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կենսաբանական գիտությունների դոկտորի
գիտական աստիճանի հայցման ատենախոսության

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

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THE ROLE OF PLANT-ORIGIN COMPOUNDS IN THE REGULATION OF CELLULAR REDOX MECHANISMS

SYNOPSIS

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

YEREVAN 2025

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական խորհրդատու՝	կ.գ.դ., պրոֆ. Կ. Ա. Թռչունյան
Պաշտոնական ընդդիմախոսներ՝	կ.գ.դ.., պրոֆ. Աստղիկ Ջավենի Փեփոյան կ.գ..դ.., պրոֆ. Ջարուհի Սամվելի Վարդանյան կ.գ.դ., Ռուզաննա Գառնիկի Պարոնիկյան
Առաջատար կազմակերպություն՝	ՀՀ ԳԱԱ Հ. Բունիաթյանի անվան կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2025թ. հունիսի 27-ին ժամը 14:30-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 2025թ. մայիսի 23-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. K. A. Trchounian
Official opponents:	D.Sc., Prof. Astghik Zaven Pepoyan D.Sc., Prof. Zaruhi Samvel Vardanyan D.Sc., Ruzanna Garnik Paronikyan
Leading organization:	H.Buniatyan Institute of Biochemistry NAS RA

The defense of the dissertation will be held on 27th June, 2025, at 14:30, 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 23thof May, 2025.

Scientific Secretary of 051 Specialized Council,

D.Sc., Associate Professor M. A. Parsadanyan

INTRODUCTION

Topic's significance. The oxidative stress, characterized by an imbalance between the production of active oxygen, nitrogen or sulfur species and the antioxidant defense mechanisms, in various living organisms, from prokaryotic single-celle organisms to eukaryotic plants and animals, is involved in the development of various pathological abnormalities, as well as in the regulation process of numerous physiological metabolic pathways. Such processes are of interest for modern biology due to their critical role for all living organisms. In this regard, it will be very important and promising to use the accumulated knowledge and constantly updated viewpoints, analyzes and information in the field of redox biochemistry, biology and medicine.

Oxidative process play a fundamental role in all living organisms, serving as important mechanism for energy production, metabolism, and cell signaling. It is associated with the activity of various enzymes and cofactors. The enzymes, such as oxidoreductases, dehydrogenases, and cytochromes, form electron transfer chains to regulate and coordinate the metabolism. In addition, the redox-active factors such as nicotinamide adenine nucleotide (NAD^+/NADH), flavin adenine nucleotide (FAD/$FADH_2$) and coenzyme Q participate in electron transport, ensuring the flow of electrons across the biological membranes (Chiang, 2012, Yuzugullu, 2020).

In addition to their role in metabolism and energy production, redox processes also serve as key regulators of cell signaling and gene expression. Reactive oxygen species, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are byproducts of aerobic metabolism and can act as signaling molecules in various cellular pathways. Free radical-mediated redox signaling affects processes such as cell division, apoptosis, and the immune response, highlighting the dual nature of the effects of these molecules (Cherkaoui Malki et al., 1990, Song et al., 2021). Moreover, redox modifications of proteins, including the oxidation of cysteine residues, redox modifications of enzymes of the antioxidant defense system play a crucial role in the regulation of protein structure and function. These post-translational changes regulate enzyme activity, transcription factor function, and protein-protein interactions, contributing to the dynamic control of cellular processes in response to environmental influences and the expression of metabolic peculiarities molecules (Cherkaoui Malki et al., 1990).

A complicated balance between oxidants and antioxidants is essential for maintaining cellular homeostasis and preventing damage caused by oxidative stress. Disruption of the superox balance in the human body can lead to the formation of various pathological conditions, including neurodegenerative, cardiovascular diseases and cancer. The excessive activation of reducing pathways, such as glutathione and thioredoxin systems, can lead to inactivation of cellular defense mechanisms (Ursini et al., 2016, Vasan et al., 2020). Furthermore, research indicates that redox processes play an important role in mediating host-pathogen relationships (Torres et al., 2022). In addition, such processes may underlie allelopathic relationships between plants (Trchounian et al., 2016, Hussain et al., 2020).

It is known that various plant-derived compounds, such as polyphenols, which are primary metabolites of plant secondary metabolism, can act as natural antioxidants, exhibiting high activity in various systems. Nowadays, both traditional and classical medicine consider medicinal plants as important components of various drugs. The flora of Armenia is rich in valuable plants with pharmacological and nutritional significance, which have been used since ancient times and about which

extensive information is recorded in ancient manuscripts (Moghrovyan et al., 2019, Sahakyan et al., 2019, Sanjian, 2012). However, this biodiversity is still not well-studied. The most promising areas for study include plants used as spices as well as agricultural plant waste, due to their availability, low cost, and wide distribution.

The presented work describes the importance of natural polymeric regulators of plant origin in controlling redox processes, acting as tools to either counteract or promote oxidation processes. The nature of the effects of these compounds in various test systems is described, along with their potential applications as well as the mechanisms by which they participate in redox processes.

Research goals and tasks. The aim of the study was to investigate the role of plant-derived polymeric compounds in the redox processes occurring in organisms belonging to different taxonomic groups (both prokaryotic and eukaryotic). The objective was to elucidate the mechanisms of these compounds' effects and explore their potential application prospects. In order to achieve that goal, the following tasks were defined in the research:

- to carry out metabolic analysis of the studied plant species,
- to clarify the reducing capacity, antioxidant and prooxidant activity of phenolic compounds isolated from the studied plant species, to clarify the possible relationship between the antioxidant activity of the studied extracts and the quantitative and qualitative composition of the polyphenolic compounds,
- to investigate the cytotoxicity of the extracts using cancer and normal cell lines of different origins,
- to investigate the possible effect of the corresponding extracts and phenolic compounds on the proton flux in bacterial membranes, H^+/K^+ exchange, and changes in ATPase activity,
- to clarify the possible mechanisms of the antibiotic-modulating effect of the studied extracts and phenolic compounds on antibiotic-sensitive and antibiotic-resistant bacterial strains and cancer cell lines, and to investigate the concentration-effect relationship of the applied extracts in the antibiotic-modulation process,
- to identify the role of the studied extracts in the processes of modulating the activity of enzymes of the antioxidant defense system and the expression of genes responsible for their synthesis in different cell lines,
- to investigate the role of the studied extracts in the processes of changing the synthesis of inflammatory markers and the expression their genes in different cell lines,
- to identify the role of the studied extracts and phenolic compounds in the regulation of redox balance in different cells and the manifestation of anti-inflammatory activity,
- to clarify the role of the studied extracts and phenolic compounds in the changes in the activity of membrane transporters of different types of cells,
- to investigate the allelopathic effect of plant polyphenolic volatile compounds and possible mechanisms using *Arabidopsis thaliana* wild-type and 2-cysPRXAB peroxiredoxin-deficient double mutant (Δ2CP) lines.

Scientific novelty and practical value of the study. For the first time, this work has provided a metabolomic characterization of the alcohol-water fractions of various plants with pharmacological and nutritional significance growing in Armenia. It has also been established, for the first time, the practical importance of agricultural plant waste. It has been shown that the main components isolated from the essential

oil-bearing plants studied are volatile compounds of mono-, di-, and sesquiterpene nature, which exhibit high biological activity in various test systems. It has been found that the main components of extracts isolated from the aerial parts of plants collected from the territory of Armenia belong to the groups of polyphenols and organic acids. However, these components vary in qualitative and quantitative composition depending on the species of the plant source and the conditions under which they grow or are cultivated.

It has been revealed that all the identified compounds exhibited pronounced antioxidant activity in chemical tests. However, the nature and mechanism of their effects varied in different living systems, depending on the concentration used and the specific plant species.

For the first time, it has been shown that extracts isolated from the aerial parts of *Vaccinium myrtillus* (bilberry*), Ribes nigrum* (blackcurrant), *Ficus carica* (fig), and *Vitis vinifera* (grape) exhibit antibiotic and antibiotic-modulating activities against various bacterial strains as well as cancer cell lines. This activity appears to be associated with the modulation of the activity of various pumps and transporters in the membranes.

The results of the study demonstrate for the first time that plant extracts affect the membrane-associated properties of different bacteria by modulating the rate of H^+ flux, H^+/K^+ exchange, and ATPase activity. It was also found that these extracts selectively inhibit the viability of cancer cells of different origins under *in vitro* conditions.

The results of the research offer new insights and approaches for the application of plant-derived phenolic compounds to overcome resistance to various antibiotics in organisms and diverse cell types, specifically in doxorubicin-resistant HT-29 cells and kanamycin-resistant *E. coli* cells. These studies could serve as another step toward understanding the mechanisms involved in the modulation of antibiotic resistance by the extracts.

As a result, it is proposed that natural compounds can be used as components in the development of new preparations and developing of strategies to combat the formation of resistance to various antibiotics, paving the way for innovative treatment approaches.

For the first time, it has been shown that sub-cytotoxic concentrations of the investigated extracts regulate the expression levels of various inflammatory markers and their synthesis-related genes *(iNOS*, *TNF-α*, *IL-1β*) and the gene *Abcd1*, which codes the ABCD1 transporter of long-chain fatty acids in BV-2 microglial wild type and Acyl-CoA oxidase 1 deficient (*Acox1*$^{-/-}$) cell lines. Additionally, certain extracts and essential oils regulate the activity of antioxidant defense enzymes and the expression levels of their corresponding genes in the same cell lines. In some cases, post-translational changes in the activity of these enzymes are observed.

For the first time, it has been demonstrated that the essential oils of *Mentha arvensis* and *Ocimum basilicum* var. *purpureum*, along with their main components, menthol and methyl chavicol, can act as allelopathins. These compounds disrupt various metabolic pathways and physiological functions in other plants, including photosynthesis and nearly all redox processes.

The obtained results make a significant contribution to the field of medicine by paving the way for the development of new antioxidant, antimicrobial, and anticancer formulations using plant-derived compounds. The findings indicate that these compounds could be applicable in the development of natural new additives and

preservatives for the cosmetics, food, and feed industries. Moreover, given the pronounced allelopathic effects of the investigated essential oils, they can be proposed for the formulation of new pesticides with potential applications in organic agriculture.

Main points to present at the defense.

1. The main components of the extracts isolated from the aerial parts of plants collected from the territory of Armenia belong to the groups of flavonoids and their derivatives, as well as phenolic acids and their derivatives.
2. Plant-derived polyphenolic compounds exhibit pronounced antioxidant activity in all chemical tests and can modulate redox processes in the cells of all living organisms by altering the balance between the synthesis and neutralization of free radicals and other oxidative markers.
3. The majority of the investigated plant metabolites suppress the viability of various bacteria and cancer cell lines and can modulate antibiotic resistance in some antibiotic-resistant bacteria and cancer cells.
4. Certain polyphenolic compounds isolated from the studied plants can selectively inhibit the viability of cancer cells of different origins under *in vitro* conditions.
5. The investigated plant extracts influence the membrane-associated properties of different bacteria by modulating the rate of H^+ flux, H^+/K^+ exchange, and ATPase activity.
6. Sub-cytotoxic concentrations of some of the extracts being studied regulate the expression levels of various inflammatory markers and their synthesis-related genes (*iNOS*, *TNF-α*, *IL-1β*) and the gene *Abcd1*, which codes for the ABCD1 protein involved in the transport of very long-chain fatty acids (VLCFA), in BV-2 microglial wild type and Acyl-CoA oxidase 1 deficient ($Acox1^{-/-}$) cell lines.
7. The sub-cytotoxic concentrations of certain extracts and essential oils regulate the activity of antioxidant defense enzymes and the expression levels of their corresponding genes in BV-2 microglial wild type and $Acox1^{-/-}$ cell lines. In some cases, post-translational changes in the activity of these enzymes are observed.
8. Polyphenolic compounds found in the essential oils of plants can act as allelopathins, disrupting various metabolic pathways and physiological functions in other plants, including photosynthesis and nearly all redox processes.

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: COST Action CA22134 meeting (Cordoba, Spain, 2025), 48th FEBS Congress (Milan, Italy, 2024), 31st International Conference of FFC (Yerevan, Armenia, 2023), Pan-Armenian conference 2023 (Yerevan, Armenia, 2023), FEMS Conference on Microbiology (Belgrade, Serbia, 2022), Pan-Armenian conference 2022 (Vanadzor, Armenia, 2022), FEBS Biochemistry global summit (Lisbon, Portugal, 2022), Biology and Biotechnology of Microorganisms ICMBB2021 (Uzbekistan, Tashkent, 2021), COST Action CA16112 conference (Gdansk, Poland, 2021), International conference “INNOVATIONS IN LIFE SCIENCES” (Belgorod, Russia, 2021), II International symposium "INNOVATIONS IN LIFE SCIENCES" (Belgorod, Russia, 2020), International conference dedicated to prof Sisakyan (Yerevan, Armenia, 2020), COST Action 16112 conference (Lisbon, Portugal, 2020), WGs Meeting COST Action CA16112 conference (Serbia, Belgrade, 2020), ASM General

Meeting ASM MICROBE 2019 (San Francisco, USA, 2019), 3rd Congress of Polish Biosciences BIO2018 (Gdansk, Poland, 2019), ASM MICROBE 2018 American Society for Microbiology General Meeting *Atlanta, USA, 2018), "Towards a Redox Heslthy Aging" COST Action CA16112 meeting (Palma de Mallorca, Spain, 2018), 42nd FEBS Congress: From molecules to cells and back (Jerusalem, Israel, 2017), NutRedOx COST Action CA16112 & Postgraduate Training Network ,'NutriOx', 2017 (Strasbourg, France, 2017), ASM MICROBE 2017 General Meeting (New Orleans, USA, 2017), "NutriOx 2016" International meeting (Kaiserslautern, Germany, 2016), ICAB 2016: 18th International Conference on Advances in Biology (Venice, Italy, 2016), General Meeting ASM MICROBE 2016 (Boston, USA, 2016), 6th International Conference on Clinical and Experimental Dermatology (Chicago, USA, 2016), 3rd International Scientific Conference on "Dialogues on Sciences" (Yerevan, Armenia, 2015), International conference on Microbiology (Frankfurt am Main, Germany, 2015), International Workshop "Trends in Microbiology and Microbial Biotechnology" (Yerevan, Armenia, 2014).

Publications. On the basis of the experimental data embaded in dissertation, 40 papers, including 30 articles in international peer-reviewed journals as well as 10 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 291 papers and books). The dissertation consists of 284 pages, 13 tables and 70 figures.

MATERIALS AND METHODS

Chemicals and Reagents: All chemicals and reagents were purchased from Sigma Aldrich (Taufkirchen, Germany). The OxiSelect™ Cellular Antioxidant Assay Kit was obtained from Cell Biolabs (Cat. No. STA-349, San Diego, California, USA). High glucose DMEM (Dulbecco's Modified Eagle Medium), EMEM (Eagle's Minimum Essential Media), McCoy's 5A, the base medium for renal epithelial cells (ATCC PCS-400-030™, Manassas, Virginia, USA), and the renal epithelial cell growth kit (AT00-040, Manassas, Virginia, USA) were from ATCC (ATCC, Virginia, USA).

Collection and Identification of Plant Material: The collection of plant material was conducted from various altitudes within the territory of Armenia. The identification of plants was carried out with the assistance of experts from the Department of Botany and Mycology at Yerevan State University (YSU) and the A.L. Takhtajyan Institute of Botany of the National Academy of Sciences of Armenia. The plant samples are preserved in the respective herbaria, and each sample has been assigned a unique voucher specimen number.

Applied Model Cultures

***Arabidopsis thaliana* Model Lines:** The *A. thaliana* wild-type (Columbia ecotype) and mutant plants were grown in soil enriched with Hoagland's solution containing various macro- and microelements (Hoagland & Snider, 1933).

Mouse Microglial BV-2 Cell Lines: The BV-2 mutant (*Acox1*$^{-/-}$) and wild-type (*Wt*) cell lines were provided by Prof. Mustafa Chérif-Malki through the BioPeroxIL laboratory at the University of Burgundy (Dijon, France) (Ginovyan et al., 2022).

Human Colorectal Adenocarcinoma HT-29 Cell Culture: The HT-29 cell culture, derived from human colorectal adenocarcinoma, was obtained from ATCC

(ATCC HTB-38, Manassas, Virginia, USA). Original HT-29 cells were sensitive to the anthracycline antibiotic doxorubicin and were labeled as HT-29S. Doxorubicin-resistant cells, labeled as HT-29R, were initially selected based on their resistance to mitoxantrone (a synthetic antitumor antibiotic analog of doxorubicin) at the Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, and provided for our studies through Prof. A. Bartoszek (Gdańsk, Poland) (Ginovyan et al., 2023).

Human Breast Cancer MCF-7 Cell Line: The human breast cancer MCF-7 cell line was obtained from ATCC (ATCC HTB-22, Manassas, Virginia, USA) and provided by Prof. A. Bartoszek (Gdańsk, Poland).

HeLa (Human Cervical Cancer) and A549 (Human Lung Adenocarcinoma) Cell Lines: These cell lines were kindly provided by the staff of the Orbeli Institute of Physiology of the National Academy of Sciences of Armenia.

Human Normal Primary Mixed Renal Epithelial Cell Line (HREC): The human normal primary mixed renal epithelial cell line (HREC) was obtained from ATCC (ATCC PCS-400-012™, Manassas, Virginia, USA).

All cultured cells were periodically tested for mycoplasma contamination using the Universal Mycoplasma Detection Kit (ATCC 30-1012K™, Manassas, Virginia, USA).

Gram-Positive Bacteria: *Bacillus subtilis WT-A1* (isolated from metal-contaminated soils of Kajaran, Microbial Culture Collection, Biotech Research Center, Armenia, lab strain), *Enterococcus hirae* ATCC 9790, *Staphylococcus aureus* MDC 5233 (Microbial Culture Collection, Biotech Research Center, Armenia, lab strain).

Gram-Negative Bacteria: *Escherichia coli* VKPM M-17 (National Collection of Industrial Microorganisms of Russia, Institute of Genetics and Selection of Industrial Microorganisms, Russia, lab strain), *Salmonella typhimurium* MDC 1754 (Microbial Culture Collection, Armenia, lab strain), ampicillin-resistant *E. coli* DH5α-pUC18, kanamycin-resistant *E. coli* pARG25 (Biotech Research Center, Armenia, lab strain), *E. coli* ATCC 25922 (obtained from ATCC, USA).

Extraction of Plant Dry Matter. Dried and powdered plant material was extracted using maceration with 80% ethanol or methanol. The dry extracts were collected into Eppendorf tubes, weighed, and stored at -20°C until use.

Essential oil extraction. Essential oils were extracted from plant material 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).

Determination of the chemical composition of 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).

Determination of Total Phenolic and Flavonoid Content. The total phenolic content of plant extracts was measured by Folin–Ciocalteu (FC) reagent employing a calibration curve of gallic acid (GA) (0–250 μg/mL) using a UV-Vis spectrophotometer. The total flavonoid content was determined employing $AlCl_3$ colorimetric assay utilizing a UV-Vis spectrophotometer (Genesys 10S, Waltham, MA, USA) (Cano-Ibáñez et al., 2022).

LC-Q-Orbitrap HRMS Analysis. The phytochemical analysis of plant extracts was performed using a Dionex Ultimate 3000 UHPLC system (Thermo Scientific ™,

Dionex, San Jose, CA, USA) equipped with Synergi ™ Hydro-RP A (150 × 4.5 mm, 4 μm, Phenomenex) column, held at a temperature of 30 °C (Chiarello et al., 2020).

Raw data from high-resolution mass spectrometry was elaborated with Compound Discoverer (v. 2.1, Thermo, Waltham, MA, USA), which facilitated the peak recognition, retention times arrangement, profile assignment, and isotope pattern. Major metabolite identification was based on accurate mass and mass fragmentation pattern spectra against MS-MS spectra of compounds available on a customized database of different classes of phytochemicals created on the basis of literature data and implemented in the software. Raw data from three experimental replicates and a blank sample were processed using a workflow presented by Kusznierewicz, Mróz, Koss-Mikołajczyk, and Namieśnik (Chiarello et al., 2020).

Post-Column Derivatization with ABTS. Profiles of polyphenols and antioxidants for plant extract were obtained employing the HPLC-DAD system (Agilent Technologies, Wilmington, DE, USA) connected with a Pinnacle PCX Derivatization Instrument (Pickering Laboratories Inc., Mountain View, CA, USA) and UV–Vis detector (Agilent Technologies, Wilmington, DE, USA). The conditions of chromatographic separation were the same as in the case of LC-HRMS analysis.

The chromatograms before derivatization were recorded at 270 nm in DAD detector. The post-column derivatization with ABTS reagent was carried out according to methods described in the literature with slight modification. A stream of methanolic ABTS solution (1 mM) was introduced to the stream of eluate at a rate of 0.1 mL/min and then directed to the reaction loop (1 mL, 130°C). The antioxidant profiles were recorded in a UV-Vis detector at 734 nm.

Evaluation of antioxidant potential of investigated solutions by ABTS or DPPH tests. Free radical scavenging assay was performed as described by Ginovyan et al., Hambardzumyan et al. (2023, 2020). Catechin was used as a standard.

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).

Hydrogen Peroxide Reduction Assay. The ability of plant metabolites to reduce hydrogen peroxide (H_2O_2) was assessed according to the method described by Ruch and colleagues (Ruch et al., 1989).

Determination of antioxidant activity with reactive thiobarbituric acid inhibition of malondialdehyde synthesis. The determination of extracts' antioxidant activity 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 (Moghrovyan et al., 2019).

Synthesis of Ag NPs Using plant Extracts and their characterization. A stock solution of plant extract was prepared by dissolving 5 mg of plant extract in 10 mL of Milli-Q water (18.2 MΩ·cm at 25 °C) and Ag NPs were synthesized by mixing the solutions of $AgNO_3$ (10 mM) and plant extract in 1:9 ratio (Hovhannisyan et al., 2022). The physical stability of the investigated samples was evaluated by the ξ-potential measurement and dynamic light scattering (DLS), employing a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The size of biosynthesized Ag NPs was determined by Scanning Electron Microscopy (SEM-ZEISS-SUPRA 40/gemini column) equipped with Electron Backscatter Diffraction (EBSD) detector and Energy Dispersive X-ray (EDX) detector -SEM-EDX analysis. The size and shape of Ag NPs were evaluated by transmission electron microscopy (LEOL JEM-1400 TEM). The total Ag content was

determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Horiba JobinYvonUltima 2).

Determination of Antimicrobial Activity. The antimicrobial activity ttesting was conducted by disk diffusion assay using bacterial strains mentioned earlier. The results were expressed by Minimum Inhibitory Concentration (MIC) and/or Minimum Bactericidal Concentration (MBC) values. Kanamycin and ampicillin (25 μg/ml) were used as positive controls (Avetisyan et al., 2017).

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

Determination of H^+/K^+-fluxes. The H^+-fluxes were determined in whole bacterial cells by employing an appropriate selective electrode (HJ1131B and HI5222 HANNA Instruments). Results were expressed in mmol min^{-1} 10^8 $cells^{-1}$ in 1 unit of volume (ml) (Hovhannisyan et al., 2022). *N*, *N*'-dicyclohexylcarbodiimide (DCCD) served as an inhibitor of the F_0F_1-ATPase and the bacterial cultures were incubated with 0.2 mmol/L of DCCD for 10 min (Taussky & Shorr, 1953).

Determination of F_0F_1-ATPase Activity in Membrane Vesicles. The impact of plant extracts on the H^+-translocating F_0F_1-ATPase activity was investigated in bacterial membrane vesicles, which were obtained by the Kaback method. F_0F_1-ATPase activity was evaluated by quantifying the amount of inorganic phosphate liberated after adding ATP to membrane vesicles of bacteria. Inorganic phosphate was detected by Tausski and Shorr method. The membrane vesicles of bacteria were incubated with 0.2 mmol/L DCCD for 10 min (Taussky & Shorr, 1953). F_0F_1-ATPase activity is expressed in nmol Pi μg^{-1} protein min^{-1}.

MTT Cytotoxicity Test. The MTT test was performed as described (Stockert et al., 2018) to assess the growth of test cells exposed to different concentrations of the plant extracts for different exposure times. Cytotoxicity was expressed as the percentage of cell growth in the presence of the plant extract, normalized to the control cells treated with the corresponding volume of solvent alone (1% ethanol in the final mixture). In the control group, cell growth was considered as 100%.

Cellular Antioxidant Activity Test. Cellular antioxidant activity (CAA) of plant extracts was tested using OxiSelect Cellular Antioxidant Activity Assay Kit (green fluorescence). The fluorescent probe 2',7'-Dichlorodihydrofluorescein diacetate (H_2DCFDA) was used. The fluorescence intensity indicative of the cellular reactive oxygen species (ROS) level was measured upon the addition of the radical initiator. The fluorescence emission at 538 nm was recorded following excitation at 485 nm, using the TECAN Infinite M200 plate reader (Tecan Group Ltd., Lyon, France).

Estimation of the NO quantity under the influence of plant extracts. The NO neutralization activity was investigated using Griess reagent (Essadek et al., 2023).

Genotoxic Effects Measured by Comet Assay. Genotoxicity of extracts was evaluated using comet assay (Sassi et al., 2016). Approximately 10^5 cells per well were seeded in 24-well tissue culture plates and exposed to various concentrations of plant extracts for 24 hours. The average percentage of DNA in the comet tail served as an indicator to assess the genotoxic potential of the tested extract.

Preparation of Cell Lysates. Cell lysates were prepared according to the described method (Minasyan et al., 2025) and using radio-immunoprecipitation assay buffer (RIPA buffer).

Catalase activity determination. The catalase activity was measured according to Cherkaoui-Malki et al. (1990) and was expressed as % of untreated

control or units. One unit is defined as 1 μmol of H_2O_2 consumed in 1 minute, and specific activity is one unit of enzyme corresponding to 1 milligram of protein.

Acetyl CoA oxidase type 1 (ACOX1) activity determination. The measurement was carried out, as described by Oaxaca-Castillo D. et al. (2007) using fresh cell lysate and was expressed as % of untreated control.

Total SOD activity determination. Total SOD activity was measured according to the Beauchamp and Fridovich (1971).

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

Immunoblotting (Western blotting) using microglial BV-2 cells. Cell protein lysate was prepared as described above. Samples were then separated on a 10% SDS-PAGE gel and transferred onto a PVDF membrane as described (Minasyan et al., 2024).

Immunoblotting using *A. thaliana* plant model. Redox gel electrophoresis followed by immunoblotting with maleimide ($H_2C_2(CO)_2NH$) labeling was used to investigate the *in vivo* redox status of selected proteins (mitochondrial (PrxIIF) and chloroplast peroxiredoxins (2-CysPrx)) in plant leaf tissue (Ratajczak et al., 2019). For this purpose, SDS-PAGE gel electrophoresis was performed.

Quantitative Reverse-Transcription-PCR

RT-qPCR was used to determine the mRNA expression in the *Wt* and *Acox1*$^{-/-}$ BV-2 microglia cell lines after the treatments with plant extracts. Cell pellets were used for total RNA extraction and purification using the RNeasy Mini kit (Qiagen, Valencia, CA, USA). cDNA was generated by reverse-transcription using an iScript cDNA Synthesis Kit (Bio-Rad). The quantitative PCR of cDNA was realized using FG Power SYBR Green (Thermo Fischer Scientific, Illkirch-Graffenstaden, France) and an iCycler iQ Real-Time Detection System (Bio-Rad, Marnes-la-Coquette, France). Melting curve analysis was performed to control the absence of non-specific products. For each transcript, the amplification efficiency was determined by the slope of the standard curve generated from two-fold serial dilutions of cDNA. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative gene expression. The results are presented as graphs of relative expression data (fold induction).

Primer Sequences Used for qPCR:

Gene Name	Accession Number	Primer Sequences
Cat-F		5′AGCGACCAGATGAAGCAGTG3′
Cat-R	NM_009804.2	5′TCCGCTCTCTGTCAAAGTGTG3′
36b4-F		5′CGACCTGGAAGTCCAACTAC3′
36b4-R	NM_007475.5	5′ATCTGCTGCATCTGCTTG3′
Tnf-α-F		5′GACGTGGAAGTGGCAGAAGAG3′
Tnf-α-R	NM_013693.3	5′TGCCACAAGCAGGAATGAGA3′
Il-1β-F		5′GAGATTGAGCTGTCTGCTCA3′
Il-1β-R	NM_008361.4	5′AAGGAGAACCAAGCAACGAC3′
iNos-F		5′CCTAGTCAACTGCAAGAGAA3′
iNos-R	NM_010927.4	5′TTTCAGGTCACTTTGGTAGG3′
Abcd1-F		5′GCCAAGTTGTGGATGTGGAG3′
Abcd1-R	NM_007435.2	5′TTCCGCAGAGTCGGGATAGA3′

The data obtained were analyzed using the StepOne software.

Quantitative Determination of Proteins. The quantitative determination of proteins in tissues and cell lysates was conducted using three colorimetric methods: Bradford, Lowry, and Smith assays. The choice of method depended on the sensitivity requirements of the experiments.

Study of Chlorophyll Fluorescence and Redox Status of Photosystems in *A. thaliana* under the influence of natural polimers. The 4–5-week-old *A. thaliana* 6–10 *mm* leaf disks were treated with different concentrations (1.0–0.07 *mM*) of *M. arvensis* and *O. basilicum* var. *purpureum* EO and their main components methyl chavicol and menthol, respectively. The concentrations of EO were recalculated based on the concentrations of the main components. The maximum quantum yield (F_v/F_m) of photosystem II of *A. thaliana* leaf disks was determined by Mini-PAM-II photosynthesis yield analyzer (chlorophyll fluorometer, Walz, Germany). The photosynthetic parameters like the redox response of ferredoxin, photosystem II, photosystem I and plastocyanin with the NIR KLAS 100 (Walz, Germany) were determined. Redox state of proteins *in vivo* the redox gel electrophoresis followed by immune analysis by extraction and labeling with maleimid was applied. Non-protein thiols (NPT) content determination was carried out by Ellman's reagent (Sahakyan, 2024).

Data processing. During the research work, all experiments were conducted independently with a minimum of three repetitions. The reported data are presented as the average of three independent biological replicates, with standard deviations (SDs) calculated using either Graphpad Prism 8.0.3 or Microsoft Office Excel, depending on the experiment's sensitivity. Detailed information about the chosen method of statistical analysis is provided in the description of each dataset. The significance of differences between experimental and control data was evaluated using the Student's t-test (P) with the T test function in Microsoft Excel 2010, considering $P < 0.05$ as significant unless otherwise specified.

RESULTS AND DISCUSSION

The main part of the research work was carried out at the institute of Biology and Department of Biochemistry, Microbiology, and Biotechnology of the Faculty of Biology at Yerevan State University (Yerevan, Armenia), the Bio-PeroxIL laboratory at the University of Burgundy (Dijon, France), the Department of Food Chemistry, Technology, and Biotechnology at Gdańsk University of Technology (Gdańsk, Poland), the Department of Plant Biochemistry and Physiology at the Faculty of Biology of Bielefeld University (Bielefeld, Germany), and the Institute of Bioorganic Chemistry at Saarland University (Saarbrücken, Germany).

During the work, the biological activities and mechanisms of action of various extracts, essential oils, or their individual components isolated from wild and cultivated plant species and agricultural plant waste from different regions of the Republic of Armenia were studied using various test systems (see the "Materials and Methods" section).

Chemical composition and antioxidant activity of plant essential oils and extracts. Among the well-known and widely distributed essential oil-bearing plants are those from the Lamiaceae family. The quantitative and qualitative analysis results of the essential oil components extracted from certain Lamiaceae family plants are presented in Table 1.

Table 1. The Main Components (%) of Essential Oils from Certain Wild and Cultivated Lamiaceae Family Plants Growing in the Highland Altitude Armenian landscape

Components	(RRI[a]	O. basilicum var.purpureum	O. basilicum var. thyrsiflora	O.× citriodorum	Z. clinopodioides	T. vulgaris	M. arvensis	O. vulgare
Sabinene	897	–	–	–	–	–	–	**3.1**
3-octanone	952	–	–	–	–	–	–	**2.6**
β-ocimene	978	–	–	–	–	–	–	**2.6**
3-octanol	979	–	–	–	–	–	–	**2.4**
p-comene	1025	–	–	–	–	18.5	–	–
1-8-cineole	1035	1.40	3.50	–	0.22	4.0	–	–
o-comene	1045	–	–	–	–	–	–	**2.5**
Eucaliptol	1059	–	–	–	–	–	0.05	**2.0**
linalool	1100	18.0	68.0	42	–	0.1	0.15	**2.9**
Isomenthon	1163	–	–	–	0.70	–	7.13	–
Neomentol	1166	–	–	–	0.90	–	–	–
Menthol	1172	–	–	–	0.90	–	69.75	–
γ-Terpinene	1078	–	–	22	0.14	15.3	–	–
L-4-terpineol	1137	–	–	–	–	–	0.48	**2.3**
Menthon	1195	–	–	–	–	–	7.1	–
Methyl chavicol	1203	57.3	20.0	.45	–	–	–	–
Nerol	1231	–	–	3.0	–	–	–	–
Pulegone	1237	–	–	–	2.1	–	0.9	–
Neral	1244	–	–	93	–	–	–	–
Geraniol	1259	–	–	20	–	–	–	–
Neomenthyl acetate	1273	–	–	–	–	–	7.05	–
Geranial	1274	–	–	5.7	–	–	–	–
Carvacrol/thymol	1298	–	–	–	0.35	36.9	–	**2.4**
Dihydroedulan II	1342	–	–	–	–	–	–	**2.0**
Piperitenon	1370	–	–	–	0.35	–	–	–
β-caryophyllene	1419	1.72	–	80	–	–	0.23	**8.2**
α-bergamotene	1433	4.34	1.34	52	–	–	–	–
Humulene	1456	–	–	–	–	–	–	**2.7**
β-cubebene	1497	–	0.75	26	–	–	–	–
β-caryophyllene oxide	1517	–	–	–	–	–	–	**13.3**
β-bisabolene	**1572**	**–**	**–**	**0.31**	**–**	**–**	**–**	**3.2**

It was found that the Ocimum varieties cultivated in Armenia belong to the methyl chavicol, linalool, or citral and nerol chemotypes. The essential oil of *Z. clinopodioides* is rich in pulegone, with a concentration of 42.1%. In *T. vulgaris* essential oil, 20 compounds were identified, primarily belonging to the groups of aromatic monoterpenes (carvacrol/thymol (36.9%), p-cymene (18.5%)) and isomeric hydrocarbons (γ-terpinene, 15.3%). *M. arvensis* essential oil is a menthol rich. Essential oil of origanum cultivated in Armenia contains over 180 compounds, mainly of terpenoid or flavonoid nature, such as terpenes, β-caryophyllene epoxide (13.3%), β-caryophyllene (8.2%), and o-cymene (5.2%). *Artemisia dracunculus* belongs to the estragole-rich chemotype, with estragole concentration reaching approximately 85%.

It has been demonstrated that the essential oils extracted from all the mentioned plants exhibit antioxidant activity in various chemical tests.

Additionally, our research showed that, for example, at the tested concentration, thyme essential oil bound 12.4% of the divalent iron present in the experimental solution, whereas the positive control's activity was 94%. This indicates a weak metal-chelating activity for this essential oil. In the reaction of preventing lipid peroxidation, the antioxidant index was 21±0.7% (compared to 91.1±1.9% for the positive control, α-tocopherol). This also suggests that this essential oil shows weak activity in almost all other antioxidant chemical tests, except for the DPPH test. A similar pattern was observed with the essential oil of *A. dracunculus*. However, this does not necessarily imply that the components of these essential oils will exhibit the same level of activity in other tests.

In recent years the biological activity of various extracts isolated from over 50 plant species have been studied. Among these, the following have garnered significant interest: *Ribes nigrum* L. (Grossulaceae), *Ficus carica* L. (Moraceae), and *Vitis vinifera* L. (Vitaceae), which are important agricultural crops and sources of pharmacologically significant compounds. Additionally, plants used in Armenian traditional medicine, such as *Hypericum alpestre* (Hypericaceae), *Agrimonia eupatoria* L. (Rosaceae), *Rumex obtusifolius* L. (Polygonaceae), and *Sanguisorba officinalis* L. (Rosaceae) have been selected for study (Torosyan, 1983). The selection of these plants was based not only on their high biological activity but also because they are widely used in everyday life. In the case of the first three species, the green biomass of these crops is considered as agricultural waste and has not been utilized until now. Additionally, the fact that the studied plant species belong to different families allowed for a demonstration of the clear differences in their chemical compositions and biological activity targets and mechanisms. The extracts isolated from these medicinal plants were analyzed for their total phenolic content, aiming to find any correlation between the quantity of active metabolites and their biological activity.

Studies have shown that there is no clear correlation between the quantitative composition of plant phenolic compounds and their levels of antioxidant activity as demonstrated in chemical tests (Fig. 1.).

The antimicrobial, antibiotic-modulating, anti-inflammatory, and other properties of phenolics are of great interest. For example, extracts of *Vaccinium myrtillus* (VM) show significant antioxidant activity according to the results of the ABTS, DPPH, and cellular antioxidant activity tests (Fig. 1. A, B, and C).

Based on the findings, the DPPH/ABTS stoichiometry values for the VM extract were 3.401 and 0.6498, respectively (Fig. 1A and B). High total phenolic and flavonoid content of ethanol extract of VM were also shown. The CAA test was performed to elucidate antioxidant potential of VM aerial part extract inside the HT29 cells. The results demonstrated that VM extract has significant antioxidant activity in HT29 cells (Fig. 1 C) relative to the reference quercetin utilized in the test. The CAA activity of the VM extract shows a direct correlation with its concentration.

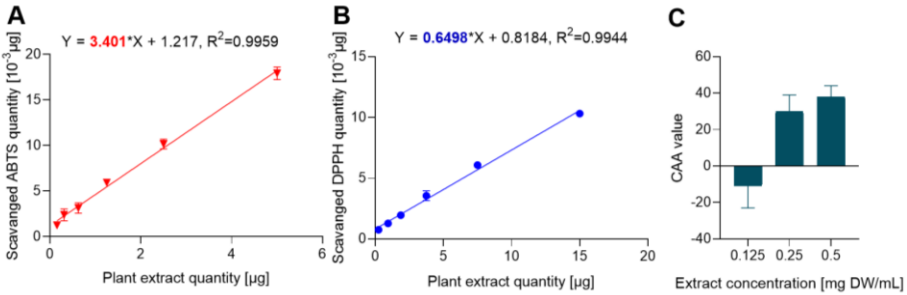

Fig. 1. Total antioxidant activity of *V. myrtillus* extract evaluated by spectrophotometric tests: A – ABTS and B – DPPH or *in vitro* cellular test C – CAA. Cellular antioxidant activity (CAA) was determined in HT29 cells exposed to VM extract for 1 h. The results are means ± SD from three independent experiments, $p < 0.05$ (for spectrophotometric tests).

Considering high content of phenolics, including flavonoids, the observed antioxidant properties of the *V. myrtillus* extract are obviously due to the presence of redox-active constituents in this herb's metabolome. Therefore, characterization of VM extracts' phenolic compounds was done using advanced chromatographic technique (LC-Q-Orbitrap HRMS). The 85 compounds were identified in *V. myrtillus* extract, from which 83 compounds were annotated, and only 2 compounds were unknown.

The identified compounds were classified as hydroxybenzoic acids and derivatives, hydroxycinnamic acids and derivatives, flavan-3-ols, hydrolysable tannins and derivatives, condensed tannins and derivatives, flavanones, flavonols, flavones, lignans, flavanonols, coumarins and derivatives, phenolic glucosides, flavonoid glucosides, flavolignans and dihydroflavonaols (Fig 2, A). Each compound was allocated to specific groups after a thorough examination of the UV-visible spectrum. According to the literature all of these substances possess high biological activity.The content of phenols and flavonoids in other plant samples has also been investigated.

A comprehensive metabolomic characterization of the extracts from the aforementioned plants was performed using LC-Q-Orbitrap HRMS analysis. In total, 144 major peaks were recorded across all samples, of which 127 were identified, while 17 compounds were documented as unknown. Flavonoids, their derivatives and phenolic acids with their derivatives primarily represented the main components. Other identified compounds were classified as hydroxycoumarins, terpenes, amino acids, peptides and their analogs, fatty acids and their conjugates, furanocoumarins, alcohols and polyols, carbohydrates and carbohydrate conjugates, linoleic acids and their derivatives, eicosanoids, lignans, naphthalene carboxylic acids and their derivatives, quinoline carboxylic acids, stilbenes, tricarboxylic acids and their derivatives (Fig. 3).

According to the obtained data, the *V. vinifera* (VV) sample showed the highest content of flavonoids and phenols, followed by *R. nigrum* (RN) and *F. carica* (FC). However, this same pattern was not observed in the DPPH test for the extracts of any of the studied plants. A comprehensive metabolomic characterization of the extracts from the aforementioned plants was performed using LC-Q-Orbitrap HRMS analysis. In total, 144 major peaks were recorded across all samples, of which 127 were identified, while 17 compounds were documented as unknown. A comprehensive metabolomic characterization of the extracts from the aforementioned plants was performed using LC-Q-Orbitrap HRMS analysis.

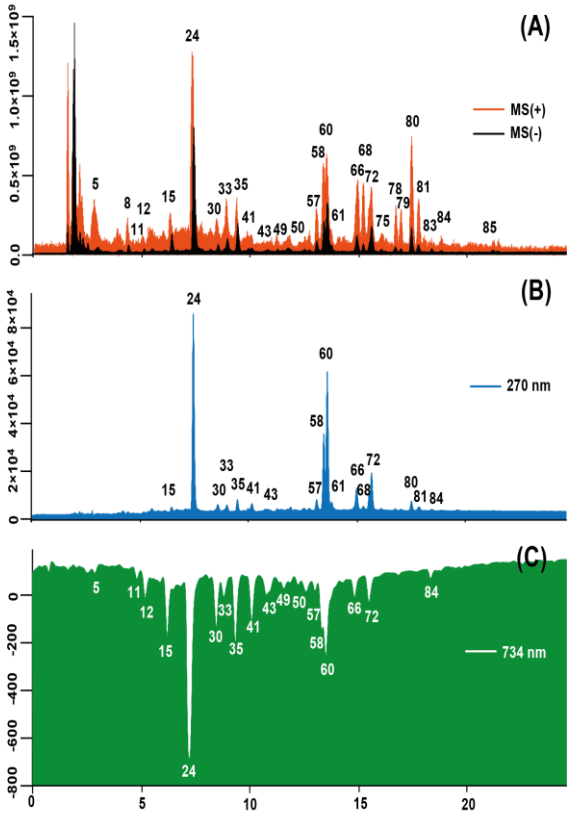

Fig. 2. HPLC-MS (A), HPLC-DAD 270 nm (B) chromatograms of phenolic constituents of *V. myrtillus* extract and the antioxidant profile (C) after (734 nm) post-column derivatization with ABTS reagent, for identity of peaks.

In the extract from *F. carica* leaves (FC), 92 peaks were recorded, and 86 compounds were identified. In the extract from *R. nigrum* leaves (RN), 91 peaks were recorded, and 78 compounds were identified. In the extract from V. vinifera leaves (VV), 66 peaks were recorded, and 56 compounds were identified. Thirty-seven peaks and 32 identified compounds were common to the extracts of all three plants. (Fig. 4). In total, 144 major peaks were recorded across all samples, of which 127 were identified, while 17 compounds were documented as unknown. Flavonoids, their derivatives and phenolic acids with their derivatives primarily represented the main components.

Other identified compounds were classified as hydroxycoumarins, terpenes, amino acids, peptides and their analogs, fatty acids and their conjugates, furanocoumarins, alcohols and polyols, carbohydrates and carbohydrate conjugates, linoleic acids and their derivatives, eicosanoids, lignans, naphthalene carboxylic acids and their derivatives, quinoline carboxylic acids, stilbenes, tricarboxylic acids and their derivatives (Fig. 3).

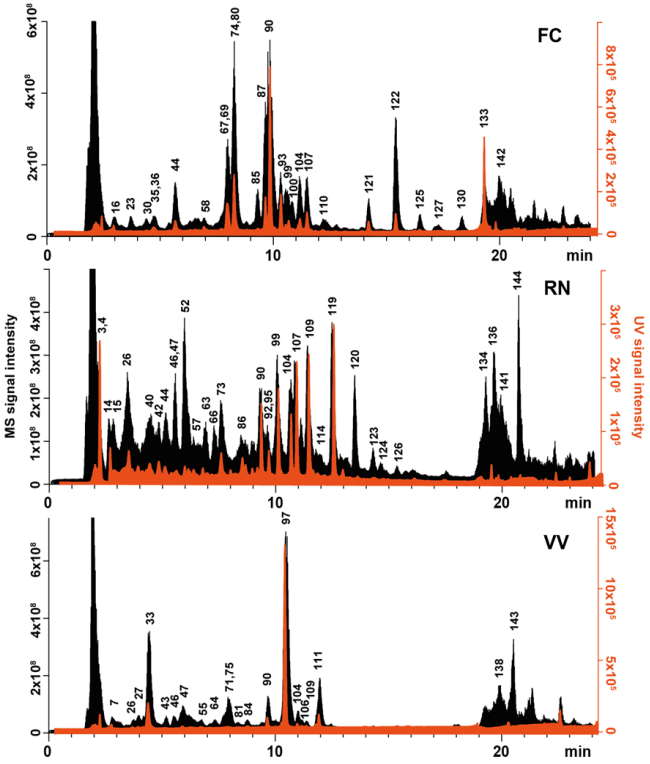

Figure 3. The LC chromatographic profiles of plant extracts monitored either by Quadrupole-Orbitrap High resolution MS (black HRMS chromatograms) or UV light (orange UV chromatograms). The panels illustrate the differences in composition between *F. carica* extract - (FC), *R. nigrum* extract - (RN), and *V. vinifera* extract - (VV).

Additionally, 11 compounds were unique to VV, 27 compounds were unique to RN, and 34 compounds were unique to FC. The recorded peaks were compared based on the intensity of their MS signals.

The antioxidant activity of the components in the extracts does not always correlate directly with their quantities.

Antibacterial and Antibiotic-Modulating Activity of Plant-Derived Compounds.

A vast number of antibiotics are used against various pathogenic bacteria and cancer cells, and each year this list is supplemented with new synthetic and natural antibiotic compounds. However, the intensification of the development of multidrug resistance among numerous bacterial strains and cancer cells of various origins has led to an increase in the severity of diseases caused or complicated by these agents. Additionally, in the context of antibiotic resistance, using higher doses of antibiotic agents can result in multiple toxic effects on the human body, leading to consequences of varying severity. In this regard, plant extracts and essential oils could serve as new sources of natural agents with antibiotic properties that are less hazardous and exhibit low toxicity.

Our screening efforts of nearly sixty plant species revealed that the majority produce metabolites that inhibit the growth and development of various bacteria and cancer cell lines to varying degrees. Notably, this includes both antibiotic-sensitive and

antibiotic-resistant forms of cells. We will discuss these findings and the possible mechanisms behind such effects in more detail.

Characteristics of Antibacterial Activity of Plant Compounds. Our research over recent years has demonstrated that essential oils and extracts isolated from various plants exhibit notably high antibacterial, anti-phage, and anti-fungal activities against numerous Gram-positive and Gram-negative bacterial strains, T4 phages, and yeast of the Candida genus. This distinctive property of the studied metabolites holds both fundamental and practical significance. However, given the urgency and relevance of the problem of antibiotic resistance, let's delve into the effects of plant-derived compounds on the growth and metabolism of certain resistant bacterial strains and explore the potential mechanisms behind these effects.

The studies revealed that the ampicillin-resistant *E. coli* DH5α-pUC18 bacterial strain, exhibited significant sensitivity to the essential oils extracted from the tested plants. Specifically, the Minimum Inhibitory Concentrations (MIC) for the essential oils from *O. × citriodorum*, *O. basilicum* var. *purpureum*, and *T. vulgaris* were found to be 6.25 µl/ml against this resistant strain. In contrast, the MIC for the *O. basilicum* var. *thyrsiflora* essential oil was 12.5 µl/ml. These same MIC values were observed for the non-resistant *E. coli* VKPM M-17 strain, which served as a control strain in this study.

A similar pattern was observed with *O. vulgare* essential oil, which exhibited an MIC value of 50 µl/ml. However, the essential oil of *Z. clinopodioides* showed a different profile: the MIC was 25 µl/ml when tested against the resistant strain of *E. coli*, while it was 50 µl/ml for the non-resistant strain (Figure 4). In this context, the MIC values were considered effective, and the action of the essential oils was evaluated as bactericidal.

Tested Gram-positive bacteria show higher sensitivity to components of *A. dracunculus* essential oil (Fig. 5).

During the testing of *A. dracunculus* essential oil against *S. aureus* and *B. subtilis* strains, the MIC value was determined to be 6.25 µL/mL.

For the *E. coli* VKPM M-17 strain, the MIC was higher, at 50 µL/mL. Interestingly, the ampicillin-resistant *E. coli* DH5α-pUC18 strain displayed significant sensitivity to the essential oil, with an MIC value of 6.25 µL/mL. Among the tested yeast strains, the highest sensitivity was observed, with the MIC value being 1.56 µL/mL (Fig. 5). The antimicrobial effects were classified as bactericidal.

The variations in the activities of essential oils extracted from different plants against the same test microorganisms can be attributed to the differences in their chemical compositions. Our studies have demonstrated that the antimicrobial activity of some essential oils is largely determined by their major components.

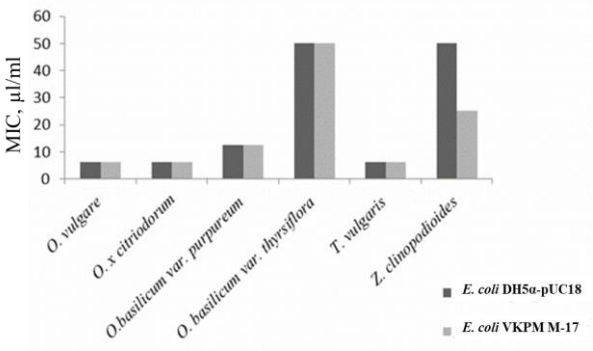

Fig. 4. MIC values of studied essential oils against *E. coli* antibiotic-sensitive and antibiotic-resistant strains.

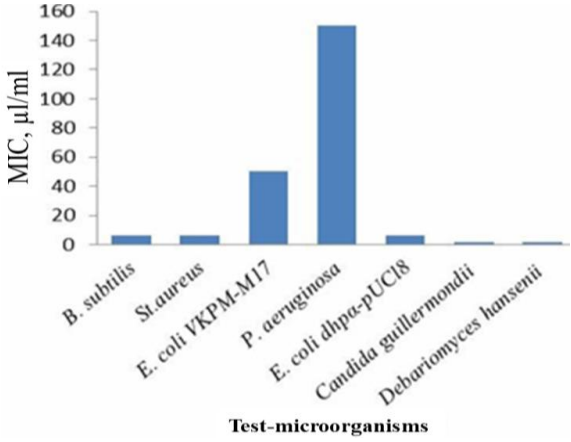

Fig. 5. MIC values of *A. dragunculus* essential oil against test microorganisms

The observed differences in the antibacterial activities of various essential oils against Gram-positive and Gram-negative bacteria suggest that these oils operate through different mechanisms of action. This is also supported by the varying activities of the oils against antibiotic-sensitive and antibiotic-resistant strains of *E. coli*.

In some cases, compounds exhibiting significant antioxidant activity in chemical tests can act as prooxidants in living cells. In such scenarios, these compounds modulate the activity of enzymatic systems within the cells by altering the expression of genes responsible for the synthesis of the enzymes.

Antibiotic-modulating activity of plant extracts. Overcoming the problem of antibiotic resistance using natural compounds is a promising approach in the treatment of infectious diseases or various types of cancer. One of the mechanisms of manifestation of antibiotic effect is the antibiotic-modulating ability of compounds. It is easy to discuss this phenomenon with the example of extracts isolated from plants belonging to different families, all of which have shown high antioxidant activity in chemical tests. In the case of VM extract, studies have been carried out using both microbes and various cancer and non-cancer cell lines as test systems.

The antimicrobial and antibiotic-modulating activity of *V. myrtillus* extract: Plant extracts or individual components can exhibit direct antimicrobial effects or act as antibiotic action modulators and modifiers and enhance the efficacy of commonly used antibiotics through various mechanisms. The antibacterial and antibiotic-modulating abilities of VM extract were investigated against two bacterial strains - *S. typhimurium* TA100 and *E. coli* BW 25113 wild-type strains. The results showed that VM extract concentrations of up to 1 mg/ml did not impair the viability of any of the tested bacteria.

In our work, the antibiotic-modulating ability of VM extract was investigated by extract/antibiotic co-treatment of bacterial cells. Ampicillin (AMP) and kanamycin (KAN) were used as test-antibiotics due to the differences in ther mechanisms of

action. Ampicillin belongs to a group of beta-lactam antibiotics that inhibit bacterial cell wall synthesis, while kanamycin is an aminoglycoside that works by irreversibly binding to the bacterial 30S ribosomal subunit.

The study revealed a synergistic interaction between VM extract and kanamycin when using *E. coli* strain (Fig. 6A). The plant extract at non-toxic concentrations (0.125 and 0.25 mg/ml) combined with kanamycin, reduced the MIC values of the antibiotics by half. However, no synergism was observed with *S. typhimurium* strain (Fig. 8 B). On the other hand, VM extract did not increase ampicillin activity against any of the tested bacteria (Fig. 8 C and D).

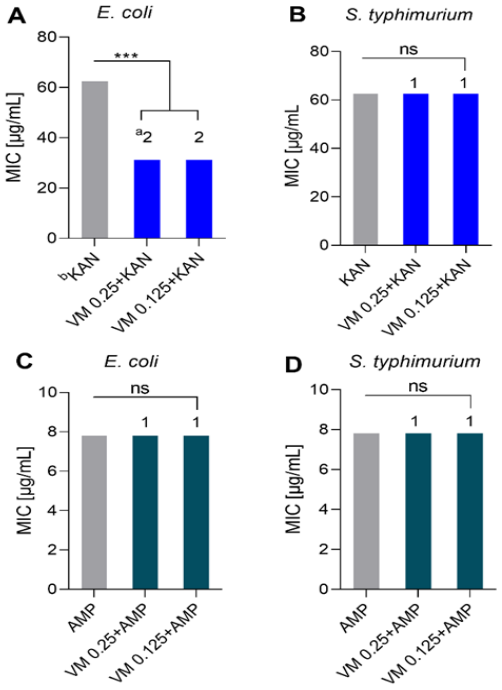

Fig. 6. Antibiotic modulatory activity of *V. myrtillus* crude ethanol extract at sub-inhibitory concentrations against some bacterial strains. Tested bacterial strains: *S. typhimurium* TA100, *E. coli* BW25113. [a](n) Modulation factor (MF) which was calculated as MF = $MIC_{antibiotic\ alone}/MIC_{antibiotic\ +\ extract}$, n ≥ 2-synergic interaction, n = 1-indifference, n≥2 - synergistic interaction. [b] Used antibiotics – KAN – kanamycin, AMP – ampicillin. ***$p < 0.001$.

Taking into account the obtained data, it can be assumed that the modulation of kanamycin activity may be the result of a change in the permeability of *E. coli* membranes under the influence of VM extract. To confirm this, we investigated the effect of VM extract on changes in proton flux across the bacterial cell membrane.

Determination of proton flux. Proton flux is directed to the extracellular environment and reaches the 2.59 mM H^+/min by 10^8 cells value during glucose utilization (Fig. 9).

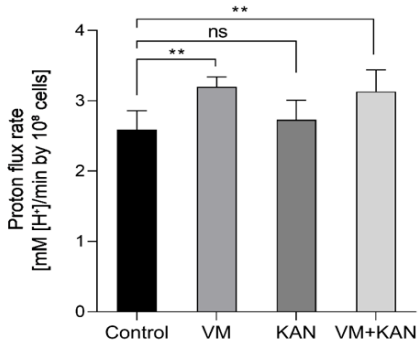

Fig. 7. VM and KAN effect on [H⁺] flux rate by 10^8 whole *E. coli* BW25113 cells within assimilation of glucose (2 g/L). Results represent means ± SD from three independent experiments. **p < 0.01.

So, although VM aqueous extracts do not have direct antibacterial activity, nevertheless, they increase the effectiveness of the kanamycin by increasing the permeability of bacterial membranes.

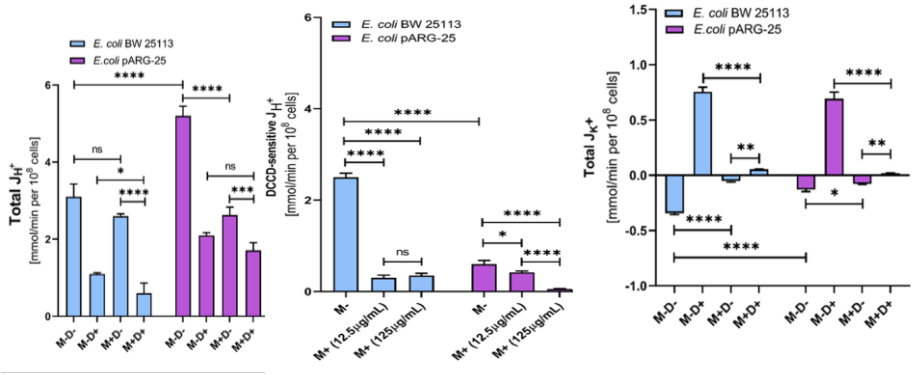

Fig.8. Effect of menthol on the change in (a) total proton, (b) DCCD sensitive proton and (c) potassium fluxes in *E. coli* BW 25113 and kanamycin-resistant *E. coli* pARG-25 bacteria (ns – not significant; *p<0.05; ***p<0.001; ****p<0.0001).

Kanamycin was not affected the proton flux rate in *E. coli* BW25113 cells, while upon the addition of VM extract, it increased by 23.6 %, which remained constant during the combined influence of KAN and VM. Since KAN needs to entry into the cell and bind to the 30S subunit of the ribosome to act, and VM stimulates membrane flux, we can conclude that the synergistic effect of VM with kanamycin is due to the effect of the extract components on the membrane permeability of bacteria. These claims are indirectly proved by the fact that the investigated extract did not show any synergistic activity when used with the antibiotic ampicillin, which inhibits the synthesis of the bacterial membrane. This feature of VM solutions is also reported for the first time. Under the influence of terpenoid alcohol menthol we observed chnges in both proton and potassium fluxes (Fig. 8).

After these studies, the question arises whether the mechanisms discovered in prokaryotic cells will also work in eukaryotic cells.

Cytotoxicity of *V. myrtillus* extracts. Human colon adenocarcinoma (HT29), human breast carcinoma (MCF-7), human cervical carcinoma (HeLa), and human normal primary renal mixed epithelial (HREC) cell lines were used as test systems. The obtained data prove that the VM extract showed a time- and concentration-dependent inhibitory effect on all tested cancer cell lines (HT29, MCF-7 and HeLa cells) (Fig. 10 A, B and Fig. 9A). In all three tested cancer cell lines, significant inhibition of cell growth was observed only at a concentration of 0.5 mg DW/ml under 24 h cultivation.

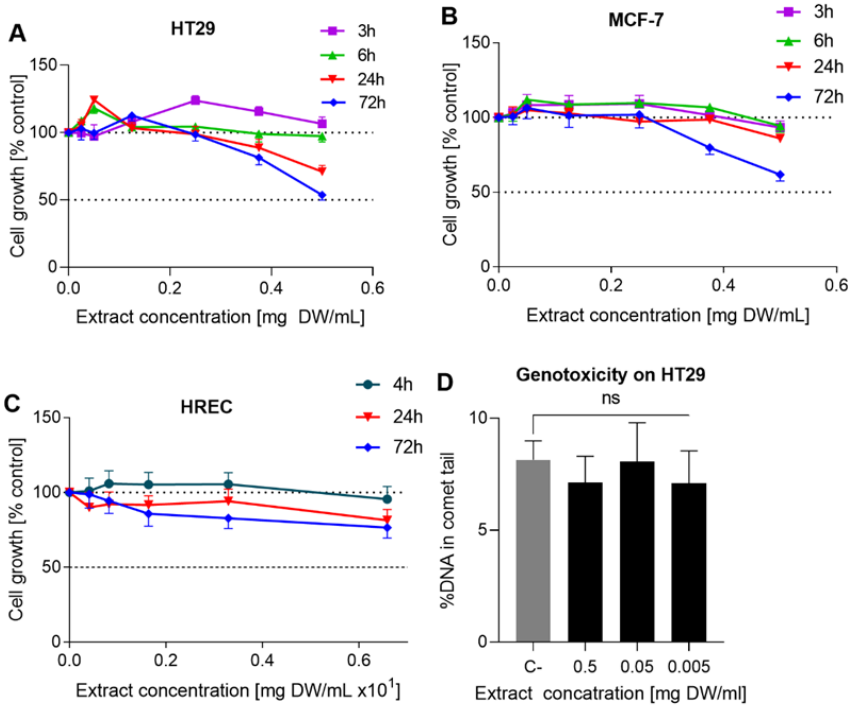

Fig. 9. Inhibition of growth of HT29 (A), MCF-7 (B) and HREC (C) cells treated by *V. myrtillus* extracts for different exposure times (determined by MTT test). Genotoxicity of VM extract expressed as %DNA in comet tail evaluated in HT29 cells (D). Results represent means ± SD from three independent experiments. C– (Negative control) non-treated cells. No significant differences between treated and non-treated cells were observed.

The strongest inhibitory effect was observed at the longest exposure (72 h) in all tested cancer cells.

However, an important fact to be highlighted is that although the VM extract shows remarkable cytotoxic activity in all cancer cells tested, it does not express cytotoxicity in normal HREC cell lines even at much higher (10-fold) concentrations (Fig. 9 C). Therefore, it can be assumed that VM extract is able to selectively affect cancer cells.

The selected plants belong to different taxonomic groups. Antimicrobial E. coli activity of extracts was studied against ampicillin-resistant and kanamycin-resistant strains. A 0.0625 to 1 mg/ml concentration range was tested. None of the plant extracts showed significant antibacterial activity against the tested strains at the indicated concentrations. The 0.25 and 0.125 mg/ml concentrations were chosen to

study the antibiotic-modulating properties. The RN and FC extracts with the tested antibiotics showed modulating activity (Fig. 13. A and B).

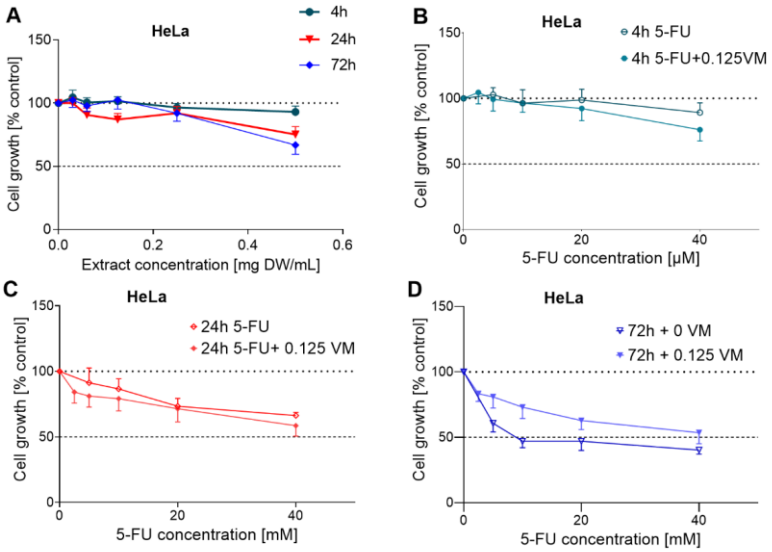

Fig. 10. Growth inhibition of HeLa cells treated with VM extract for 4, 24, and 72h (A). The growth inhibiting effect of 5-flurouracil separately and in combination with subinhibitory concentration of VM extract on HeLa cells for 4 (B), 24 (C), and 72h (D). Results represent means ± SD from three independent experiments; SD values did not exceed 15%.

The same pattern was observed also using extracts of three popular agricultural crops, grape (VV), blackcurrant (RN) and fig (FC), in both bacterial and human *in vitro* cell cancer models.

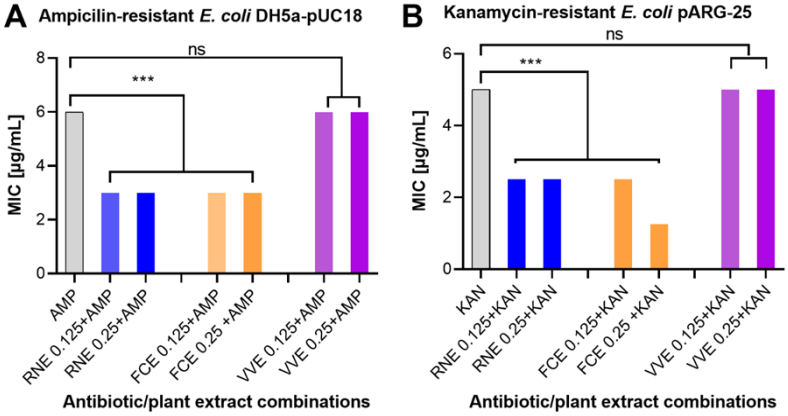

Fig. 11. Modulatory effect of plant extracts at non-inhibitory concentrations on the antibiotic activity against ampicillin or kanamycin resistant *E. coli* strains. (A) - Modulatory activity of plant extracts on ampicillin against ampicillin-resistant *E. coli* strain. (B) - Modulatory activity of plant

extracts on kanamycin against kanamycin resistant *E. coli* pARG-25 strain. The abbreviations refer to: RNE – *R. nigrum* extract, FCE – *F. carica* extract, VVE – *V. vinifera* extract, KAN – kanamycin, AMP – ampicillin.

VV was an exception in this case, as it did not show any modulating effect on the antibiotics tested. As shown in Fig. 10, RN and FC at least two-fold reduced the MIC values of ampicillin and kanamycin against the tested antibiotic-resistant strains, indicating the existence of a synergistic relationship with these antibiotics (MF≥2). Moreover, at a concentration of 0.25 mg/ml, FC reduced the MIC value of kanamycin by 4 times (MF=4). Interestingly, in the case of RN, similar changes in MIC values were observed at two tested concentrations (0.25 and 0.125 mg/ml), suggesting that the modulating effect of this solution reached its maximum value at the lower concentrations tested or that the correlation relationship was not strong with concentration.

In order to understand the mechanisms underlying the antibiotic resistance-modulating effect of the extracts, the changes in the H^+ fluxes under the influence of extracts and kanamycin in *E. coli* pARG-25 membranes were determined (Fig. 11, A).

It was not possible to assess the combined effect of kanamycin and extract on H^+ fluxes, as bacterial biomass formation was stopped under these conditions. As expected, given the kanamycin-resistance of this strain, no significant change in proton flux was observed under the influence of kanamycin. The RNE caused the highest increase in H^+-flux across the bacterial membrane, followed by the VVE and FCE.

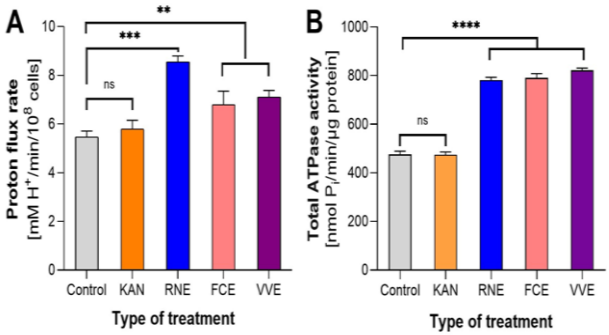

Fig. 12. The H^+-fluxes across membrane - (A) and ATPase activity - (B) during assimilation of glucose (22 g/L) determined in the intact kanamycin-resistant *E. coli* cells treated with kanamycin (KAN) or plant extracts. (**p=0.0036, ***p=0.0008, ****p < 0.0001; ns, not significant).

All tested extracts at a concentration of 10 µg/ml stimulate ATP-ase activity (Fig. 12, B). These data also correspond to changes in the intensity of the H^+ flow rate (Fig. 12, A). In our research, we have also shown that extracts isolated from some plants also change the H^+/K^+ ratio.

Cytotoxicity and chemotherapeutic agent modulating ability of *R. nigrum*, *F. carica* and *V. vinifera* extract: *In vitro* growth inhibiting properties of RNE, FCE and VVE were explored in human colon adenocarcinoma HT-29 cancer cells after 72 h exposure time in order to choose proper concentrations for experiments. Based on the obtained data, all three extracts exhibited considerable growth inhibiting properties towards HT-29 cells. FCE possessed the strongest growth inhibiting properties, followed by RNE and VVE leaf extracts (Fig. 13). Their EC50 values are lining up in the same way and were 0.18 mg/mL in the case of FCE, 0.33 mg/mL for RNE and 0.51 mg/mL for VVE. There is clear linear correlation between concentration and growth inhibiting properties for all extracts.

However, the inhibitory effect of all tested plant extracts was detected only at high exposure time (72 h). Therefore, during the further experiments, only 72-h exposure time was applied.

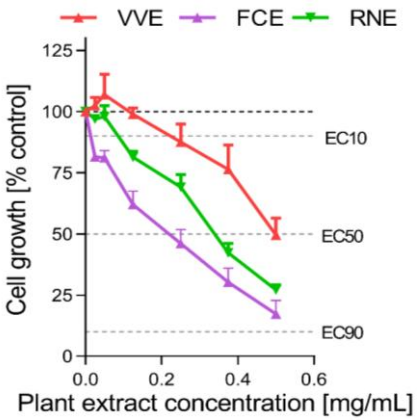

Fig. 13. Growth inhibitory properties of RNE, FCE and VVE towards human colon adenocarcinoma HT-29 cells determined by MTT test after 72 h exposure. The grey broken lines in the graphs mark the level of EC10, EC50, and EC90 values corresponding to respectively 90%, 50%, and 10% cell growth compared to control (100%). Results represent means ± SD from three independent experiments carried out in triplicates.

Further resistance modifying properties of test plant extracts were explored on doxorubicin (DOX) susceptible and resistant HT-29 lines - HT-29S and HT-29R, respectively.

For these, we selected 0.5, 0.25, and 0.05 mg/mL concentrations of the extracts based on their growth inhibiting properties towards HT29 DOX sensitive cell line. In general, the interaction of DOX and plant extracts in susceptible HT29S cells turned out generally antagonistic or just additive, particularly in the case of FCE and VVE (See Fig. 14 and 15). At the lowest tested concentration none of the plant extracts affected the cytotoxic properties of DOX (Fig. 16). In case of DOX resistant HT29R cells, the effects of extracts on activity of DOX were different and they seemed to act as modulating agents. RNE enhanced the effect of DOX more than FCE. Particularly some captivating results were obtained regarding DOX combination with 0.5 mg/mL of RNE or with 0.25 and 0.5 mg/mL concentrations of VVE, where DOX resistant cells became significantly more susceptible to the treatment than sensitive cells (Fig. 14 A and C).

As one of the main objectives of the study was to check if the reversal of antibiotic resistance of cancer cells to doxorubicin using plant extracts would be possible, therefore, the survival and resistance indexes (SI and RI) of plant extract/DOX combinations were calculated (Fig. 14 and 15). In our experiments, RI values of all combinations were lower than RI value calculated for DOX alone. Moreover, RI values were below 1 in case of the treatments of cells with all plant extract samples at 0.25 and 0.5 mg/mL concentrations combined with DOX. Importantly, although there were differences between the levels of effect of extracts, all of them reversed the HT-29R cell resistance to DOX and made them more susceptible than HT-29S cells in a concentration dependence manner. The RNE showed resistance modifying properties in all tested concentrations (Fig 14 A). Despite the fact that FCE displayed high cell growth inhibiting activity (Fig. 15), it demonstrated the lowest resistance modifying properties and had effect only at highest concentration and slight effect (Fig 16, B). VVE+DOX had the highest SI, it

showed significant resistance modifying properties at 0.25 and 0.5 mg/mL concentrations (Fig. 14 C, 15).

Since the modulatory activity of RNE was the most pronounced compared to other tested plants, the genotoxicity of this extract in HT-29 cell lines was also investigated by comet assay, according to which, the RNE showed a weak genotoxic effect on HT-29 cells only at a high concentration (0.5 mg/ml DW).

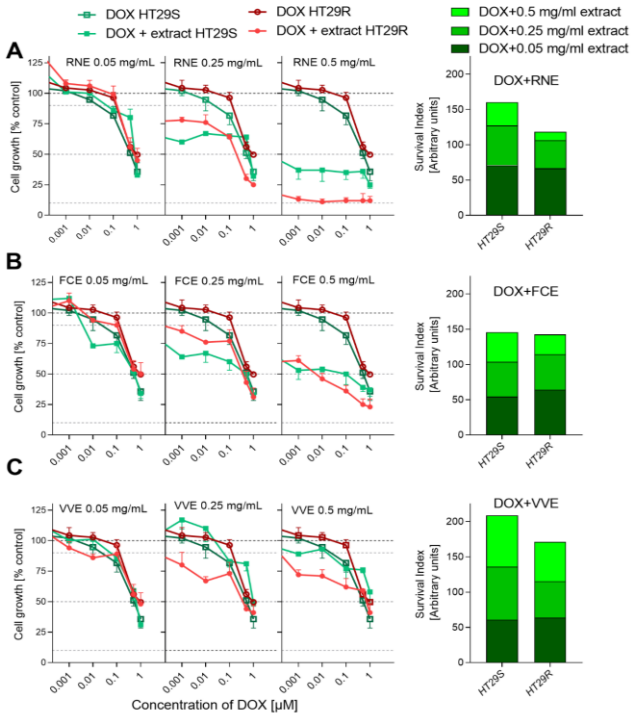

Fig. 14. Growth inhibitory properties of combinations of DOX with plant extracts towards human cancer colon HT-29 cells, both DOX-sensitive (HT-29S) and DOX-resistant (HT-29R), after 72 h treatment with indicated concentrations of FCE - A, RNE - B, or VVE - C. The grey broken lines in the graphs mark the level of EC10, EC50, and EC90 values corresponding to respectively 90%, 50%, and 10% cell growth compared to control (100%). The SI values were calculated as the sums of areas under survival curves for combinations of DOX and plant extracts applied at three concentrations (0.05, 0.25 and 0.5 mg DW/mL).

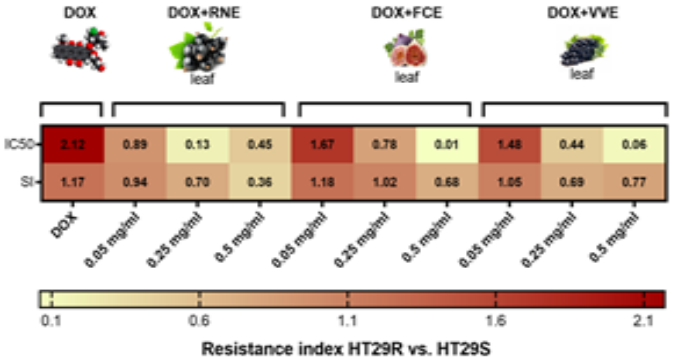

Fig. 15. The heat map illustrating modifying effects of studied plant extracts on DOX activity towards DOX-resistant vs. DOX-sensitive human cancer colon cells HT-29. The value of RI >1 indicates that DOX-resistant HT-29R cell line is less sensitive to this antibiotic than DOX-sensitive

HT-29S cell line; RI <1 indicates that DOX-resistant HT-29R cell line as a result of combined treatment with DOX and plant extracts became more sensitive to this antibiotic than DOX-sensitive HT-29S cell line.

Antioxidant potential of *R. nigrum* leaf extract and regulation of redox balance in microglial cells: Compounds of plant origin, including those isolated from agricultural waste, may find application not only in the development of approaches to overcome antibiotic resistance, but also in the correction of other metabolic disorders. This approach can be considered as a model of oxidative stress using different test systems. This can be discussed based on data of the study of antioxidant and anti-inflammatory activity of RNE on BV-2 microglial *Wt* and *Acox1*$^{-/-}$ deficient cell line models, which are commonly used to study some neurodegenerative disorders. This extract contains a large amount of phenolic compounds, including flavan-3-ols, flavonols, furanocoumarins, hydroxycinnamates, quercetin and derivatives, which are known for their high biological activity in various test systems.

We showed marked antioxidant potential of RNE in chemical tests. Based on this, it was decided to study its activity in different cellular test systems in order to understand the processes in the mentioned two microglial BV-2 cell lines. ACOX1 is the rate-limiting enzyme of β-oxidation of peroxisomal very long chain fatty acids. Deficiency of this enzyme is a peroxisomal neurogenerative disorder characterized by the accumulation of VLCFA in the cytoplasm of cells, leading to oxidative damage to cells. Lipopolysaccharides (LPS) were used to induce additional oxidative stress in the two cell lines under study. The role of enzymes involved in antioxidant defense mechanisms, SOD, catalase and ACOX1 is very important. So, it is of scientific and practical interest to study the effect of RNE on these enzymes.

In order to evaluate the effect of RNE on the mitochondrial function and cell viability of BV-2 *Wt* and *Acox1*$^{-/-}$ cells, an MTT test was performed. The effect of RNE on cell viability was evaluated with its concentration of 0.0625 - 1 mg/ml for 4, 24 or 48 hours. According to the data obtained, all concentrations of RNE above 0.25 mg/ml were cytotoxic, regardless of cell genotype, and cell viability suppression reached 70% or more when cells were treated with higher concentrations of extract (Fig. 16 (a) and (b)).

As the sub-cytotoxic and working the concentration of RNE 0.125 mg/mL was chosen, which was applied in almost all further experiments.

Effect of RNE on LPS-induced intracellular ROS accumulation: The effect of RNE on LPS-induced (1 μg/mL) intracellular ROS accumulation was studied. For this, H_2DCFDA was applied as a ROS probe.

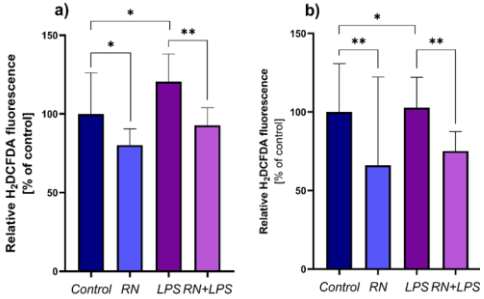

Fig. 16. Effects of RNE on ROS production induced by LPS in the *Wt* (a) and *Acox1*$^{-/-}$ (b) BV-2 microglial cells assessed by the H_2DCFDA (5 μM) dye test. Cells were incubated for 24 h with RN (0.125 mg/mL) in the absence or presence of LPS. All values are presented as means ± SD of two independent experiments performed in triplicate (*p < 0.1; and **p < 0.01). Statistical significance was determined by one-way ANOVA followed by Tukey's test.

In contrast to the *Acox1*$^{-/-}$ cells, LPS treatment induced oxidative stress with the intracellular ROS production in the *Wt* cells (Fig. 16a). The observed LPS-induced

increase of ROS production in BV2-*Wt* cells which was significantly overturned by the co-treatment with RN. LPS treatment did not stimulated the excessive production of ROS in *Acox1*$^{-/-}$ BV-2 cells, but the treatment with RN decreased ROS accumulation both in the absence or presence of LPS (Fig. 16b).

***R. nigrum* extract effects on LPS-induced nitric oxide generation, on the expression of genes encoding different inflammation markers or peroxisomal ABCD transporter protein:** To evaluate the anti-inflammatory effects of RN, we measured the level of NO production and the expression of iNOS gene in the absence or the presence of LPS (Fig. 17, Fig. 18).

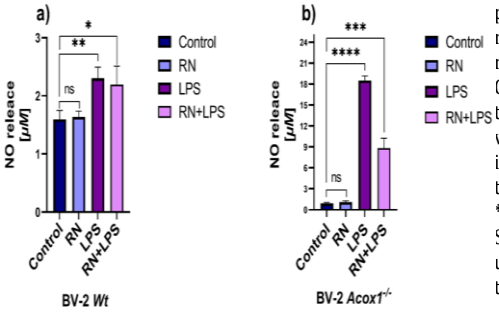

Fig. 17. Effect of RNE on the NO production induced by LPS in the culture media of the *Wt* (a) and *Acox1*$^{-/-}$ (b) BV-2 microglial cells assessed by the Griess test. Cells were incubated for 24h with RN in the absence or the presence of LPS. All values are presented as means ± SD of two independent experiments performed in triplicate (*p < 0.1; **p < 0.01; ***p < 0.001; ****p < 0.0001 and ns, not significant). Statistical significance was determined using one-way ANOVA followed by Tukey's test.

The treatment with RN alone had no any influence on NO production in both BV-2 cell lines (Figure 17 a, b). After the incubation with LPS, the level of NO was significantly increased in comparison to the control cells (Figure 17). In *Wt* cells the treatment with RN reduced the LPS-induced NO level (Fig. 17a). The more expressed LPS-induced NO level reduction was observed in the *Acox 1*$^{-/-}$ BV-2 cell line after the treatment with RN (Fig. 17b).

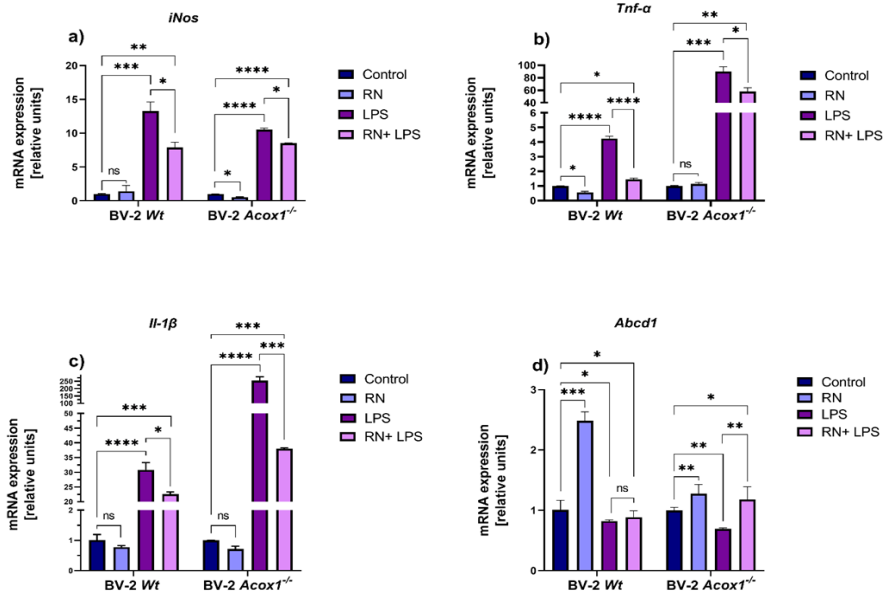

Fig. 18. Effect of *R. nigrum* leaf extract on the gene expression of *iNos* (a), *Tnf-α* (b), *Il-1β* (c) and *Abcd1* (d) with or without the LPS influence. All values are presented as means ± SD of two independent experiments performed in triplicate (*p < 0.1; **p < 0.01; ***p < 0.001; ****p < 0.0001 and ns, not significant). Statistical significance was determined using two-way ANOVA followed by Tukey's test for multiple comparisons.

The expression level of iNOS mRNA was increased by LPS treatment concomitantly to the increase in NO production in both cell lines. In response to LPS treatment, the mRNA expression level of iNOS was significantly increased in both cell lines. The combined treatments with RN+LPS significantly attenuated the LPS-induced iNOS mRNA level increase (Figure 18a).

The expression of other inflammatory marker encoding genes was also change accordingly. The treatment with RN kept the *Tnf-α* and *Il-1β* gene expression levels constant in both cell lines. Expectedly, the treatment with LPS increases the *Tnf-α* and the *Il-1β* gene expression significantly. The co-treatments with RN+LPS limited the increased mRNA levels of both cytokine genes (Figure 18 b, c). But the treatment with the RN increases the *Abcd1* gene expression level; meanwhile LPS treatment decreases the expression level of this *Abcd1 mRNA* encoding peroxisomal protein transporter. The treatment with RN+LPS combination elevated this gene expression level to the level of control (Fig. 18 d).

Effects of *R. nigrum* extract on the activity of peroxisomal enzymes and the expression of these protein-encoding genes: The evaluation of activity of the main antioxidant enzymes in peroxisomes of BV-2 cells (ACOX1—the rate-limiting enzyme of β-oxidation process in cells; catalase—antioxidant enzyme, quenching hydrogen peroxide) under the treatment of RN is of interest in order to fully understand the antioxidant capacity of test-extract.

The catalase activity in *Wt* cells remained almost unchanged during the 24-hour treatment, meanwhile further treatment lead to significant decrease of this parameter. The long-term treatment of Wt cells with RN had no significant effect on catalase activity.

In case of Acox1$^{-/-}$ BV-2 cells, during the first 24 hours of treatment with RN, a strict decrease in catalase activity was observed. Then, the activity of the enzyme almost equaled to its activity in control cells, and the long-term treatment did not have any effect (Fig. 19a).

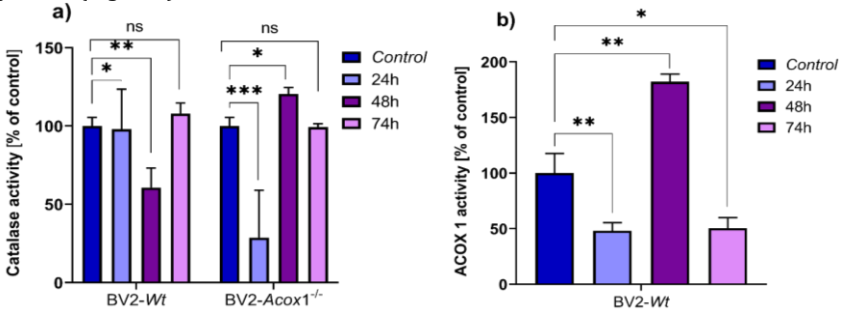

Fig. 19. Effect of RNE on catalase activity in the *Wt* and *Acox1*$^{-/-}$ BV-2 microglial cells (a), and on ACOX1 activity (b) in *Wt* BV-2 microglial cells (*p < 0.1; **p < 0.01; ***p < 0.001; ns, not significant).

The ACOX1 activity in *Wt* BV-2 microglial cells decreased by almost 50% during the 24 hours of treatment with RN. The further treatment resulted in an increase in the activity of this enzyme by almost 70%. In case of the long-

term treatment (72-hour), the activity of ACOX1 decreased more than 3 times compared to the second stage of the treatment (Fig. 19b).

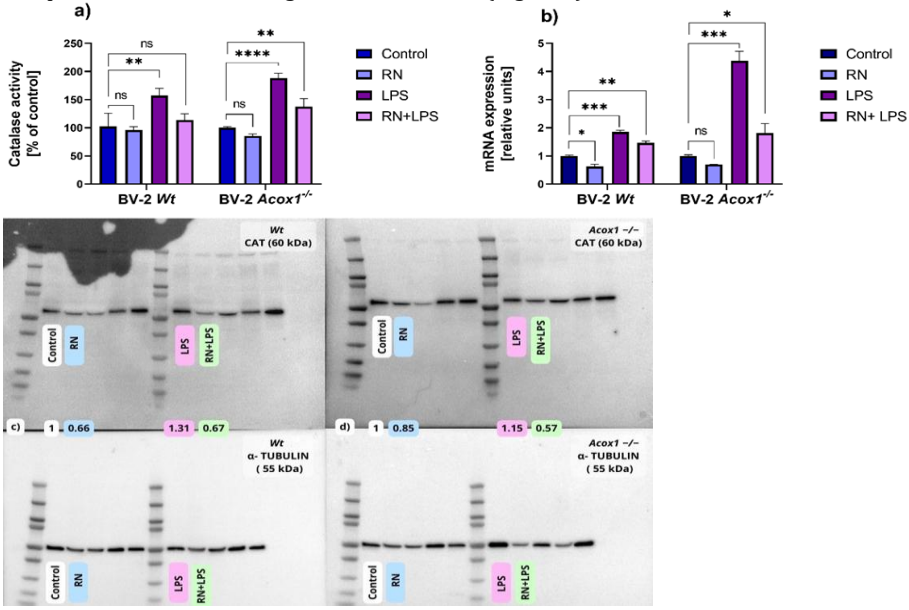

Fig. 20. Effect of *R. nigrum* extract on the level of catalase activity (a) and on the expression of the peroxisomal Cat mRNA (b) in the *Wt* and in *Acox1*$^{-/-}$ BV-2 microglial cells. Panels (c) and (d) show the results of immunoblotting assay of RN effect on the expression of the peroxisomal protein CAT in *Wt* (c) and in *Acox1*$^{-/-}$ (d) BV-2 microglial cells. Cells were incubated for 24 h with RN (0.125 mg/mL) in the absence or presence of LPS (1 μg/mL). Band intensities were analyzed by densitometry and standardized to α-tubulin expression level.

To further analyze the change in catalase activity under the treatment of RN, the expression of its mRNA and protein levels have been investigated. Next the triggering the oxidative stress and ROS formation in both cell lines by LPS and further treatment with RN was carried out to observe the possible mitigating effect of this extract on the LPS-induced oxidative deviations in cell models (Fig. 20).

Our investigations showed that 1 μg/mL LPS significantly trigger catalase activity in both cell lines at up to 60% (Fig. 20a). The LPS treatment also increased the Cat mRNA level in both *Wt* and *Acox1*$^{-/-}$ cells (Fig. 20b). The co-treatments with RN+LPS decreased the LPS-induced catalase activity as well as the CAT gene expression in both cell lines (Fig. 20 a, b). The immunoblotting assay shows that the CAT protein quantity change replicates the changes in catalase activity (Fig. 20 c,d).

Effect of natural polymeric compounds on redox regulation mechanisms in plants: Understanding of the molecular mechanisms allowing formation of response to adverse environmental factors, including the influence of different allelochemicals, such as plant essential oils, is one of the issues that will help to rule these relationships between the species. Maintenance of cellular homeostasis in plants under the influence of various external factors is provided by a number of protective systems. Normally, ROS are generated by metabolic activity of the plants and act as signaling molecules for regulating plant metabolic pathways. To combat the harmful effect of increased ROS accumulation, plants are equipped with effective ROS-scavenging tools: enzymes (SOD, catalase, peroxiredoxines and others) and antioxidant molecules like ascorbic acid, α-

tocopherols, glutathione, proline, phenolic substanses and carotenoids. However, under environmental stresses, as well as the influence of different toxins, generation of ROS increases in various compartments of plant cell such as chloroplasts, peroxisomes and mitochondria. Let's discuss the allelopathic influence of essential oils, extracted from *O. basilicum* var. *purpureum* and *M. arvensis* and their main active components.

According to our previous results the concentration of methyl chavicol in *O. basilicum* var. *purpureum* EO cultivated in high altitude Armenian flora was 57.3%, and concentration of menthol in *M. arvensis* EO reached 69%. PAM-fluorometric data (F_v/F_m) showed that the optimal treatment period of *A. thaliana* leaf disks was 3-hour treatment. EC50 concentration data were used to further treatment of investigated plants in order to reveal some action mechanisms of influence of the selected potential allelopchemicals (Table 2).

In order to reveal the redox state of proteins of interest *in vivo* the redox gel electrophoresis followed by immune analysis by extraction and labeling was applied. Chloroplast peroxiredoxin (2-CysPrx) has decisive role in regulating of photosynthesis. So, it has been investigated the redox status of this enzyme in *A. thaliana Wt* under the treatment of essential oils and their main components.

Table 2. EC_{50} values of *O. basilicum* EO, methyl chavicol, *M. arvensis* EO, and menthol.

EC_{50} values, *mM*							
Ocimum basiicum var. *purpureum* EO							
0-hour treatment		3-hour treatment		6-hour treatment		24-hour treatment	
0.946±0.3	1.024±0.5	0.38±0.09	0.57±0.1	0.35±0.1	0.37±0.08	0.28±0.05	0.25±0.08
Methyl chavicol							
3.5±1.4	2.97±1.3	0.59±0.1	0.59±0.08	0.58±0.08	0.63±0.07	0.55±0.06	0.56±0.05
Mentha arvensis EO							
1.164±0.9	2.38±1.8	0.6±0.04	0.6±0.08	0.6±0.07	0.66±0.09	0.54±0.08	0.4±0.07
Menthol							
1.69±0.3	5.16±3.2	0.59±0.06	0.61±0.12	0.55±0.06	0.58±0.06	0.56±0.07	0.6±0.03

The results showed that the treatment with menthol lead to the oxidation of 2-CysPrx, which leaded to the dimerization of part (around 48%) of the enzyme (Fig. 21). Data, represented in Fig. 22, show strong influence of menthol on PrxIIF. The deviation in the concentration of non-protein thyols (NPT) in plant tissue also occurred (Fig. 23).

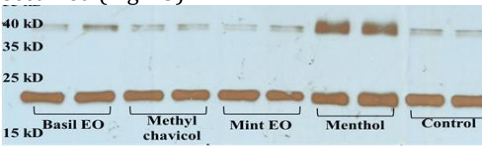

Fig. 21. Immune analysis of peroxiredoxins of *A. thaliana* WT plants chloroplasts, under the treatment of basil EO, methyl chavicol, minth EO and menthol.

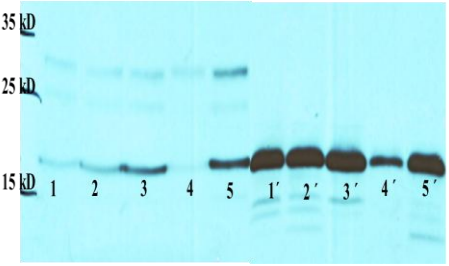

Fig. 22. Immune analysis of mitochondrial peroxiredoxins (PrxIIF) of A. thaliana WT, treated with EC50 concentrations of investigated allelochemicals: 1, 2, 3, 4 – treatment with basil EO, methyl chavicol, mint EO, menthol, respectively; 5 – control (untreated plant). PrxIIF were labeled with maleimid. 1′, 2′, 3′, 4′ – are not labeled, but treated samples; 5′ – control (labeled, but untreated plant).

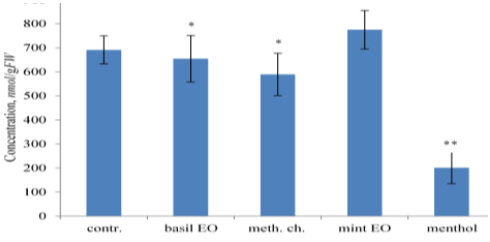

Fig. 23. Content of NPT in A. thaliana WT under the treatment of basil EO, methyl chavicol (meth. ch.), mint EO and menthol (* $p > 0.05$; ** $p < 0.05$).

This data confirming the above-mentioned results sating that menthol can bring to the deviation in plant cellular homeostasis. NIR KLAS 100 data also showed some influence of investigated allelochemicals on photosynthetic apparatus of *A. thaliana Wt* and mutant plants (Table 3).

Table 3. Photosynthetic parameters of the photosynthetic apparatus of *A. thaliana* (Wt) and (Δ2CP) under normal and menthol treated conditions.

Experimental conditions	Fv/Fm*(relative units)	Y(I)(relative units)	Y(II)(relative units)	ETR(I) (μmol e^{-} $m^{-2}s^{-1}$)	ETR(II) (μmol e^{-} $m^{-2}s^{-1}$)
Control, *A. thaliana* (*Wt*)	0.69±0.08	0.62±0.07	0.32±0.01	32.01±2.2	19.27±1.4
Control *A. thaliana* (Δ2CP)	0.39±0.06	0.21±0.09	0.00±0.00	11.12±2.9	0.00±0.00
Menthol treated, *A. thaliana* (*Wt*)	0.22±0.05	0.12±0.02	0.07±0.02	18.85±0.9	11.90±1.1
Menthol treated, *A. thaliana* (Δ2CP)	0.12±0.02	0.09±0.01	0.00±0.00	11.78±2.88	0.00±0.00

*** Fv/Fm - potential quantum yield, Y(I) - effective quantum yield of photosystem I; Y(II) - effective quantum yield of photosystem II; ETR(I) and ETR(II) – electron transfer rate in photosystem I and photosystem II.**

CONCLUDING REMARKS

It is well known that many plant-derived compounds, such as polyphenols, which are major products of plant secondary metabolism, can exhibit quite pronounced biological activity in various organisms. This characteristic underpins the approach in traditional and classical medicine, wherein medicinal plants are considered important, and sometimes essential and irreplaceable, components of various drugs. However, it remains not fully understood why the extracts of these plants and their isolated components are so active in many tested biological systems. One possible reason could be their strong antioxidant activity due to the high content of phenolic compounds. Our research, along with the analysis of scientific literature published in recent years, has shown that these natural compounds, with their pronounced reducing potential, can act as either antioxidants and protective agents or as prooxidants in different test systems.

As previously mentioned, flavonoids and other phenolic compounds demonstrate high reducing potential. They may play a crucial role in anticancer therapy and exhibit antibiotic-modulating properties by disrupting, for example, the redox balance in cancer cells or acting through other mechanisms. According to our research, more than 22 main components identified in the FC and RN extracts show antioxidant properties. The number of main antioxidant components found in the VV

extract was 19. LC-Q-Orbitrap HRMS analysis also confirmed these results. The main components of the extracts from all three plants primarily consist of flavonoids and phenolic acids and their derivatives. These main compounds of the extracts show high biological activity (antimicrobial, antioxidant, anticancer, anti-inflammatory, and antibiotic-modulating/resistance-altering properties), likely by acting as modulators of redox processes in cells. These key active components may also contribute to the manifestation of antibiotic-resistance-modulating abilities.

Kanamycin, being an aminoglycoside antibiotic, works by irreversibly binding to the bacterial 30S ribosomal subunit, while ampicillin, a beta-lactam antibiotic, disrupts the cell wall formation process in bacteria. In the case of bacterial strains, RN and FC extracts modulate the efficacy of the applied antibiotics, reducing their MIC values by two to four times. Interestingly, the effectiveness of the resistance-modulating ability of the tested extracts is not the same in bacteria and cancer cell tests. In bacterial cells, RN and FC extracts showed significant antibiotic-modulating properties, whereas in cancer cell models, the extracts exhibit varying efficacy. Therefore, it can be inferred that different mechanisms might be at play.

Further studies have elucidated the potential effects of plant extracts on the H+/K+ fluxes across bacterial membranes, the F0F1-ATPase and antioxidant enzyme activity, revealing possible mechanisms of their antibiotic-modulating effects (Fig. 24).

It is known that the efficacy of bactericidal drugs can increase with the intensification of the bacterial respiration process. Respiration in bacterial cells serves as an important source of energy (ATP) and reducing equivalents. The activation of the proton pump can be associated with an increase in ATPase activity, which might also be linked to the activation of ATP-binding transporters (ABC proteins) involved in the transport of various molecules, including antibiotics. Moreover, as described earlier, ATP hydrolysis mediated by ATPase leads to the expulsion of protons from the cell, creating certain pH and potential gradients around the membrane, activating secondary transporters.

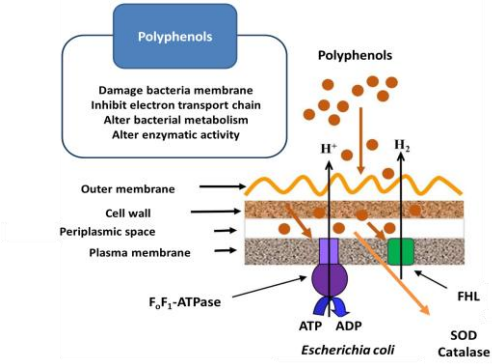

Fig. 24. Possible mechanisms of action of plant phenolic compounds in *E. coli*

Summarizing all the obtained data, it can be concluded that plant extracts affect membrane-associated properties of bacteria, which in turn contributes to the modulation of the respective antibiotic's effect, thereby overcoming bacterial resistance to antibiotics. Furthermore, the biological activity of the phenolic fraction of the studied extracts may vary depending on the source of these compounds. A similar effect has been observed during the testing of essential oils from different plants.

The antibiotic-modulating activity of the extracts has also been studied in cancer cell models. Cytotoxicity tests have shown that the aqueous-alcoholic extracts of

VV and RN exhibit strong cytotoxic effects on the human colon cancer carcinoma HT29 cell lines. The inhibition of HT29 cell growth by the extracts of FC, RN, and VV may be related to the presence of numerous anticancer components in these extracts. According to the literature, the aforementioned 19 main components identified in the FC extract exhibit anticancer or growth-inhibiting properties in cancer cells. The approximate number of main anticancer compounds found in RN and VV extracts was 14 and 13, respectively. Interestingly, FC, which contains the largest number of main components with potential anticancer properties, showed the highest growth-inhibiting activity on the tested cell lines, followed by RN and VV.

Despite the growth-inhibiting activity on DOX-sensitive HT29S cells, an interesting phenomenon was observed when these cells were treated in combination with DOX and plant extracts. The combined effect of DOX and plant extracts on DOX-sensitive HT29S cells varied depending on the treatment parameters. This can be explained by the fact that oxidative stress is the main factor in the manifestation of DOX cytotoxicity on cancer cells.

On the other hand, since RN, FC, and VV extracts contain many antioxidant components and can reduce ROS formation in cells, during the combined treatment of HT29S cancer cells with plant extracts, these extracts, depending on the applied concentration, can act as antioxidants and reduce the cytotoxicity of DOX.

Further investigation into the antibiotic-resistance modulating properties of the plant extracts on the DOX-resistant HT-29R cell line revealed that all three extracts overcame antibiotic resistance to doxorubicin in HT29R cells and made them sensitive to this compound in a dose-dependent manner. Moreover, there was a certain correlation between the ability of the extracts to modulate antibiotic resistance. The resistance-modulating properties were primarily observed with RN and VV extracts, which also demonstrated high antioxidant potential in chemical tests. Phytochemical characterization of the RN, FC, and VV extracts indicated that the phenolic compounds mainly consisted of hydroxycoumarins, terpenes, and furanocoumarins.

Additionally, the study extended to explore the relationship of plant extracts with antibiotics of known action mechanisms, ampicillin and kanamycin, in the test systems. Interestingly, RN and FC extracts notably enhanced the effects of these two antibiotics even at low concentrations, indirectly suggesting that the extracts impact the membrane-associated properties of bacteria.

Another type of research also supports the importance of antioxidant activity in plant-derived polymeric compounds in regulating metabolic processes in various cell lines. Our previous studies demonstrated that the RN extract from leaves cultivated in the highlands of Armenia had a high content of total phenols and flavonoids, and LC-Q-Orbitrap HRMS analyses revealed over 30 phenolic compounds, including flavan-3-ols, dimeric glycosides, hydroxycinnamates, tannins, and flavonols. All identified components are known for their biological activity in various test systems. Furthermore, these substances can interact with each other and other metabolites through mechanisms of synergy or antagonism.

The most studied characteristic of the RN extract is its antioxidant activity. In our studies, it exhibited high antioxidant potential in chemical tests. Moreover, RN treatment significantly altered SOD activity in BV-2 *Wt* and *Acox1*$^{-/-}$ microglial cells.

As discussed earlier, in response to stress factors such as the accumulation of high concentrations of ROS, microglial cells produce pro-inflammatory cytokines (TNF-α, IL-1β, IL-18). This can induce the activation of iNOS and the secretion of NO. NO, in turn, is a free radical toxic to all cells and, at high concentrations, non-specifically

reacts with proteins, lipids, and nucleic acids, accelerating inflammatory and neurodegenerative processes. Recently, plant-derived metabolites have garnered interest as potential sources for developing new drugs to treat various neurological disorders. Our research revealed that RN treatment significantly altered the activity of all investigated enzymes involved in the antioxidant defense system in BV-2 *Wt* and *Acox1*$^{-/-}$ microglial cells at sub-inhibitory concentrations. It is known that peroxisomal proteins, such as catalase, SOD, and ACOX1, play a crucial role in developing resistance to oxidative stress in cells, particularly when there is a decrease in glutathione peroxidase activity or an increase in H_2O_2 levels. A slight decrease in catalase and ACOX1 activity during the initial phase of treatment in *Wt* cells may indicate that RN acts as an antioxidant. Additional evidence shows a reduction in intracellular ROS concentration under treatment with both RN alone and in combination with LPS (LPS induces oxidative stress in cells).

In *Acox1*$^{-/-}$ cells, the scenario might be different, as this cell type can be viewed as an oxidative stress model a priori due to ACOX1 deficiency and thus impaired β-oxidation of fatty acids. Nevertheless, in both cell lines, there is a decrease in NO production and ROS formation as indicated by the H_2DCFDA assay, suggesting a strong antioxidant activity of RN. The decrease in iNOS gene expression can also be seen as further evidence of RN's antioxidant activity.

In RN+LPS treated cells, the regulation of *Tnf-α* and *IL-1β* gene expression, linked to iNOS gene expression and NO production, supports RN's oxidative stress-modulating potential and anti-inflammatory effects. The activation of the *Abcd1* gene expression in both RN and RN+LPS-treated cells indicates an enhancement of VLCFA transport and β-oxidation in peroxisomes of both cell lines, reducing oxidative stress levels. Furthermore, the expression of CAT gene, aligned with changes in catalase activity, confirmed by both RT-qPCR and immunoblot analysis.

Overall, RN affects multiple metabolic pathways in microglial cells, as schematically depicted below (Fig. 26).

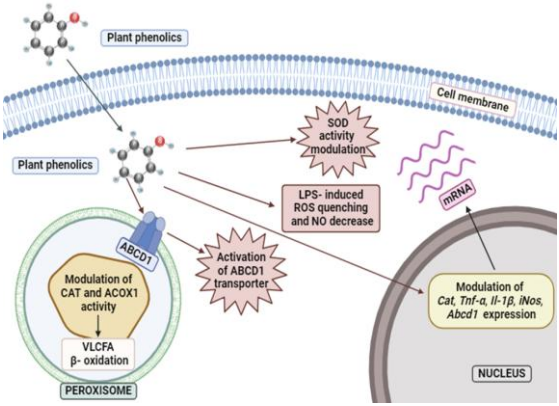

Fig 25. The schematic representation of influence of the phenolic components of RN extract on microglial BV-2 Wt cells.

All the aforementioned data, including the fact that RN extract exhibits low genotoxicity, suggest that this plant could serve as an invaluable source of biologically active metabolites. These metabolites could be especially significant in the development of new approaches for the prevention and treatment of various disorders associated with oxidative stress, including neurodegenerative diseases.

Additionally, the interactions between plants through polyphenolic compounds are intriguing. This was discussed with a specific example illustrating how plants use

different protective mechanisms to combat environmental stresses. These mechanisms help maintain cellular homeostasis or disrupt the redox balance of other plant species, potentially leading to significant impairment in the growth, development, and reproduction of those plants. Thus, environmental stress factors, allelochemicals, and the cellular responses of plants are engaged in complex interactions.

CONCLUSIONS

The following conclusions were made based on experimentall results:

1. The main components of the extracts isolated from the aerial organs of plants harvested from the territory of the Republic of Armenia belong to the group of flavonoids and their derivatives and phenolic acids and their derivatives.
2. Phenolic compounds isolated from various plant species demonstrate pronounced antioxidant capacity in all chemical tests, and in the cells of living organisms, depending on their composition, they can modify the redox processes.
3. A significant part of the studied plant compounds suppresses the viability of various cancer cell lines and Gram-positive and Gram-negative bacteria and can demonstrate antibiotic-modulating ability against antibiotic-sensitive and antibiotic-resistant bacteria and cancer cells. Moreover, in the antibiotic-modulation process, in most cases, there is a concentration-effect dependence.
4. Plant phenolic compounds can increase sensitivity to kanamycin by increasing bacterial membrane permeability and suppressing ATPase activity.
5. Plant phenolics exhibit cytotoxic effects in tested cancer cells at low concentrations, without suppressing the viability of normal HREC cell lines, thus demonstrating a selective effect. A significant number of the studied plant compounds inhibit the viability of various bacteria and cancer cell lines and can exhibit antibiotic-modulating capabilities against both antibiotic-sensitive and resistant strains of bacteria and cancer cell lines. Additionally, in most cases, there is a concentration-effect dependency in the process of antibiotic modulation.
6. The biological activity of phenolic components isolated from plants of different families can be manifested by different mechanisms, both when applied to bacterial and human cancer *in vitro* cell models.
7. The majority of plant compounds with antibacterial or antibiotic-modulating properties affect the H^+ flux rate, H^+/K^+ exchange and ATPase activity in the membranes of the studied *E. coli* strains.
8. Compounds isolated from plant waste regulate the activity of antioxidant defense system enzymes (SOD, catalase, Acyl-CoA oxidase 1), various inflammatory markers and genes responsible for their synthesis (*iNOS*, *Tnf-α*, *Il-1β*) and the ABCD1 protein transporter *Abcd1* in BV-2 microglial *Wt* and Acyl-CoA oxidase 1 enzyme deficient ($Acox1^{-/-}$) cell lines.
9. Polyphenolic compounds of plant essential oils can act as allelopathic agents, disrupting metabolic pathways and physiological functions in other plants.
10. Plant phenolic compounds affect the redox processes of cells of organisms belonging to all taxonomic groups, ranging from simple metabolite recovery to complex metabolic processes.

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ՍԱՀԱԿՅԱՆ ՆԱԻՐԱ ԺՈՐԱՅԻ

ԲՈՒՍԱԿԱՆ ԾԱԳՄԱՆ ՄԻԱՑՈՒԹՅՈՒՆՆԵՐԻ ԴԵՐԸ ԲՋՋԱՅԻՆ ՕՔՍԻԴԱՎԵՐԱԿԱՆԳՆՈՂԱԿԱՆ ԿԱՐԳԱՎՈՐՄԱՆ ՄԵԽԱՆԻԶՄՆԵՐՈՒՄ

Ամփոփագիր

Հանգույցային բառեր՝ բուսական ֆենոլներ, հակաօքսիդանտային ակտիվություն, հակաբիոտիկ-մոդուլացնող ակտիվություն, ԱԵՖ-ազ, պրոտոնի և կալիումի հոսք, *Escherichia coli*, միկրոգլիա, հակաբորբորքային մարկերներ, ալելոպաթիա, *Arabidopsis thaliana*

Պոլիֆենոլները՝ բույսերի երկրորդային նյութափոխանակության հիմնական արգասիքները, հայտնի են բարձր կենսաբանական ակտիվությամբ, ինչի շնորհիվ այս պոլիմերային միացությունները հաճախ կիրառվում են ավանդական և դասական բժշկությունում: Այս միացությունները բուսական լուծամզվածքներում կարող են հանդես գալ և՛ որպես հակաօքսիդանտներ, և՛ որպես պրոօքսիդանտներ՝ կախված կիրառման պայմաններից: Տարբեր կարգաբանական խմբերի պատկանող բույսերից անջատված լուծամզվածքների նյութափոխանակային հետազոտությունները ցույց են տալիս, որ պոլիֆենոլները մեծապես նպաստում են բուսական լուծամզվածքներում կենսաբանական ակտիվության դրսևորմանը: Հայտնաբերվել են բարձր հակաօքսիդանտային պոտենցիալ ունեցող բազմաթիվ ֆլավոնոիդային բնույթի միացություններ և ֆենոլային թթուներ: Այսպիսի միացությունները հատկապես շատ են *V. vinifera*, *R. nigrum* և *F. carica* նմուշներում, որոնք հիմնականում պարունակում են ֆլավոնոիդներ, ֆենոլային թթուներ և դրանց ածանցյալներ: Բույսերի լուծամզվածքների հիմնական բաղադրիչները դրսևորում են արտահայտված կենսաբանական ակտիվություն՝ հակամանրէային, հակաօքսիդանտային, հակաքաղցկեղային, հակաբորբոքային և հակաբիոտիկ կայունությունը մոդուլացնող հատկություններ:

Ուսումնասիրությունը ցույց տվեց, որ մասնավորապես, *V. myrtillus*, *R. nigrum* և *F. carica* բույսերի լուծամզվածքները դրսևորել են հակաբիոտիկ-մոդուլացնող հատկություններ՝ զգալիորեն նվազեցնելով կիրառված հակաբիոտիկների նվազագույն արգելակող կոնցենտրացիաների (ՆԱԿ) արժեքները համապատասխան բակտերիաների նկատմամբ: Կանամիցինը և ամպիցիլինը, որոնք հանդիսանում են հակաբիոտիկների տարբեր խմբերի ներկայացուցիչներ, տարբեր մեխանիզմներով են ազդում բակտերիաների բջիջների վրա: Դրանց նկատմամբ կայունությունը կիրառված լուծամզվածքների ազդեցությամբ հաղթահարվել է նշված հակաբիոտիկների նկատմամբ կայուն բակտերիաների շտամերում: Այս տիպի ազդեցությունը կարող է պայմանավորված լինել բակտերիայի բջիջների թաղանթների վրա այս միացությունների ազդեցությամբ, ինչպես նաև բջջային օքսիդավերականգնողական հավասարակշռության փոփոխմամբ:

Ուսումնասիրությունները պարզեցին նաև, որ որոշ բույսերի լուծամզվածքները, ինչպիսիք են *V. vinifera*-ն, *R. nigrum*-ը և *F. carica*-ն ցուցաբերել են հակաքաղցկեղային ազդեցություն HT29 մարդու հաստ աղիքի կարցինոմայի բջիջների գծերի վրա՝ անկախ այն հանգամանքից թե դեղամիջոցների նկատմամբ զգայուն են, թե՝ կայուն: Բույսերի լուծամզվածքների և դոքսորուբիցինի (DOX) համակցված կիրառումը ցույց է տվել, որ DOX-ի նկատմամբ կայուն՝ HT29R, բջիջներում լուծամզվածքները կարող են մոդուլացնել կայունությունը և բարձրացնել DOX-ի ակտիվությունը՝ կախված լուծամզվածքների կոնցենտրացիայից: Այսինքն, *R. nigrum* և *V. vinifera* լուծամզվածքների բարձր հակաօքսիդանտային պոտենցիալը նպաստում է DOX-ի նկատմամբ կայուն բջիջների DOX-զգայունության բարձրացմանը:

Վերջին տարիներին բուսական պոլիմերային միացությունների հակաօքսիդանտային ակտիվության ուսումնասիրությունները նոր լույս են սփռում բջջային նյութափոխանակային գործընթացների կարգավորման վրա: Մեր հետազոտության շրջանակներում մենք ուսումնասիրել ենք *R. nigrum* տերևների լուծամզվածքի (RN) ազդեցությունը:

Թեստերը ցույց են տվել RN-ի բարձր հակաօքսիդիչ պոտենցիալը: BV-2 *Wt* և *Acox1*$^{-/-}$ միկրոգլիալ բջիջներում RN-ով մշակման ընթացքում բացահայտվել են օքսիդային սթրեսի և բորբոքային գործընթացների կարգավորման նոր ուղիներ: Սթրեսային գործոնների ազդեցությամբ միկրոգլիային բջիջները արտադրում են նախաբորբոքային ցիտոկիններ՝ TNF-α, IL-1β և IL-18, ինչը ակտիվացնում է ինդուկցված ազոտական օքսիդ սինթազի (iNOS) և ազոտի օքսիդի (NO) արտադրությունը: Մեր հետազոտությունները պարզել են, որ RN-ով մշակումը փոխում է BV-2 *Wt* և *Acox1*$^{-/-}$ բջիջների հակաօքսիդանտային պաշտպանության համակարգի ֆերմենտների ակտիվությունը (կատալազի, սուպերօքսիդ դիսմուտազի (ՍՕԴ), և ACOX1-ի): BV-2 *Wt* բջիջներում, RN-ի ազդեցությամբ կատալազի և ACOX1-ի ակտիվության որոշակի նվազումը մշակման նախնական շրջանում վկայում է RN-ի հակաօքսիդիչ դերի մասին: Լրացուցիչ հետազոտությունները ցույց են տվել, որ RN-ով մշակումը նվազեցնում է բջիջներում ԹԱՁ-ի կոնցենտրացիան և iNOS-ի ու NO-ի արտազատումը: *Acox1*$^{-/-}$ բջիջներում RN-ի ազդեցությունը տարբերվում է: RN-ի ազդեցությամբ կատալազի ակտիվությունը ավելանում է միայն 48-ժամյա մշակման արդյունքում, մինչդեռ մեր նախորդ հետազոտությունները ցույց են տվել, որ ՍՕԴ-ի ակտիվությունը բարձրանում է 24-ժամյա մշակման ընթացքում:

Մեր ուսումնասիրություններում RN-ի ազդեցությամբ BV-2 *Wt* և *Acox1*$^{-/-}$ բջիջներում նկատվել է NO-ի արտազատման և ԹԱՁ-ի ձևավորման նվազում: iNOS-ի գենի էքսպրեսիայի նվազումը RN-ի հակաօքսիդանտային ակտիվության մեկ այլ ապացույց է: RN+ԼՊՍ մշակման ենթարկված բջիջներում *Tnf-α* և *IL-1β* գեների էքսպրեսիայի բարձրացումը, որը փոխկապակցված է iNOS-ի գենի էքսպրեսիայի և NO-ի արտազատման հետ, վկայում է RN-ի կարգավորող ներուժի և հակաբորբոքային ազդեցության մասին: *Abcd1* գենի էքսպրեսիայի ակտիվացումը RN և RN+ԼՊՍ-ով մշակված բջիջներում վկայում է պերօքսիսոմներում ճարպաթթուների փոխադրման և β-օքսիդացման ակտիվացման մասին, ինչը բերում է օքսիդային սթրեսի մակարդակի նվազման: *R. nigrum*-ի լուծամզվածքի ազդեցությամբ BV-2 *Wt* միկրոգլիային բջիջներում օքսիդավերականգնողական հավասարակշռության կարգավորման և հակաբորբոքային ակտիվության սխեման ցույց է տալիս, թե ինչպես կարող է այս բույսը ծառայել որպես կենսաբանորեն ակտիվ մետաբոլիտների աղբյուր՝ օքսիդային սթրեսի և

նեյրոդեգեներատիվ խանգարումների կանխարգելման ու բուժման նոր ուղիների մշակման համար:

Բացի այդ, պոլիֆենոլային միացությունները կարևոր դեր ունեն բույս-բույս փոխհարաբերություններում՝ այլ բույսերում օքսիդավերականգնողական հավասարակշռության փոփոխման ունակության շնորհիվ:

СААКЯН НАИРА ЖОРАЕВНА

РОЛЬ СОЕДИНЕНИЙ РАСТИТЕЛЬНОГО ПРОИСХОЖДЕНИЯ В МЕХАНИЗМАХ КЛЕТОЧНОЙ ОКИСЛИТЕЛЬНО-ВОССТАНОВИТЕЛЬНОЙ РЕГУЛЯЦИИ

РЕЗЮМЕ

Ключевые слова: растительные фенолы, антиоксидантная активность, антибиотико-модулирующая активность, АТФ-аза, поток протонов и калия, *Escherichia coli*, микроглия, противовоспалительные маркеры, аллелопатия, *Arabidopsis thaliana*.

Полифенолы, являясь основными продуктами вторичного метаболизма растений, известны своей высокой биологической активностью, что делает их часто используемыми как в традиционной, так и в классической медицине. В растительных экстрактах эти соединения могут действовать как антиоксиданты или прооксиданты в зависимости от условий их применения. Метаболомические исследования экстрактов растений различных таксономических групп показали, что полифенолы значительно способствуют проявлению биологической активности растительных экстрактов. В составе исследуемых экстрактов были обнаружены многочисленные флавоноиды и фенольные кислоты с высоким антиоксидантным потенциалом. Такие соединения особенно в большом количестве были найдены в экстрактах образцов *V. vinifera*, *R. nigrum* и *F. carica*.

Экстракты этих растений содержат флавоноиды, фенольные кислоты и их производные, которые демонстрируют значительную биологическую активность: антимикробные, антиоксидантные, противоопухолевые, противовоспалительные и антибиотико-модифицирующие свойства.

Исследование модулирующих свойств растительных экстрактов на действие антибиотиков показало, что, в частности, экстракты *V. myrtillus*, *R. nigrum* и *F. carica* обладают антибиотико-модулирующими свойствами, значительно снижая минимальные ингибирующие концентрации (МИК) применяемых антибиотиков по отношению к соответствующим бактериям. Канамицин и ампициллин, представители различных групп антибиотиков, воздействуют на бактериальные клетки разными механизмами, и устойчивость к ним преодолевалась под воздействием исследуемых экстрактов у штаммов бактерий, устойчивых к этим антибиотикам. Этот вид активности может быть обусловлен воздействием соединений на мембраносвязанные свойства бактериальных клеток, а также модуляцией клеточного окислительно-восстановительного баланса.

Изучение биологической активности растительных экстрактов показало, что экстракты таких растений, как *V. vinifera*, *R. nigrum* и *F. carica*, обладают противоопухолевым эффектом в отношении клеточных линий карциномы толстой кишки человека HT29, как чувствительных, так и устойчивых к противоопухолевым препаратам.

Комбинированное использование растительных экстрактов и противоопухолевого препарата доксорубицина (DOX) показало, что в клетках HT29R, устойчивых к DOX, экстракты могут модулировать устойчивость и увеличивать активность DOX в зависимости от концентрации экстрактов. Это означает, что высокий антиоксидантный потенциал экстрактов *R. nigrum* и V. *vinifera* способствует увеличению чувствительности устойчивых к DOX клеток.

В последние годы изучение антиоксидантной активности растительных фенолов проливает новый свет на регуляцию метаболических процессов в клетках. Исследования показали высокий антиоксидантный потенциал RN. Наиболее интересные результаты были получены при обработке микроглиальных клеток BV-2 *Wt* и *Acox1*$^{-/-}$. Исследования на этих клетках выявили новые пути регуляции окислительного стресса и воспалительных процессов. Под воздействием стрессовых факторов микроглиальные клетки вырабатывают прововоспалительные цитокины: TNF-α, IL-1β и IL-18, что активирует синтазу оксида азота (iNOS) и продукцию оксида азота (NO). Наши исследования показали, что обработка RN в субингибирующих концентрациях изменяет активность ферментов антиоксидантной защиты в клетках BV-2 *Wt* и *Acox1*$^{-/-}$ в частности, пероксисомальных белков: каталазы, супероксиддисмутазы (СОД) и ACOX1. В клетках BV-2 *Wt* снижение активности каталазы и ACOX1 под воздействием RN на начальной стадии обработки свидетельствует об антиоксидантной роли RN. Дополнительные исследования показали, что обработка RN снижает внутриклеточную концентрацию ROS, а также секрецию iNOS и NO. В клетках *Acox1*$^{-/-}$ воздействие RN несколько отличается: активность каталазы увеличивается только после 48-часовой обработки, в то время как наши предыдущие исследования показали, что активность СОД повышается уже через 24 часа обработки.

В наших исследованиях под воздействием RN в клетках BV-2 *Wt* и *Acox1*$^{-/-}$ наблюдалось снижение секреции NO и образования ROS в тесте H_2DCFDA. Снижение экспрессии гена iNOS является еще одним доказательством антиоксидантной активности RN. В клетках, обработанных RN+ЛПС, наблюдается увеличение экспрессии генов *Tnf-α* и *IL-1β*, что связано с экспрессией гена iNOS и секрецией NO, указывает на потенциал RN в регуляции окислительного стресса и противовоспалительный эффект. Активизация экспрессии гена *Abcd1* в клетках, обработанных RN и RN+ЛПС, свидетельствует об усилении транспорта и β-окисления жирных кислот в пероксисомах, что приводит к снижению уровня окислительного стресса.

Схема регуляции окислительного стресса и противовоспалительной активности под воздействием экстракта *R. nigrum* в микроглиальных клетках BV-2 *Wt* показывает, как это растение может служить источником биологически активных метаболитов для разработки новых подходов к профилактике и лечению окислительного стресса и нейродегенеративных нарушений.

Кроме того, полифенольные соединения играют важную роль во взаимодействиях между растениями. Для борьбы с воздействием стрессов окружающей среды растения используют различные защитные механизмы, такие как поддержание клеточного гомеостаза или нарушение окислительно-восстановительного равновесия других видов растений.