ՀՀ ԿՐԹՈՒԹՅԱՆ, ԳԻՏՈՒԹՅԱՆ, ՄՇԱԿՈՒՅԹԻ ԵՎ ՍՊՈՐՏԻ
ՆԱԽԱՐԱՐՈՒԹՅՈՒՆ
ԵՐԵՎԱՆԻ ՊԵՏԱԿԱՆ ՀԱՄԱԼՍԱՐԱՆ

ԲԱԲԱՅԱՆ ԱՆՈՒՇ ՄԱՐՏԻՆԻ

ORIGANUM VULGARE L. և *OCIMUM BASILICUM* VAR. *PURPUREUM* ԲՈՒՅՍԵՐԻՑ ԱՆՋԱՏՎԱԾ ՄԻԱՑՈՒԹՅՈՒՆՆԵՐԻ ԿԵՆՍԱԲԱՆԱԿԱՆ ԱԿՏԻՎՈՒԹՅՈՒՆՆ ՈՒ ԱԶԴԵՑՈՒԹՅԱՆ ՄԵԽԱՆԻԶՄՆԵՐԸ

Գ.00.04 – Կենսաքիմիա մասնագիտությամբ
կենսաբանական գիտությունների թեկնածուի
գիտական աստիճանի հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

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MINISTRY OF EDUCATION, SCIENCE, CULTURE AND SPORTS OF RA
YEREVAN STATE UNIVERSITY

BABAYAN ANUSH MARTIN

BIOLOGICAL ACTIVITY AND MECHANISMS OF ACTION OF COMPOUNDS ISOLATED FROM *ORIGANUM VULGARE* L. AND *OCIMUM BASILICUM* VAR. *PURPUREUM* PLANTS

SYNOPSIS

of dissertation for conferring of science degree of
Candidate of Biological Sciences
In the specialty of 03.00.04-Biochemistry

YEREVAN 2024

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝ կ.գ.դ., պրոֆ. Կարեն Արմենի Թռչունյան

Պաշտոնական ընդդիմախոսներ՝ կ.գ.դ., դոց. Գայանե Յուրիի Մարմարյան
կ.գ.թ., Աննա Հովհաննեսի Թադևոսյան

Առաջատար կազմակերպություն՝ ՀՀ ԳԱԱ Գ.Ս. Դավթյանի անվան հիդրոպոնիկայի պրոբլեմների ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2024թ. հուլիսի 16-ին ժամը 12^{00}-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲԿԳԿ-ի Կենսաֆիզիկայի 051 մասնագիտական խորհրդի նիստում (0025, Երևան, Ալեք Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:

Ատենախոսության սեղմագիրն առաքված է 2024թ. հունիսի 12-ին:

051 մասնագիտական խորհրդի գիտական
քարտուղար, կ.գ.դ., դոցենտ՝ Մ.Ա. Փարսադանյան

The theme of dissertation has been approved at Yerevan State University

Academic advisor: D.Sc., Prof. Karen Armen Trchounian

Official opponents: Dr.Sc., Assoc. Prof. Gayane Yuri Marmaryan
Ph.D., Anna Hovhannes Tadevosyan

Leading organization: G.S. Davtyan Institute of Hydroponics Problems of NAS RA

The defense of the dissertation will be held on 16th July, 2024, at 12:00, at the session of 051 Scientific Specialized Council on Biophysics of HESC of RA at Yerevan State University (0025, Yerevan, Alex Manoogian str. 1, YSU, Faculty of Biology).

The dissertation is available at the library of Yerevan State University.

The synopsis has been sent on 12th June, 2024.

Scientific Secretary of 051 Specialized Council,
D.Sc., Associate Professor M. A. Parsadanyan

INDRODUCTION

Topic's significance. Over the world, there is ongoing research and development of drugs with antioxidant and antimicrobial effects that can be effective for both the prevention and adjuvant therapy of various diseases (Staszowska-Karkut et al., 2020). A number of synthetic antioxidants widely used in food and medicine cause or stimulate various deviations in metabolic pathways in the body, so there is an increasing interest in the development of natural preparations with antioxidant and antimicrobial properties (Avetisyan et al., 2017; Sahakyan et al., 2019; Kosakowska et al., 2021; Babayan et al., 2022). In recent years, plant extracts have attracted significant scientific interest due to their potential as a source of natural biologically active compounds. Medicinal and aromatic plants constitute a large part of the natural flora and are considered an important resource in various fields. Moreover, the use of plant extracts in the food, perfumery, cosmetic, and pharmaceutical industries is continuously increasing (Moghrovyan et al., 2022; Babayan et al., 2022). Currently, more than 80% of the world's population uses herbal medicines to treat various health problems (Babayan et al., 2022). According to the literature (Laws et al., 2019; Gorlenko et al., 2020), another prospect for plant compounds is their ability to increase the sensitivity of bacteria to antibiotics. The discovery of such compounds can be particularly useful in the process of overcoming the antibiotic resistance in bacteria resistant to various antibiotics. From this point of view the study of the biological activity of the products of plants secondary metabolism is up-to-date and has practical and fundamental importance.

Inevitable by-products of aerobic metabolism are reactive oxygen species (ROS), which have toxic effects on all types of cells, cellular structures, and biomolecules, including DNA, RNA, proteins, and lipids (Imlay, 2008; Savchenko et al., 2021). Living organisms, including plants, have developed various protective and regenerative mechanisms to keep free radical concentrations at low levels and repair damages caused by the oxidative stress (Sharma et al., 2019). At the same time, ROS serve as regulators of signals, causing increased or decreased expression of many genes and changes in metabolic pathways. Many human diseases, such as cancer, diabetes, neurodegenerative diseases, and aging process of the body, are associated with a disturbance in the balance of oxidation and repair reactions, leading to an increase of ROS levels. It is known that a number of plant-derived substances, such as polyphenolic compounds, which are the main products of plant secondary metabolism, can act as natural antioxidants and free radical scavengers, exhibiting high biological activity.

Since ancient times people have been using plants for the treatment of various infectious diseases. Nowadays medicinal plants continue to be used in traditional medicine in many countries, including Armenia. The flora of Armenia is rich in edible and medicinal plants that are valuable for health. However, this biodiversity is still not well studied (Sahakyan et al., 2019; Moghrovyan et al., 2019, 2022; Ginovyan et al., 2022). One of the most popular medicinally valuable plants are the plant species belonging to Lamiaceae family. In general, essential oil-bearing plants of the Lamiaceae family are rich in polyphenolic compounds, and most of them are known for their antioxidant properties and are widely used as spices. Among the plants of this family,

Origanum vulgare L. and *Ocimum basilicum var. purpureum* were selected as research objects.

Research goals and tasks. The aim of the research was to study the biological activities, biochemical features, and mechanisms of action of ethanol extracts and essential oils of *Origanum vulgare* L. and *Ocimum basilicum var. purpureum* plants, belonging to the Lamiaceae family growing in Armenia.

Constituted tasks of the research were:

- Determine the biochemical composition of the ethanol extracts and essential oils of *O. vulgare* and *O. basilicum var. purpureum* plants aerial parts, determine the content of total phenols and flavonoids in ethanol extracts of plants,
- Investigate the antimicrobial activity of plants extracts and essential oils,
- Study the antibiotic-modulating activity of plant extracts toward different antibiotics,
- Evaluate the antioxidant activity of ethanol extracts and essential oils through different chemical tests,
- Evaluate the metal chelating activity of plants extracts,
- Determine the quantity of NO in *E. coli* NM111 cells under the influence of ethanol extracts of plants,
- Evaluate the effects of ethanol extracts and essential oils of plants on tyrosinase, superoxide dismutase and catalase activities,
- Determine the β-galactosidase activity in cells of *E. coli* NM111 carrying the *katG:lacZ* gene fused under the influence of plant ethanol extracts.

Scientific novelty and practical value of the study. The antimicrobial activity of *O. vulgare* and *O. basilicum* plants belonging to the Lamiaceae family growing in Armenia was studied. The biochemical composition of ethanol extracts and essential oils of *O. vulgare* L. and *O. basilicum var. purpureum* plants growing in Armenia was determined for the first time. High antibacterial, antifungal, antiradical, and metal-chelating activities of the ethanolic extracts and essential oils of the investigated plants were shown. The analysis of the obtained results and literature data allowed us to evaluate the potential of *O. vulgare* L. and *O. basilicum var. purpureum* plants as an alternative source of substances with high antibacterial and antioxidant activity. These plants have great application importance in cosmetics, food (as nutritional supplements), medicine, feed production, and other fields. Ethanol extracts of *O. vulgare* L. and *O. basilicum var. purpureum* plants have been shown to contain high levels of phenols and flavonoids. Ethanol extracts and essential oils of *O. vulgare* and *O. basilicum* were first evaluated for their tyrosinase inhibitory potential as a natural treatment for skin hyperpigmentation. The determination of tyrosinase inhibitory activity, apart from its practical value, can also serve as one of the additional methods for assessing the antioxidant activity of ethanolic extracts and essential oils. The essential oils of the investigated plants were found to have tyrosinase inhibitory activity. Thus, *O. vulgare* and *O. basilicum* plants can be considered as a source of natural bleaching agents. The evaluation of the antioxidant activity of the ethanolic extracts of *O. vulgare* and *O. basilicum* plants was

conducted for the first time using reactive thiobarbituric acid to inhibit the synthesis of malondialdehyde. It was demonstrated that the ethanolic extracts of the studied plants exhibit high activity in inhibiting malondialdehyde synthesis. The change of the NO quantity was measured in *E. coli* NM111 cells under the influence of ethanol extracts of the plants. It was observed that the NO concentration increases in *E. coli* cells under the influence of the investigated extracts. An increase in the activity of superoxide dismutase and catalase was shown in *E. coli* cells under the influence of ethanol extracts of *O. vulgare* and *O. basilicum* plants. The ability to modulate β-galactosidase activity of the ethanol extracts of the studied plants was determined. Most of the studied extracts expressed a protective effect against the bacteriostatic effect of H_2O_2, increasing the cell growth rate.

Main points to present at the defense.

1. The ethanol extracts and essential oils of *O. vulgare* and *O. basilicum var. purpureum* plants have antimicrobial, antiradical, malondialdehyde synthesis inhibitory and metal-chelating activity, in which the content of phenols and flavonoids in ethanol solutions has an important role.
2. Under the effect of ethanol extracts of *O. vulgare* and *O. basilicum var. purpureum* plants, the NO quantity in *E. coli* NM111 cells increased.
3. Ethanol extracts and essential oils of *O. vulgare* and *O. basilicum var. purpureum* plants have the ability to inhibit tyrosinase, change of superoxide dismutase, catalase, β-galactosidase activity and *katG:lacZ* gene expression.
4. Ethanol extracts of *O. vulgare* and *O. basilicum var. purpureum* plants act as prooxidants in the cells of *E. coli* NM111, and thereby determine the antibacterial activity of the components of these extracts.

Work approbation. Main results of the dissertation were discussed at seminars at the Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty of Yerevan State University and at scientific conferences: 31st International Conference of FFC (Yerevan, Armenia, 2023), ASM Microbe 2016 (Boston, USE, 2016), 3rd International Scientific Conference on "Dialogues on Sciences" (Yerevan, Armenia, 2015), "Trends in microbiology and microbial biotechnology" International Conference (Yerevan, Armenia, 2014).

Publications. On the basis of the experimental data observed in dissertation, 7 papers, including 3 articles in peer-reviewed journals and 4 abstracts were published.

Volume and structure of dissertation. Dissertation contains the following chapters: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and discussion (Chapter 3), concluding remarks, conclusions and cited literature (totally 158 papers and books). The dissertation consists of 130 pages, 8 tables and 28 figures.

MATERIALS AND METHODS

During the study, ethanol extracts and essential oils from the aerial parts of culinary plants belonging to the Lamiaceae family, specifically *O. vulgare* L. and *O. basilicum*, growing or cultivating in Armenia were studied. *O. vulgare* herbs were collected from Gegharkunik province, v. Chkalovka, 1930 m above the sea level, during

the blossoming period (July, 2016), and cultivated *O. basilicum var. purpureum* plant were collected from Kotayk province, at an altitude of 1600 m above sea level, during the flowering period (July-August, 2014). The identification of plant was carried out at the Department of Pharmacognosy, Yerevan State Medical University, Yerevan (Armenia). The plants of *Ocimum* genus were not included in the herbarium as there were cultivated species and were not typical for the flora of Armenia. The samples of basil are available at the Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty, Yerevan State University, Yerevan, Armenia.

Extraction of plant dry matter. Extraction of dry plant material was performed using 80% ethanol as a solvent. 10-15 ml of ethanol (80%) was added to the dried and powdered plant material and crushed for 15 - 20 minutes until a homogeneous mass was obtained and stored in a refrigerator for 24 hours (5 - 6 °C). Centrifugation was performed at 5000 rpm for 10 min. The supernatant was separated, the precipitate was treated similarly. Processing and centrifugation of the pellet was repeated 3 times to achieve complete dissolution of the active substances. The supernatants of all phases were combined and dried at room temperature without exposure to direct sunlight. The combined dry material was collected in Eppendorf-type test tubes, weighed and stored in a freezer (-18 to -20 °C) for further studies (Babayan et al., 2022).

Essential oil extraction. Essential oils were extracted from air dried plant material (aerial parts only) by hydro-distillation, using a Clevenger-type apparatus and lasted 3 h. The distilled essential oils had been dehydrated with anhydrous sodium sulphate and stored at 4 °C in dark airtight bottles until further analysis (Avetisyan et al., 2017).

Test organisms and culture conditions used. Test microorganisms used in the investigations: *Escherichia coli* VKPM M-17, *Pseudomonas aeruginosa* GRP3, *Bacillus subtilis* WT-A1, *Enterococcus hirae* ATCC 9790, *Salmonella typhimurium* MDC 1754, *Staphylococcus aureus* MDC 5233, ampicillin resistant *E. coli* DH5α-pUC18, kanamycin resistant *E. coli* pARGS-25, *E. coli* NM111 as well as *Candida albicans* WT-174, *Debariomyces hansenii* WT, *Saccharomyces cerevisiae* ATCC 9804 and *S. cerevisiae* ATCC 13007 yeasts. Nutrient broth and nutrient agar media (peptone 20 g/l, NaCl 5 g/l, K2HPO4 2 g/l, glucose 2 g/l, agar 15 g/l) were used for bacterial growth. 2% glucose was added to the nutrient broth for yeast growth.

Determination of the chemical composition of plant essential oils and ethanol extracts. The gas chromatography (GC) mass selective (MS) analysis of the essential oils was performed using a Hewlett–Packard 5890 Series II gas chromatograph, fitted with a fused silica HP – 5MS capillary column (30 m×0.25 mm, in thickness 0.25 μm) (Avetisyan et al. 2017). The identification of the phytochemical composition of investigated plant extracts was performed using a Dionex Ultimate 3000 UHPLC system (Thermo Scientific TM, Dionex, San Jose, USA) equipped with Synergi TM Hydro-RPA (150 × 4.5 mm, 4 μm, Phenomenex) column (Koss-Mikołajczyk et al., 2019, Kusznierewicz et al., 2021). Profiles of phenolic compounds and antioxidants for plant extracts were obtained employing the HPLC-DAD system (Agilent Technologies, Wilmington, USA) connected with a Pinnacle PCX Derivatization Instrument (Pickering Laboratories Inc., Mountain View, USA) and UV–Vis detector (Agilent Technologies, Wilmington, USA) (Hovhannisyan et al., 2022, Ginovyan et al., 2023).

Investigation of essential oil and ethanol extract antimicrobial and antifungal activity. The antimicrobial activity of the extracts and essential oils were determined using the disk-diffusion method in agar. The final concentrations of the essential oils reached 150, 100, 50, 25, 12.5, 6.25 μl/mL. Dimethyl sulfoxide (DMSO) was used as the solvent. The following concentrations of the extracts were used: 125, 250, 500, 1000 and 1500 μg/mL. Ampicillin (25 μg/mL), kanamycin (25 μg/mL) and fluconazole (25 μg/mL) were used as positive controls (Babayan et al., 2023).

To determine the minimum bactericidal and fungicidal concentration of the test samples, the selected pieces of nutrient medium from the zones of microorganism growth absence were transferred to the nutrient medium corresponding to each microorganism (without essential oil and ethanol extract). This was confirmed after the re-cultivation of the suspension on the fresh nutrient medium (meat peptone agar) (Moghrovyan et al., 2019).

Determination of antibiotic modulatory activity. The antibiotic modulatory activity was explored by determining the minimal inhibitory concentrations (MICs) of antibiotics in the presence and absence of extracts at non-inhibitory concentrations (Ginovyan et al., 2023).

Determination of radical scavenging activity. Free radical scavenging potential of the test sampled was determined by DPPH assay (1,1-diphenyl-2-picrylhydrazyl). Catechin was applied as standard. The radical scavenging activity was calculated using the following formula: Radical scavenging activity (%) = $A_c - A_s$ / A_c x 100. IC_{50} calculated denote the concentration of investigated samples required to decrease the DPPH absorbance at 517 nm by 50% (Hambardzumyan et al., 2020).

Determination of total phenolic content. The concentration of phenolics in plant extracts was determined using Folin-Ciocalteu assay. Solutions with different concentrations of gallic acid (250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml, 15.625 μg/ml, 7.8125 μg/ml and 3.9 μg/ml) were used to construct a calibration curve (Vijay et al., 2014, Ginovyan et al., 2021).

Determination of total flavonoid content. The total flavonoid content in plant extracts was determined employing $AlCl_3$ colorimetric assay. Solutions with different concentrations of quercetin (1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml and 15.625 μg/ml) were used to construct a calibration curve (Moghrovyan et al., 2019).

Chelating capability of ethanol extracts. Fe^{2+} chelating capability of ethanolic extracts was determined using 0.2 mM $FeSO_4$ and 0.5 mM ferrozine. Ethylene diamine tetraacetic acid (EDTA) was used as a positive control (Moghrovyan et al., 2019).

Determination of antioxidant activity with reactive thiobarbituric acid inhibition of malondialdehyde synthesis. The determination of the antioxidant activity of the ethanol extract obtained from the leaves of the investigated plants by the inhibition of malonaldehyde synthesis was carried out using 0.375% thiobarbituric acid (THB) and 15% trichloroacetic acid (TCA). α-tocopherol was used as positive control (Kulisic et al., 2004, Moghrovyan et al., 2019).

Estimation of the NO quantity under the influence of plant extracts. The NO neutralization activity was investigated using Griess reagent. Solutions with different

concentrations of $NaNO_2$ (25 μM, 125 μM, 6.25 μM, 3.125 μM, 1.5625 μM) were used to construct a calibration curve (Essadek et al., 2023).

Tyrosinase activity inhibition assay. Tyrosinase activity inhibition colorimetric assay was carried out according to the method described by Wang et al. (2015). Arbutin was used as a positive control.

Determination of superoxide dismutase and catalase activity. Determination of the total superoxide dismutase activity was carried out by the Beauchamp and Fridovich method (1971). The determination of catalase activity was performed using the spectrophotometric measurement method of the dynamics of hydrogen peroxide quantity at 240 nm (Essadek et al., 2023).

Determination of β-galactosidase activity in *E. coli* cells containing *katG::lacZ* fused genes. Determination of β-galactosidase activity was performed by Miller's method (Smirnova et al., 2010, Samoylova et al., 2014).

Data processing. The average data obtained from three independent experiments were presented, and the standard deviation of the values did not exceed 5%. The statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, USA) (antiradical activity, metal chelating activity, determination of antioxidant activity with reactive thiobarbituric acid inhibition of MDA synthesis, estimation of the NO quantity, determination of tyrosinase inhibitory activity, determination of superoxide dismutase and catalase activity) and Microsoft Office Excel software (determination of the chemical composition, antimicrobial and antiradical activity of the ethanol extracts and essential oils of the researched plants, determination of total phenols and flavonoid content, determination of the amount of proteins, determination of β-galactosidase activity in *E. coli* cells containing the fused *katG::lacZ* gene, determination of catalase activity), and a $p<0.05$ was considered significant. The validity of differences between experimental and appropriate control data were evaluated by Student's criteria using Microsoft Excel 2010 with the T test function, $P<0.05$ (if not indicated).

RESULTS AND DISCUSSION

Chemical composition of ethanol extracts and essential oils.

The application of hydro-distillation method allows getting 1% yield of *O. vulgare* essential oil. According to GC data, essential oil extracted from Armenian *O. vulgare* contained more than 180 types of substances, which were mainly of terpenoid nature. The substances exceeding 2% of the total content, (which makes 73.6% of all components) are included in Table 1. The remaining 26.4% are minor components and not included in the Table 1. The greatest part of major components was different sesqui- and monoterpenes (β-caryophyllene epoxide - 13.3%; β-caryophyllene - 8.2%; o-cymene - 5.2%). Other components represent only a minor part: carvacrol, which is described by huge number of authors as the main component of oregano essential oil (El Babili et al., 2011), were only 2.9 %, α-terpineol - 2.3 %, 1,8-cineole - 2.9 % of essential oil distilled from *O. vulgare* growing in Armenian flora. These differences might be important for the explanation of antioxidant and antibacterial activity of Armenian *O. vulgare* essential oil.

Table 1. The chemical composition of the essential oil isolated from the aerial parts of *O. vulgare* L., characteristic of the flora of Armenia

Compounds	*O. vulgare* L., %	Retention Index (iu)[1]
Sabinene	3.1	897
3-Octanone	2.8	952
trans-β-Ocimene	2.6	978
3-Octanol	2.4	979
(E)-2-Hexenal diethyl acetal	3.8	993
o-Cymene	5.2	1045
1,8-Cineole	2.0	1059
Linalool	2.9	1083
Terpinen-4-ol	2.3	1137
α-Terpineol	2.6	1158
Carvacrol	2.4	1262
β-Bourbonene	2.2	1339
α-Humulene	2.7	1456
β-Caryophyllene	8.2	1494
ß-Bisabolene	3.2	1501
Germacrene D	3.8	1515
β-Caryophylleneepoxid	13.3	1517
Spathulenol	3.2	1536
α-Humulene epoxide II	2.4	1579
Palmitic acid	2.5	1968

[1]for HP-5 capillary column

In spite of the great amount of data concerning the chemical composition of *O. vulgare* essential oil, the composition and biological properties of the oregano essential oil growing in Armenia are of scientific and practical interest (Abrahamyan et al., 2014, Swamy et al., 2016, Őzkan et al., 2017). The sample distilled from Armenian *O. vulgare* belongs to another chemotype and contains β-caryophyllene epoxide and β-caryophyllene, as the major components (see Table 1). In addition, according to the literature data, these terpenoids display analgesic, anticancer, antinociceptive activity (Fidyt et al., 2016). These data may explain the moderate antiradical and antimicrobial activity of essential oil derived from Armenian *O. vulgare*.

The GC-MS analysis showed that the main volatile components of *O. vulgare* extract were catechol (6.2%), ethyl catechol (4.45%), angelicin (1.52%), isovaleric acid (4.31%), and palmitic acid (1.2%). The HPLC analysis of *O. vulgare* extract revealed that the concentration of some well-known flavonoids with a plethora of biological activities was present in high levels. These compounds were: tannic acid (19.63%), luteolin (29.21%), rutin (29.68%), and catechin (3.66%) in flavonoid fraction.

Quantitative and qualitative analysis of essential oil components isolated from *O. basilicum* resulted in more than 40 compounds being isolated, detected and most of them identified for each essential oil sample. The dominant components were identified to be linalool, methyl chavicol, citral and nerol. According to the data obtained, *O. basilicum* contains 57.3% methyl chavicol, with the second largest component being linalool (18%) (Table 2). This places the given variety of *O. basilicum* into methyl chavicol-rich chemotype.

Table 2. Chemical composition of essential oil of *O. basilicum*

Compounds	*O. basilicum var.purpureum*, %	Retention Index (iu)[1]
1-octen-3-ol	0.2	979
1-8- Cineole	1.40	1035
Linalool	18.00	1100
Camphor	1.30	1146
Methyl chavicol	57.3	1203
Bornyl acetate	0.13	1291
β-Elemene	3.62	1387
β -Caryophyllene	1.72	1419
β –Copaene	0.28	1428
trans-α-Bergamotene	4.34	1433
α-Humulene	0.55	1455
cis- β-Farnesene	0.31	1472
Germacrene d	0.68	1482
α-Bulnesene	1.39	1502
α-Amorphen	1.54	1510
Aromadendrene	1.67	1529
Spathulenol	0.68	1544
Caryophyllene oxide	0.57	1550

[1]for HP-5 capillary column

Antibacterial activity of ethanol extracts and essential oils.

Both essential oil and ethanol extract of *O. vulgare* were able to suppress the growth of tested microorganisms. It was revealed that there were no significant differences between the antibacterial activity of both *O. vulgare* essential oil and ethanol extract against Gram-positive and Gram-negative bacterial strains. Our investigations showed that antibacterial activity of investigated essential oil against ampicillin- and kanamycin-resistant strains of *E. coli* was almost similar to the activity against non-resistant one. In both cases, MIC of essential oil was 50 μl/mL However, in contrast to essential oil, extracts were not active against ampicillin- and kanamycin-resistant *E. coli* strains, whereas non-resistant *E. coli* strain was sensitive to ethanol extract. The values of MIC and MBC were 250 μg/mL and 500 μg/mL, respectively. These data show that the investigated plant material could be also used against antibiotic-resistant bacterial strains. Armenian oregano essential oil has antimicrobial activity against *E. hirae.* The essential oil and ethanol extract showed no antifungal activity. The ethanol extract exhibits moderate activity against non-pathogenic *Salmonella* and *Pseudomonas* bacteria (Table 3).

Our research showed that the Gram-positive bacteria tested were more sensitive to basil essential oil than the Gram-negative bacteria. Such tendency is also observed by other authors (Sarrazin et al., 2012). The essential oil of *O. basilicum* showed quite high antimicrobial activity against *B. subtilis*, with the MIC of 3.125 μl/ml, which was 16 times higher than that of the essential oil of *O. vulgare*. The MIC of *O. basilicum* against *St. aureus* was 6.25 μl/ml, which was 8 times higher than essential oil of *O. vulgare*. the MICs of essential oil of *O. basilicum* against *P. aeruginosa*, *S. typhimurium* and *E. hirae*, (25, 12.5, 6.25 μl/ml) was 4, 8 and 16 times higher than that of essential oil *O. vulgare*, accordingly. The ampicillin- and kanamycin-resistant *E. coli*

bacteria also displayed sensitivity against the essential oil tested. The MIC values of *O. basilicum* against those bacteria were 6.25 μL/mL. The essential oil of *O. basilicum* also showed high antifungal activity (Table 3).

Table 3. MIC and MBC values of essential oils and ethanol extracts from *O. basilicum* and *O. vulgare* L.

Test-bacteria	*O. basilicum var. Purpureum*				*O. vulgare* L.				Positive control*, μg/mL
	Essential oil, μl/mL		Ethanol extract, μg/mL		Essential oil, μl/mL		Ethanol extract, μg/mL		
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
St. aureus MDC 5233	6.25	12.5	-	-	50	100	500	1000	12.5
E. hirae ATCC 9790	12.5	25	-	-	100	100	500	500	25
B. subtilis WT-A1	3.125	6.25	250	250	50	100	250	250	12.5
E. coli VKPM M-17	25	25	500	1000	50	100	250	500	25
P. aeruginosa GRP3	25	50	-	-	100	100	250	500	25
S. typhimurium MDC 1754	12.5	25	125	250	100	150	500	500	25
E. coli DH5a-pUC18	6.25	6.25	-	-	50	50	-	-	6
E. coli pARG25	6.25	6.25	-	-	50	50	-	-	5
D. hansenii WT	6.25	12.5	-	-	-	--	-	-	6
C. albicans WT-174	3.125	6.25	-	-	-	-	-	-	5

* ampicillin was used as a positive control, and kanamycin for ampicillin-resistant *E. coli.*

The essential oil from *O. basilicum* has high antibacterial activity against *St. aureus* bacteria, which makes possible to consider using this oil as active natural ingredient for the treatment of skin irritations, since *S. aureus* is extremely common on the skin of patients with certain dermatological diseases and it is often considered to be a major culprit in causing skin irritation and soft tissue infections (Kong et al., 2012).

In case of ampicillin and kanamycin resistant *E. coli* strains ethanol extract of *O. basilicum* did not shown any activity. It also did not have any activity against *St. aureus* and *E. hirae* strains. The MIC value of *O. basilicum* was 250 μg/mL against *B. subtilis*, 125 μg/mL against *S. typhimurium* and 500 μg/mL against *E. coli*. The investigated extracts did not show any suppressing effect on tested yeasts.

Studies have shown that the ethanol extracts of the investigated plants have no antibiotic-modulating activity against all four investigated bacteria (*St. aureus* MDC 5233, *E. coli* VKPM M-17, ampicillin-resistant *E. coli* DH5a-pUC18 and kanamycin-resistant *E. coli* pARG25), with the use of appropriate antibiotics.

Antioxidant activity of ethanol extract and essential oil.

The antiradical activity of the ethanolic extracts and essential oils of the studied plants was expressed as IC_{50} value. According to obtained results the IC_{50} value for the positive control (catechin) was determined to be 13.08±0.035 μg/mL ($y = 3.4343x + 5.0693$, $R^2 = 0.99$). The DPPH assay indicated the high antiradical activity of both ethanol extract and essential oil of *O. basilicum*. The IC_{50} value of essential oil of *O. basilicum* was 22±0.37 μl/ml. In case of ethanol extract of *O. basilicum* this parameter had the following value: 19.37±0.38 μg/mL ($y = 2,196x + 7,4702$, $R^2 = 0.99$). The The IC_{50}

value of *O. vulgare* ethanol extract and essential oil was 19.97±0.51 μg/ml and 1057±17.61 μl/ml, respectively.

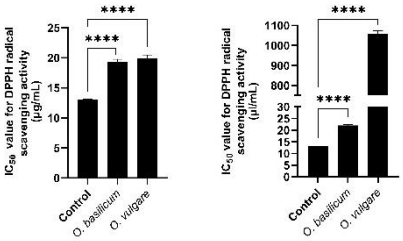

Fig. 1. The radical scavenging activity of *O. basilicum* and *O. vulgare* ethanol extracts ((μg/ml) left) and essential oils ((μl/ml) right) with IC_{50} value presented, (****p<0.0001).

The radical scavenging activity of the investigated extracts was in strong correlation with the total phenolic content. Our studies confirmed that the ethanol extracts of *O. basilicum* and *O. vulgare* growing in Armenia contain a high amount of polyphenolic compounds (317.75 ± 4.105, 555.08 ± 5.598 μg of GAE/mg, respectively). The amount of total flavonoids in the ethanol extracts of *O. basilicum* and *O. vulgare* growing in Armenia was 46.9±0.884 and 31.39±1.16 μg of QE/mg, respectively. The rather high content of total phenols and flavonoids content in the investigated extracts explains the high antiradical and antioxidant activity.

Table 4. Total phenolic (expressed in terms of gallic acid equivalent (μg of GAE/mg of extract)) and the total flavonoid (expressed in terms of quercetin equivalent (μg QE/mg)) contents of *O. basilicum* and *O. vulgare* extracts

Plants	Total phenolic content (μg of GAE/mg)	Total flavonoid content (μg QE/mg)
O. basilicum	317.75 ± 4.105	46.9±0.884
O. vulgare	555.08 ± 5.598	31,39±1,16

Chelating capability of ethanol extracts.

Plant compounds can act as metal chelating agents, helping to reduce the amount of free radicals due to their ability to regenerate iron divalent ions. The metal chelating activity of ethanol extracts of *O. basilicum* and *O. vulgare* was explored. According to the obtained data, the ethanol extract of *O. vulgare* showed significant metal chelating activity (74.5±0.9 %). The same concentration of the positive control (EDTA) brought this parameter to a value of 96.27 ± 0.8% for the chelation of ferrous ions. According to the results of our studies, the metal chelating ability of the ethanol extract of *O. basilicum* was weakly expressed and was about 10% (Fig. 2).

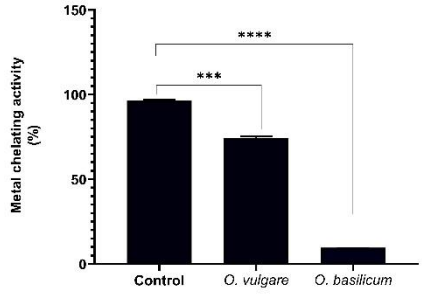

Fig. 2. Metal chelating activity of ethanol extracts of *O. basilicum* and *O. vulgare* (***p<0.001, ****p<0.0001).

Given that the tested samples exhibited high antiradical activity and a rich phenolic content, their ability to suppress lipid peroxidation was assessed by measuring the inhibition of malondialdehyde (MDA) synthesis. Analysis with the reactive thiobarbituric acid revealed that the MDA

synthesis inhibitory activity of the *O. vulgare* ethanol extract was 77.3 ± 1.5%. In comparison, at the same concentration the positive control α-tocopherol showed an inhibition rate of 91.1 ± 1.9% (Fig. 3). Our study found that the MDA synthesis inhibitory activity of the *O. basilicum* ethanol extract was relatively weak, at approximately 8%.

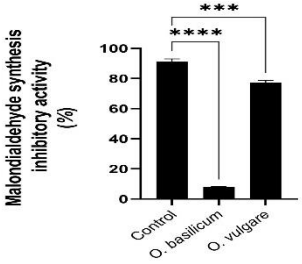

Fig. 3 The ability of ethanol extracts of *O. basilicum* and *O. vulgare* to inhibit malondialdehyde synthesis, (***p<0.001, ****p<0.0001).

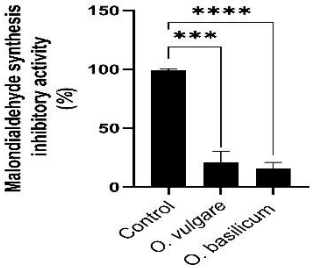

Fig. 4 The ability of ethanol extracts of *O. basilicum* and *O. vulgare* to inhibit malondialdehyde synthesis in *E. coli* NM111 cells (***p<0.001, ****p<0.0001).

In the case of using *E. coli* NM111 cells, the malondialdehyde synthesis inhibitory activity of the ethanol extract of *O. vulgare* plant was 20.715±2.47%, and the ethanol extract of *O. basilicum* was 15.77±1.5% (Fig. 4).

To evaluate the effects of ethanol extracts of *O. basilicum* and *O. vulgare*, the level of NO production in the presence and absence of the plant extract was determined in *E. coli* NM111 cells. Thus, according to the results of the experiment, the amount of NO in *E. coli* NM111 cells under the influence of the ethanol extract of *O. basilicum* (8.74 μM) is higher than that under the influence of the ethanol extract of *O. vulgare* (5.94 μM). In the case of the control, this value was 3.34 μM (Fig. 5).

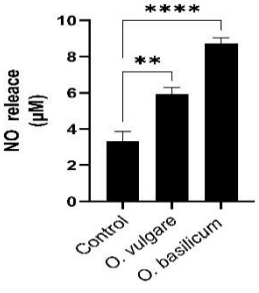

Fig. 5 Determination of the amount of NO radical in *E. coli* NM111 cells under the influence of *O. basilicum* and *O. vulgare* ethanol extracts, (**p<0.01, ****p<0.0001).

Tyrosinase inhibition activity of ethanol extracts and essential oils.

Ethanol extracts and essential oils of *O. vulgare* and *O. basilicum* have been evaluated for their ability to inhibit tyrosinase enzyme as a natural treatment for skin pigmentation disorder, as the overproduction or accumulation of melanin pigment in the epidermal cells of human skin can lead to many skin disorders such as freckles, hyperpigmentation, even melanoma or skin cancer.

Tyrosinase inhibitory activity determination, besides its practical value, could serve also as one of the additional methods of revealing the ethanol extracts and essential oils antioxidant activity, because for the inhibition of tyrosinase, the copper ions in its active center should be reduced, for which the presence of compounds with reducing

potential is necessary. Arbutin was used as a positive control. The values for tyrosinase inhibitory activity of arbutin, ethanolic solution of *O. vulgare* and essential oil were calculated to be 81.5 ± 2.6%, 6.5 ± 0.2%, 26.5 ± 0.3%, respectively. The tyrosinase inhibitory activity values of *O. basilicum* essential oil and ethanol extract were 11.5 ± 0.3% and 4.7 ± 0.2%, respectively (Fig. 6).

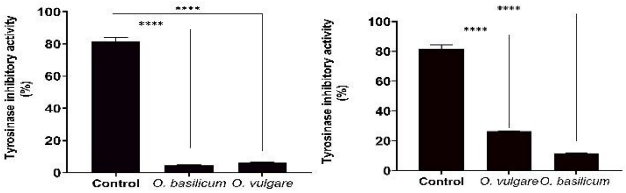

Fig. 6 Tyrosinase inhibitory activity of ethanol extracts (left) and essential oils (right) of *O. basilicum* and *O. vulgare* (****p<0.0001).

Determination of superoxide dismutase and catalase activity in *E. coli* cells.

Superoxide dismutase activity was evaluated in *E. coli* NM111 cells with and without treatment of ethanol extracts of *O. basilicum* and *O. vulgare*. Thus, according to the results of the experiment, the activity of superoxide dismutase in *E. coli* cells under the influence of the ethanol extract of *O. vulgare* (141.39±1.21%) is higher than that which was under the influence of the ethanol extract of *O. basilicum* (112.3±1.1%). The addition of 4 mM of H_2O_2 after the pretreatment with the extracts caused an additional increase in SOD activity. The activity of SOD under the influence of the ethanol extract of *O. vulgare* (165.5±1.35%) compared to the control was 1.65 times higher, and under the influence of the ethanol extract of *O. basilicum* (153.5±1.3%) it was 1.53 times (Fig. 7).

Catalase activity was 1.35 times higher under the influence of the ethanol extract of *O. vulgare* (135±1.27%) compared to the control, and 1.3 times higher, and under the influence of the ethanol extract of *O. basilicum* (130±0.98%). The addition of 4 mM of H_2O_2 after the pretreatment with the extracts caused an additional increase in catalase activity. Catalase activity under the influence of ethanol extract of *O. vulgare* (168±1.4%) was higher than that under which was the influence of ethanol extract of *O. basilicum* (157±1.31%) (Fig. 8).

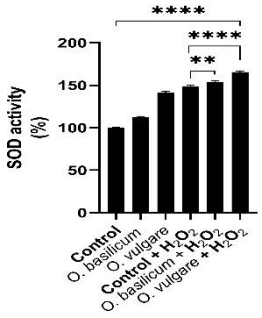

Fig. 7 Superoxide dismutase activity in *E. coli* NM111 cells before and after treatment with plant extracts and 4 mM H_2O_2 (**p<0.01, ****p<0.0001).

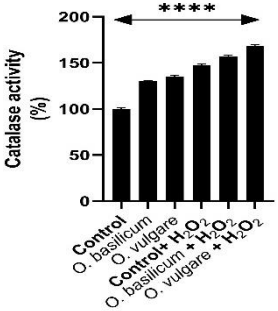

Fig. 8 Catalase activity in *E. coli* NM111 cells before and after treatment with plant extracts and 4 mM H_2O_2 (****p<0.0001).

β-galactosidase activity in *E. coli* cells containing a *katG::lacZ* fusion gene.

Catalase-hydroperoxidase I is encoded by the H_2O_2-inducible gene katG and mediates the detoxification of endogenous H_2O_2 in aerobically grown *E. coli* cells. The expression level of the katG gene can be used as a significant indicator of the antioxidant defense activity of the cell. In order to evaluate the effect of plant extracts on the expression of the katG gene, β-galactosidase activity was measured in *E. coli* cells under the conditions of the *katG::lacZ* gene fusion. *E. coli* cells produce β-galactosidase, the biosynthesis of which is controlled by the *lacZ* gene of the lac operon.

Treatment of *E. coli* cells growing under aerobic conditions with 4 mM of H_2O_2 resulted in marked growth inhibition. Most of the studied extracts increased the cell growth rate under the oxidative stress compared to the untreated cells by 2.34-fold - for *O. basilicum* and 5.74-fold - for *O. vulgare* extracts.

After the addition of the tested extracts to the medium, a statistically significant 1.27–1.41-fold increase in *katG::lacZ* expression was induced. A significant increase in gene expression (1.41-fold) was observed after treatment of cells with *O. vulgare* extract and 1.27-fold when bacteria were treated with basil extract (Table 5). Because the *katG* is an H_2O_2-inducible gene, the addition of 4 mM of H_2O_2 after the pretreatment with the extracts caused an additional increase in *katG::lacZ* expression. The induction index ranged from 1.44 (*O. basilicum*) to 1.9 (*O. vulgare*).

Table 5. Expression of the *katG::lacZ* antioxidant gene in *E. coli* NM111 cells before and after treatment with test extracts.

Plants	*katG::lacZ* gene expression	
	Before H_2O_2	+4 mM H_2O_2
O. basilicum	1.27[1]	1.44[2]
O. vulgare L.	1.41	1.9

[1]the β-galactosidase activity values of the extract-treated samples were divided by the sane values of the untreated samples,

[2]extracts and the values of samples treated with 4 mM of H_2O_2 were divided by the values of samples treated with H_2O_2 alone.

CONCLUDING REMARKS

Origanum vulgare and *Ocimum basilicum* plants are used both in Armenian cuisine and in folk medicine. Despite of the large amount of data on these plants in the literature, the identification of the chemical composition and biological properties of essential oils and ethanol extracts of *O. vulgare* and *O. basilicum* growing in Armenia are of great scientific and practical interest as the potential of *O. vulgare* and *O. basilicum* plants has not yet been fully explored.

The aim of the work was to study the biological activity, biochemical features, and mechanisms of action of ethanol extracts and essential oils of plants *O. vulgare* and *O. basilicum*. According to GC data, essential oil extracted from Armenian *O. vulgare* contained more than 180 types of substances basically of terpenoid nature. The greatest part of major components was different sesqui- and monoterpenes (β-caryophyllene epoxide, β-caryophyllene, o-cymene). These data may explain the moderate antiradical and antimicrobial activity of *O. vulgare* essential oil. The GC-MS analysis showed that

the main volatile components of *O. vulgare* extract were catechol, ethyl catechol, angelicin, isovaleric acid and palmitic acid. According to the obtained data, essential oil of *O. basilicum* contains a large quantity of methyl chavicol, with the second largest quantity being linalool. This places the given variety of *O. basilicum* into methyl chavicol-rich chemotype.

Both essential oil and ethanol extract of *O. vulgare* were able to suppress the growth of test-microorganisms. It was revealed, that there were no significant differences between the antibacterial activity of *O. vulgare* essential oil and its ethanol extract against Gram-positive and Gram-negative bacterial strains. The essential oil and ethanol extract did not show any antifungal activity at tested concentrations.

Our research showed that the tested Gram-positive bacteria were more sensitive to basil essential oil than the Gram-negative bacteria. The essential oil of *O. basilicum* also showed high antifungal activity. In case of the ampicillin and kanamycin resistant *E. coli* strains ethanol extract of *O. basilicum* did not showed any growth inhibiting activity. It also did not expressed any activity against *St. aureus*, *E. hirae* strains and tetsed yeasts. Results have shown that the ethanol extracts of the investigated plants had no antibiotic-modulating activity against all the bacteria investigated, with the use of appropriate antibiotics.

The DPPH assay revealed that the ethanol extracts of *O. vulgare* and *O. basilicum* have quite high antiradical activity and showed the result equivalent to the positive control. The antiradical activity of *O. basilicum* essential oil was also high and that of *O. vulgare* essential oil is moderate. The rather high content of total phenols and flavonoids in the investigated extracts explains their high antiradical and antioxidant activity. Plant compounds can act as metal chelating agents, helping to reduce the amount of free radicals due to their ability to reduce iron divalent ions. According to the results of our study, the ethanol extract of *O. vulgare* exhibited significant metal chelating activity in contrast to the ethanol extract of *O. basilicum*. The analysis of the synthesis of malondialdehyde using reactive thiobarbituric acid showed that the ethanol extract of *O. vulgare* has a high activity of inhibiting the synthesis of this compound, and the activity of inhibiting the synthesis of malondialdehyde of the ethanol extract of *O. basilicum* was weakly expressed. In addition, the amount of NO in *E. coli* cells under the influence of the ethanol extract of *O. basilicum* was higher than that which were under the influence of the ethanol extract of *O. vulgare*, which indicats that ethanolic extract of *O. basilicum* induced oxidative stress in *E. coli* cells. The tyrosinase inhibitory activity of both *O. vulgare* and *O. basilicum* essential oil was higher than that of the ethanol extracts. The study showed that the activity of SOD and catalase in *E. coli* cells increased under the influence of ethanol extracts of the studied plants. After the addition of the tested extracts to the medium, a statistically significant increase in *katG::lacZ* gene expression was induced. Because *katG* is an H_2O_2-inducible gene, the addition of 4 mM H_2O_2 after pretreatment with the solutions caused an additional increase in *katG::lacZ* expression.

Thus, essential oils and ethanol extracts of *O. basilicum* and *O. vulgare* are potential sources of antimicrobial agents. At the same time, the combination of marked antibacterial and antioxidant properties with antiradical, metal chelating, tyrosinase

inhibitory activities, makes the *O. basilicum* and *O. vulgare* essential oils and ethanol extracts an applicable source for medicinal, pharmaceutical and for use in the cosmetic industry as an antimicrobial, analgesic, antioxidant, and skin whitening agent. Being a widely used spice and having the biological effects mentioned above, the *O. basilicum* and *O. vulgare* can also be suggested to be used as a food preservative.

CONCLUSIONS

The following conclusions were made based on experimentally obtained results:

1. The essential oil isolated from *O. vulgare* has been shown to contain more than 180 types of substances basically of terpenoid nature. The greatest part of the major components was different sesqui- and monoterpenes (β-caryophyllene epoxide, β-caryophyllene, o-cymene). The main components of *O. vulgare* extract were catechol, ethyl catechol, angelicin, isovaleric acid, and palmitic acid. The main component of the *O. basilicum* essential oil was methyl chavicol.
2. Both essential oil and ethanol extract of *O. vulgare* were able to suppress the growth of test-microorganisms. The essential oil and ethanol extract did not show any antifungal activity at tested concentrations. The tested Gram-positive bacteria were more sensitive to basil essential oil than the Gram-negative bacteria. The essential oil of *O. basilicum* also showed high antifungal activity. In case of ampicillin and kanamycin resistant *E. coli* strains ethanol extract of *O. basilicum* did not shown any growth inhibiting activity. Ethanol extracts of the investigated plants have no antibiotic-modulating activity against all the investigated bacteria, with the use of appropriate antibiotics.
3. The antiradical activity of the ethanol extracts and essential oils of *O. vulgare* and *O. basilicum* was shown to be equivalent to result of the positive control. Ethanol extract of *O. vulgare* exhibited significant metal chelating and malondialdehyde synthesis inhibitory activity in contrast to the ethanol extract of *O. basilicum*. The high antioxidant activity of the ethanol extracts of *O. basilicum* and *O. vulgare* can be explained by the high content of phenols and flavonoids.
4. The quantity of NO in *E. coli* cells under the influence of the ethanol extract of *O. basilicum* was higher than that which was under the influence of the ethanol extract of *O. vulgare.*
5. The tyrosinase inhibitory activity of both *O. vulgare* and *O. basilicum* essential oil was higher than that of the ethanol extracts. The activity of SOD and catalase in *E. coli* cells also increased under the influence of ethanol extracts of the studied plants.
6. After the addition of the tested extracts to the medium, a significant increase in *katG::lacZ* gene expression was induced, which is due to their prooxidant activity in *E. coli* NM111 cells. This can explain the antibacterial activity of the components of these extracts.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. **Babayan A.** (2023), Antimicrobial and antioxidant activities of *Ocimum basilicum var. purpureum* ethanol extract, ***Proceedings of the YSU B: Chemical and Biological Sciences***, 57(3), 258-268.
2. Shirvanyan A., **Babayan A**., Minasyan A., Petrosyan M., Poladyan A., Sahakyan N. (2023), The oxidative stress and possible preventive action of plant born polyphenols, **31st International Conference of FFC**, 246-248.
3. Moghrovyan A., Sahakyan N., **Babayan A.**, Chichoyan N., Petrosyan M., Trchounian A. (2019) Essential Oil and Ethanol Extract of Oregano (*Origanum vulgare* L.) from Armenian Flora as a Natural Source of Terpenes, Flavonoids and other Phytochemicals with Antiradical, Antioxidant, Metal Chelating, Tyrosinase Inhibitory and Antibacterial Activity, ***Current Pharmaceutical Design***, 25 (16), 1809-1816.
4. Avetisyan A., Markosian A., Petrosyan M., Sahakyan N., **Babayan A.**, Aloyan S., Trchounian A. (2017) Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars, ***BMC Complementary and Alternative Medicine***, 17 (1), 1-8.
5. Petrosyan M., Avetisyan A., Markosian A., Sahakyan N., **Babayan A**., Aloyan S., Trchounian A. (2016), Essential Oils of Basil Plants as Antimicrobial Agents, **ASM MICROBE 2016**, 189.
6. Muradyan M., Sahakyan N., Petrosyan M., **Babayan A**., Trchounian A. (2015), Free radical scavenging activity and total flavonoid content of essential oil and ethanol extracts of *Origanum vulgare* L., represented in Armenian flora**, 3rd International Scientific Conference on "Dialogues on Sciences"**, 61.
7. Sahakyan N., Petrosyan M., Moghrovyan A., Chichoyan N., Hovhannisyan N., **Babayan A.**, Mouradyan M., Trchounian A. (2014), Essential oil of *Origanum vulgare* L. as a source with anyibacterial activity, **Trends in microbiology and microbial biotechnology**, 84.

ԲԱԲԱՅԱՆ ԱՆՈՒՇ ՄԱՐՏԻՆԻ

Origanum vulgare L. և *Ocimum basilicum var. purpureum* բույսերից անջատված միացությունների կենսաբանական ակտիվությունն ու ազդեցության մեխանիզմները

Ամփոփագիր

Հանգուցային բառեր՝ *Origanum vulgare*, *Ocimum basilicum,* հակառադիկալային ակտիվություն, հակամանրէային ակտիվություն, ամպիցիլին- և կանամիցին-կայուն *E. coli*, ֆենոլ, ֆլավոնոիդ, սուպերօքսիդ դիսմուտազ (ՍՕԴ), կատալազ, *katG::lacZ* միաձուլված գենի էքսպրեսիա:

Origanum vulgare և *Ocimum basilicum* բույսերն օգտագործվում են ինչպես հայկական խոհանոցում, այնպես էլ ժողովրդական բժշկության մեջ: Չնայած, գրականության մեջ այս բույսերի վերաբերյալ տվյալների մեծ քանակին, Հայաստանում աճող խնկածաղկի և ռեհանի եթերայուղերի և էթանոլային լուծամզվածքների քիմիական կազմը և կենսաբանական հատկությունները գիտական և գործնական մեծ հետաքրքրություն են ներկայացնում, քանի որ *O. vulgare* և *O. basilicum* բույսերի ներուժը դեռ ամբողջությամբ ուսումնասիրված չէ:

Այս հետազոտության նպատակն էր պարզել Հայաստանում աճող խնկածաղկի և ռեհանի եթերայուղերի և էթանոլային լուծամզվածքների

քիմիական կազմի առանձնահատկությունները, կենսաբանական ակտիվությունն ու ազդեցության մեխանիզմները: Հայկական ֆլորային բնորոշ *O. vulgare*-ից արդյունահանված եթերայուղը համաձայն ԳՔ-ՋՍ-ի տվյալների, պարունակում է հիմնականում տերպենոիդային բնույթի ավելի քան 180 տեսակի նյութեր: Հիմնական բաղադրիչների մեծ մասը կազմում են տարբեր սեսքվի- և մոնոտերպեններ (β-կարիոֆիլեն էպօքսիդ, β-կարիոֆիլեն, օ-ցիմեն): *O. vulgare* էթանոլային լուծամզվածքի քիմիական կազմի ԳՔ-ՋՍ վերլուծությունը ցույց է տվել, որ այս բույսի բաղադրիչներից են կատեխոլը, էթիլ կատեխոլը, անգելիցինը, իզովալերիանաթթուն և պալմիտինաթթուն: Ստացված տվյալների համաձայն՝ *O. basilicum* -ի եթերայուղը պարունակում է մեծ քանակությամբ մեթիլ խավիկոլ, որից հետո քանակությամբ երկրորդ բաղադրիչը լինալոլն է: Այս տվյալների հիման վրա կարելի է համարել, որ *O. basilicum* -ը պատկանում է մեթիլ խավիկոլով հարուստ քեմոտիպին:

Խնկածաղկի և՛ էթանոլային լուծամզվածքը, և՛ եթերայուղը ճնշել են փորձնական միկրոօրգանիզմների աճը: Բացահայտվել է, որ էական տարբերություններ չկան խնկածաղկի էթանոլային լուծամզվածքի և եթերայուղի հակաբակտերիական ակտիվության միջև Գրամ դրական և Գրամ բացասական բակտերիաների նկատմամբ: Եթերայուղը և էթանոլային լուծամզվածքը չեն ցուցաբերել հակասնկային ակտիվություն: Մեր հետազոտությունները ցույց տվեցին, որ փորձարկված Գրամ-դրական բակտերիաները ավելի զգայուն են ռեհանի եթերայուղի նկատմամբ, քան Գրամ-բացասական բակտերիաները: *O. basilicum*-ի եթերայուղը ցուցաբերել է նաև բարձր հակասնկային ակտիվություն: *O. basilicum*-ի էթանոլային լուծամզվածքը ամպիցիլին- և կանամիցին-կայուն *E. coli* շտամերի դեպքում որևէ ակտիվություն չի ցուցաբերել: Այն որևէ ակտիվություն չի ցուցաբերել նաև *St. aureus*, *E. hirae* շտամերի և խմորասնկերի նկատմամբ:

ԴՖՊՀ վերլուծությունը ցույց է տվել, որ *O. vulgare*-ի և *O. basilicum*-ի էթանոլիային լուծամզվածքներ ունեն բավականին բարձր հակառադիկալային ակտիվություն և ցուցաբերել են դրական ստուգիչին համարժեք արդյունք: *O. basilicum*-ի եթերայուղի հառադիկալային ակտիվությունը ևս բարձր է, իսկ *O. vulgare*-ի եթերայուղինը՝ չափավոր: *O. basilicum*-ի և *O. vulgare*-ի Էթանոլային լուծամզվածքում բարձր ընդհանուր ֆենոլների և ֆլավոնոիդների պարունակությունը բացատրում է բարձր հակառադիկալային և հակաօքսիդանտային ակտիվությունը: Բուսական միացությունները կարող են հանդես գալ որպես մետաղ խելատացնող միջոցներ՝ նպաստելով ազատ ռադիկալների քանակության նվազեցմանը՝ երկաթի երկարժեք իոնների վերականգնման ունակության շնորհիվ: Մեր ուսումնասիրության արդյունքների համաձայն՝ *O. vulgare*-ի էթանոլային լուծամզվածքը դրսևորել է զգալի մետաղ խելատացնող ակտիվություն, ի տարբերություն *O. basilicum* -ի էթանոլային լուծամզվածքի: Ռեակցիոնունակ թիոբարբիտուրաթթվի կիրառմամբ մալոնային երկադեհիդի սինթեզի վերլուծությունը ցույց է տվել, որ *O. vulgare* -ի Էթանոլային լուծամզվածքն ունի այդ միացության սինթեզի արգելակման բարձր ակտիվություն, իսկ *O. basilicum* -ի էթանոլային լուծամզվածքի մալոնդիալդեհիդի սինթեզի արգելակման ակտիվությունը եղել է թույլ արտահայտված: Բացի այդ, *O.*

basilicum-ի էթանոլային լուծամզվածքի ազդեցությամբ NO-ի քանակը *E. coli* բջիջներում ավելի բարձր է, քան *O. vulgare*-ի էթանոլային լուծամզվածքի ազդեցությամբ, ինչը ցույց է տալիս, որ *O. basilicum* -ի էթանոլային լուծամզվածքը *E. coli* բջիջներում առաջացրել է օքսիդային սթրես: Ե՛վ *O. vulgare* –ի և՛ *O. basilicum* -ի եթերայուղի թիրոզինազ արգելակող ակտիվությունը ավելի բարձր է, ի տարբերություն էթանոլային լուծամզվածքների: Ուսումնասիրությունը ցույց տվեց, որ հետազոտված բույսերի էթանոլային լուծամզվածքների ազդեցությամբ բարձրանում է ՍՕԴ-ի և կատալազի ակտիվությունը *E. coli*-ի բջիջներում: Ուսումնասիրված լուծամզվածքները միջավայր ավելացումից հետո առաջացրել է նաև *katG::lacZ* էքսպրեսիայի նշանակալի աճ: Քանի որ *katG*-ը H_2O_2-ինդուկտիվ գեն է, 4 մՄ H_2O_2 ավելացումը լուծամզվածքների հետ նախնական մշակումից հետո առաջացրել է *katG::lacZ* արտահայտման լրացուցիչ աճ:

Այսպիսով, *O. basilicum*-ի և *O. vulgare*-ի եթերայուղերն ու էթանոլիային լուծամզվածքները հակամանրէային նյութերի պոտենցիալ աղբյուրներ են: Միևնույն ժամանակ, ընդգծված հակաբակտերիական և հակաօքսիդանտային հատկությունների համադրությունը հակառադիկալային, մետաղ խելատացնող, թիրոզինազային արգելակող ակտիվությունների հետ, *O. basilicum*-ի և *O. vulgare*-ի եթերայուղերն ու էթանոլային լուծամզվածքներ դարձնում է լավ աղբյուր բժշկության, դեղագործության և կոսմետիկ արդյունաբերության մեջ օգտագործելու համար՝ որպես հակամանրէային, անալգետիկ, հակաօքսիդիչ և մաշկը սպիտակեցնող միջոց: Լինելով լայնորեն օգտագործվող համեմունք և ունենալով վերը նշված կենսաբանական ազդեցությունները, *O. basilicum*-ը և *O. vulgare*-ը կարող են կիրառվել նաև որպես սննդի կոնսերվանտ:

БАБАЯН АНУШ МАРТИНОВНА

БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ И МЕХАНИЗМЫ ДЕЙСТВИЯ СОЕДИНЕНИЙ, ИЗОЛИРОВАННЫХ ИЗ РАСТЕНИЙ *ORIGANUM VULGARE* L. И *OCIMUM BASILICUM VAR. PURPUREUM*

РЕЗЮМЕ

Ключевые слова: *Origanum vulgare*, *Ocimum basilicum*, антирадикальная активность, антимикробная активность, ампициллин- и канамицин резистентная *E. coli*, фенол, флавоноид, супероксид дисмутаза (СОД), каталаза, экспрессия слитого гена *katG::lacZ*.

Origanum vulgare и *Ocimum basilicum* используются как в армянской кухне, так и в народной медицине. Несмотря на большое количество данных об этих растениях в литературе, химический состав и биологические свойства эфирных масел и этаноловых экстрактов *O. vulgare* и *O. basilicum*, выращиваемых в Армении, представляют большой научный и практический интерес, поскольку потенциал растений *O. vulgare* и *O. basilicum* еще не полностью изучен.

Целью работы было изучение биологической активности, биохимических особенностей и механизмов действия этанольных экстрактов и эфирных масел растений *O. vulgare* и *O. basilicum*. Согласно данным ГХ, эфирное масло, полученное из *O. vulgare*, содержало более 180 видов веществ, в основном терпеноидного происхождения. Большая часть основных компонентов представляла собой различные сескви- и монотерпены (β-кариофиллен эпоксид, β-кариофиллен, о-цимен). Анализ ГХ-МС показал, что основными летучими компонентами экстракта *O. vulgare* были катехол, этилкатехол, ангелицин, изовалериановая кислота и пальмитиновая кислота. Согласно полученным данным, *O. basilicum* содержал большое количество метилхавикола, при этом вторым по содержанию компонентом являлся линалоол. Это относит данный вид *O. basilicum* к химотипу, богатому метилхавиколом.

Как эфирное масло, так и этанольный экстракт *O. vulgare* смогли подавлять рост тест-микроорганизмов. Было выявлено, что не было значительных различий в антибактериальной активности как эфирного масла *O. vulgare*, так и этанольного экстракта против грамположительных и грамотрицательных штаммов бактерий. Эфирное масло и этанольный экстракт не проявили антигрибковую активность. Наши исследования показали, что грамположительные бактерии, протестированные на чувствительность, были более чувствительны к эфирному маслу базилика, чем грамотрицательные бактерии. Эфирное масло *O. basilicum* также проявило высокую антигрибковую активность. В случае ампицилин- и канамицин-резистентных штаммов *E. coli* этанольный экстракт *O. basilicum* не проявил никакой активности. Он также не проявил активность против штаммов *St. aureus*, *E. hirae* и дрожжей.

Анализ ДФПГ показал, что этанольные экстракты *O. vulgare* и *O. basilicum* обладали довольно высокой антирадикальной активностью и показали результат, эквивалентный позитивному контролю. Антирадикальная активность эфирного масла *O. basilicum* была также высока, а у *O. vulgare* - умеренна. Довольно высокое содержание общих фенолов и флавоноидов в исследуемых экстрактах объясняет высокую антирадикальную и антиоксидантную активность. Растительные соединения могут действовать как хелатирующие агенты металлов, помогая снизить количество свободных радикалов за счет их способности регенерировать ионы двухвалентного железа. Согласно результатам нашего исследования, этанольный экстракт *O. vulgare* проявил значительную хелатирующую активность в отличие от этанольного экстракта *O. basilicum*. Анализ синтеза малондиальдегида с использованием реактивного тиобарбитуровой кислоты показал, что этанольный экстракт *O. vulgare* обладает высокой активностью в ингибировании синтеза этого соединения, а активность в ингибировании синтеза малондиальдегида этанольного

экстракта *O. basilicum* была слабо выражена. Кроме того, значение NO в клетках *E. coli* под воздействием этанольного экстракта *O. basilicum* выше, чем при воздействии этанольного экстракта *O. vulgare*, что указывало на то, что этанольный экстракт *O. basilicum* вызывает оксидативный стресс в клетках *E. coli*.

Способность ингибирования активности тирозиназы как эфирного масла *O. vulgare*, так и *O. basilicum* оказалась выше, чем у этанольных экстрактов. Исследование показало, что активность СОД и каталазы в клетках *E. coli* увеличивалась под воздействием этанольных экстрактов изучаемых растений. После добавления в среду тестируемых растворов произошло статистически значимое увеличение экспрессии гена *katG::lacZ*. Поскольку *katG* - это ген, индуцируемый H_2O_2, добавление 4 мМ H_2O_2 после предварительной обработки растворами вызвывало дополнительное увеличение экспрессии гена *katG::lacZ*.

Таким образом, эфирные масла и этанольные экстракты *O. basilicum* и *O. vulgare* являются потенциальными источниками антимикробных средств. В то же время сочетание выраженных антибактериальных и антиоксидантных свойств с антирадикальной, металл-хелатирующей, ингибирующей тирозиназу активностью, делает эфирные масла и этанольные экстракты *O. basilicum* и *O. vulgare* хорошим источником для применения в медицине, фармацевтической и косметической промышленности в качестве антимикробных, обезболивающих, антиоксидантных и отбеливающих средств. Будучи широко используемыми специями и обладая упомянутыми выше биологическими эффектами, *O. basilicum* и *O. vulgare* также могут быть использованы в качестве консервантов пищевых продуктов.