. (Parkin et al., 2005).

Serious side effects are observed in people due to the use of chemotherapy drugs in cancer treatment. Today, methods such as chemotherapeutic drugs, radiotherapy, surgical treatment and hormone therapy used in the treatment of cancer have made it much more important to search for alternative methods that can be used in treatment because of the low probability of success and side effects (Tekin et al., 2012).

The importance of introducing alternative and complementary drugs and treatment methods for the treatment of cancer is emphasized in clinical, epidemiological and experimental studies. Plants are the leading natural resources preferred in the traditional treatment of different types of cancer diseases. About 60% of the sources used for anticancer treatment consist of plants, seafood or microorganisms (Jain et al., 2011).

According to another study; It was concluded that Niaouli essential oil inhibited all microorganisms except *P. aeruginosa* (Hammer et al., 1998).

As a result of examining the antimicrobial effect of various essential oils on the Swiss microflora; They concluded that essential oils of *Eucalyptus globulus* Labill., *Melaleuca alternifolia* (Maiden & Betche) Cheel, *Pimpinella anisum* L. and *Syzygium aromaticum* (L.) Merr. & L.M.Perry offered the most effective antimicrobial efficiency (Ponce et al., 2003).

Byahatti et al; They evaluated the anticancer activity of *M. alternifolia* on the Leukemia cancer cell line (K562) with the MTT test and mentioned the existence of statistically significant results (Byahatti et al., 2018).

The in vitro antitumor activity of *M. alternifolia* plant belonging to the genus Melaleuca was analyzed according to long-term exposure to doxorubicin to human melanoma M14 WT cells and their drug-resistant counterparts, M14 ADR cells. As a result of the experiment, it was revealed that the presence of antioxidant and cytotoxic effects was significant (Bhalla et al., 2013).

According to the study of Chabir et al.; *Melaleuca armillaris* (Sol. ex Gaertn.) Sm. essential oil has been observed to have high cytotoxic activity on MCF-7 cells (Chabir et al., 2011).

In another study; Antimicrobial and in vitro anti-tumor activities of essential oil obtained from *M. alternifolia* plant were tested. The essential oil has important antimicrobial efficiency against other tested microorganisms beyond *Aspergillus niger*; It was also concluded that it exhibits potent cytotoxicity against the human lung cancer cell line (A549), the human breast cancer cell line (MCF-7) and the human prostate cancer cell line (PC-3) (Xia et al., 2009).

With the study of Rapper et al.; It has been revealed that viridiflorol and furanoeudesma-1,3-diene, which are compounds found in the essential oil of plants of the genus *Melaleuca*, have activity against the pathogen *Haemophilus influenzae* (Rapper et al., 2021).

Viridiflorol in the composition of essential oils obtained from plants of the genus *Melaleuca*; It is a phytochemical that can work as an anti-cancer compound. The effects of viridifluoro at various concentrations were investigated in vitro on breast, lung and brain cancer cells and these cell lines were found to exhibit cytotoxic and apoptotic abilities (Akiel et al., 2022).

According to the GC-MS analysis in our study, alpha pinene, alpha terpineol, beta pinene, cineol 1.8, gamma terpinene, limonene, viridiflorol components were determined in the content of Nioli essential oil. It was determined that cineol 1.8 (52,63%) was in the first place and alpha pinene (8.28%) was second in the content of our foundation oil. In our study; The content of our Nioli essential oil sample is very similar to the results obtained from studies conducted by different researchers in the literature.

According to disk diffusion test (Table 2), Nioli essential oil formed an inhibition zone against *K. pneumoniae* bacteria at 1,2,3,4 and 5 concentrations, and against *C. albicans* yeast at 1st and 2nd concentrations.

Table 2. Disc diffusion results of Nioli essential oil

Mikroorganizma	Nioli Essential Oil -- Disc Diffusion Zone Diameters (mm)									
	Concentrations (µg/mL)								Positive Control	Negative Control
	1	2	3	4	5	6	7	8		
Escherichia coli	-	-	-	-	-	-	-	-	19	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	17	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	19	-
Klebsiella pneumoniae	9	8	8	8	7	-	-	-	22	-
Bacillus cereus	-	-	-	-	-	-	-	-	20	-
Candida albicans	8	6	-	-	-	-	-	-	18	-

Table 3. MIC results of Nioli essential oil

Microorganisms	Nioli Essential Oil (µg/mL)
Escherichia coli	100
Pseudomonas aeruginosa	>200
Staphylococcus aureus	100
Klebsiella pneumoniae	100
Bacillus cereus	6.25
Candida albicans	>200

Table 4. IC_{50} values of Nioli essential oil

IC_{50}(µg/mL)	MCF-7	DU-145	WI-38
Nioli	18.49 ±0.98	15.85 ±0.15	39.45 ± 0.85

The antimicrobial test results (MIC) of Nioli essential oil obtained from the *M. viridiflora* plant are shown in (Table 3). Nioli essential oil provided the maximum antibacterial effect against *B. cereus* (MIC: 6.25 µg/mL). While it was moderately effective against *P. aeruginosa* and *C. albicans*, it reached MIC values effective against other bacteria.

Solutions of Nioli essential oil was used to determine antitumor activities on DU-145 and MCF-7 cell lines, It was prepared at concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.56 µg/mL. Viability of cells was determined by XTT test. % Inhibition was determined by making the calculations specified in the reference sources and the relevant tables and figures were obtained.

Nioli essential oil was determined to have a quite activity on MCF-7 cells in the first 4 concentrations. A cell killing rate of over 100% was found at the 2 highest concentrations (200 µg/mL and 100 µg/mL). The determination of the IC_{50} value as 18.49 µg/mL shows the effectiveness of Nioli essential oil even at low concentrations (Table 4).

It was determined that the first 6 concentrations of Nioli essential oil showed a quite activity (80% and above) on DU-145 cells. At the highest concentration of the essential oil (200 µg/mL), a cell killing rate of over 100% was found. Even at the lowest concentration (1.56 µg/mL), the rate of killing cancer cells, which is 77.14%, is quite high. The determination of the IC_{50} value as 15.85 µg/mL shows the effectiveness of Nioli essential oil even at low concentrations (Table 4).

Eight different concentrations of Nioli essential oil was tested on the WI-38 cell line, a normal human lung fibroblast cell, and cytotoxic activity results were evaluated as % cell viability. Cell viability was determined as 100% at the last 5 concentrations. It was observed that Nioli essential oil (Table 4) had no significant toxicity in the healthy WI-38 cell line at the effective doses (IC_{50} = 39,45 µg/mL).

Conclusion

In this study; the antitumor activities of Nioli essential oil obtained from *M. viridiflora* plant on DU-145 and MCF-7 cell lines and cytotoxic activities on WI-38 cell line were investigated. In addition, its antimicrobial effects on various bacteria and yeast cells were investigated. It was observed that the inhibitory effect of Nioli essential oil on the DU-145 cell line was higher than that of the MCF-7 cell line. It was determined that the essential oil had no toxic effect on healthy WI-38 cell lines at concentrations effective on DU-145 and MCF-7 cells. Results have been obtained that Nioli essential oil has the capacity to be used as an alternative product in anticancer treatments. At the next stage, it may be suggested that this essential oil be tested in animal experiments and then directed to clinical research and developed as a chemotherapy drug.

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References

Adukwu EC, Bowles M, Edwards-Jones V, Bone H. 2016. Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (*Cymbopogon flexuosus)* and pure citral. Appl Microbiol Biotechnol, 100: 9619–9627

Akgül H, Mohammed FS, Kına E, Uysal İ, Sevindik M, Doğan M. 2022. Total Antioxidant and Oxidant Status and DPPH Free radical activity of *Euphorbia eriophora*. Turkish Journal of Agriculture-Food Science and Technology, 10(2): 272-275.

Akiel MA, Alshehri OY, Aljihani SA, Almuaysib A, Bader A, Al-Asmari AI, Alamri HS, Alrfaei BM, Halwani MA. 2022. Viridiflorol induces anti-neoplastic effects on breast, lung, and brain cancer cells through apoptosis. Saudi Journal of Biological Sciences, 29: 816–821

Awouafack MD, Mcgaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, Eloff JN. 2013. Antibicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds ısolated from *Eriosema robustum*. BMC Complementary Medicine and Therapies, 13: 289

Bayram E, Kırıcı S, Tansı S, Yılmaz G, Arabacı O, Kızıl S, Telci İ. 2010. Possibilities to Increase Production of Medicinal and Aromatic Plants. Turkey Agricultural Eng. 7th Technical Congress, 11-15 January 2010 Ankara, Proceedings Book I, pp.437-456

Bhalla Y, Gupta VK, Jaitak V. 2013. Anticancer activity of essential oils. J Sci Food Agric, 93:3643–3653

Bombarda I, Raharivelomanana P, Ramanoelina P, Faure R, Bianchini JP, Gaydou EM. 2001. Spectrometric identifications of sesquiterpene alcohols from niaouli (*Melaleuca quinquenervia*) essential oil. Analytica Chimica Acta, 447 113–123

Byahatti S, Bogar C, Bhat K, Dandagi G. 2018. Evaluation of anticancer activity of *Melaleuca alternifolia* (i.e. tea tree oil) on leukemia cancer cell line (K562): An in vitro study. Journal of Medicinal Plants Studies, 6(5): 01-06

Chabir N, Romdhane M, Valentin A, Moukarzel B, Marzouq H, Brahim N, Mars M, Bouajila J. 2011. Chemical study and antimalarial, antioxidant and anticancer activities of *Melaleuca armillaris* (sol ex gateau) sm essential oil. Journal of Medicinal Food vol. 14, NO. 11 | https://doi.org/10.1089/jmf.2010.0168

Chao W, Sub CC, Pengc HY, Choub ST. 2017. *Melaleuca quinquenervia* essential oil inhibits α-melanocyte-stimulating hormone-induced melanin production and oxidative stress in B16 melanoma cells. Phytomedicine, 34: 191–201

CLSI. 2018. Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard. 13th Edition, CLSI document M02. Clinical and Laboratory Standards Institute Clinical and Laboratory Standards Institute, Pennsylvania USA.

CLSI. 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard. 9th Edition, CLSI document M07-A9. Clinical and Laboratory Standards Institute, Pennsylvania USA.

CLSI. 2012. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard. 2th Edition, CLSI document M27-A2. Clinical and Laboratory Standards Institute, Pennsylvania USA.

Çelik E, Çelik GY. 2007. Antimicrobial properties of plant essential oils. Orlab On-Line Journal of Microbiology, 2:(5), 1-6

Daştan SD. 2016. Evaluation of in vitro anticancer effect of *Plantago major L.* and *Plantago lanceolata L.* leaf extracts from Sivas. Cumhuriyet University. Journal of the Institute of Health Sciences, (1)1: 07-14

Elliot WR, Jones DL. 1993. Encyclopaedia of Australian Plants Suitable for Cultivation. Melbourne: Lothian Publishing Co. Pty. Ltd., vol:6, pp. 315-317, 359

Hammer KA, Carson CF, Riley TV. 1998. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 86, 985–990

Jain D, Murti Y, Khan WU, Hossain R, Hossain MN, Agrawal KK, Ashraf RA, Islam MT, Janmeda P, Taheri Y, Alshehri MM, Daştan SD, Yeskaliyeva B, Kipchakbayeva A, Sharifi-Rad J, Cho WC. 2021. Roles of therapeutic bioactive compounds in hepatocellular carcinoma. Oxidative Medicine and Cellular Longevity, p:31 https://doi.org/10.1155/2021/9068850

Jain D, Pathak N, Khan S, Raghuram GV, Bhargava A, Samarth R, Mishra PK. 2011. Evaluation of cytotoxicity and anticarcinogenic potential of mentha leaf extracts. International Journal of Toxicology, 30: (2), 225-236

Kına E, Uysal İ, Mohammed FS, Doğan M, Sevindik M. 2021. In-vitro antioxidant and oxidant properties of *Centaurea rigida*. Turkish Journal of Agriculture-Food Science and Technology, 9(10): 1905-1907.

Kiltie A, Knowles MA, Selby PJ. 2005. Radiotheraphy and molecular radiotheraphy. Introduction to The Cellular and Molecular Biology of Cancer Oxford University Press USA, 414

Kuete V. 2010. Potential of cameroonian plants and derived products againts microbial infections. Planta Med., 76: 1479–1491

Mohammed FS, Kına E, Uysal İ, Mencik K, Dogan M, Pehlivan M, Sevindik M. 2022. Antioxidant and Antimicrobial Activities of Ethanol Extract of *Lepidium spinosum*. Turkish Journal of Agriculture-Food Science and Technology, 10(6): 1116-1119.

Mouhssen L. 2004. Methods to study the phytochemistry and bioactivity of essential oils. Phytotherapy Research, 18: 435–448

Oussalah M, Caillet S, Saucier L, Lacroix M. 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control, 18, 414–420

Parkin DM, Bray F, Ferlay J, Pisani P. 2005. Global cancer statistics 2002. A Cancer Journal for Clinicians, 55 (2): 74-108

Ponce AG, Fritz R, Valle RC, Roura RC. 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensm.-Wiss. U.-Technol., 36: 679–684

Rapper SL, Tankeu SY, Kamatou G, Viljoen A, Vuuren SV. 2021. The use of chemometric modelling to determine chemical composition-antimicrobial activity relationships of essential oils used in respiratory tract infections. Fitoterapia, 154: 105024

Sevindik M, Akgul H, Pehlivan M, Selamoglu Z. 2017. Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresen Environ Bull, 26(7): 4757-4763.

Sevindik M. 2019. The novel biological tests on various extracts of *Cerioporus varius*. Fresenius Environmental Bulletin, 28(5): 3713-3717.

Sharifi-Rad J, Salehi B, Varoni EM, Sharopov F, Yousaf Z, Ayatollahi SY, Mehdi Sharifi-Rad FK, Afdjei MH, Sharifi-Rad M, Iriti M. 2017. Plants of the melaleuca genus as antimicrobial agents: From farm to pharmacy. Phytother Res., 31(10):1475-1494

Tanker M, Tanker N, Şarer E, Atasu E, Şener B, Kurucu S, Meriçli F. 1990. Result of Certain İnvestigation on the Volatile Oil Centaining Plants of Turkey. Essential Oils for Perfumery and Flavours. Preceedings of an International Conference, May, Antalya

Tanker N, Koyuncu M, Coşkun M. 2016. Pharmaceutical Botany. Ankara University Faculty of Pharmacy Publications, Ankara, P: 247

Tekin A, Kaya E, Yazıcı S. 2012. Content analysis of cancer-related alternative medicine. Mehmet Akif Ersoy University Journal of Social Sciences Institute, 4: (6), 14-34

Toroğlu S, Çenet M. 2006. Usage areas of some plants used for therapeutic purposes and methods used for determination of antimicrobial activities. KSU Journal of Science and Engineering, 9: (2), 12-21

Unal O, Eraslan EC, Uysal I, Mohammed FS, Sevindik M, Akgul H. 2022. Biological activities and phenolic contents of *Rumex scutatus* collected from Turkey. Fresenius Environmental Bulletin, 31(7): 7341-7346.

Uysal İ, Mohammed FS, Şabik AE, Kına E, Sevindik M. 2021. Antioxidant and Oxidant status of medicinal plant *Echium italicum* collected from different regions. Turkish Journal of Agriculture-Food Science and Technology, 9(10): 1902-1904.

Xia L, Yuangang Z, Yujie F, Liping Y, Chengbo G, Wei W, Thomas E. 2009. Antimicrobial activity and cytotoxicity towards cancer cells of *Melaleuca alternifolia* (tea tree) oil. Eur Food Res Technol, 229: 247–253.