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MINISTRY OF EDUCATION, SCIENCE, CULTURE AND SPORTS OF RA

YEREVAN STATE UNIVERSITY

MARGARYAN LUSINE YURI

BIOCHEMICAL MECHANISMS OF THE EFFECTS OF ESSENTIAL OILS
OF SOME MEDICINAL PLANTS MENTIONED IN ARMENIAN MEDIEVAL
MANUSCRIPTS

SYNOPSIS

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

YEREVAN 2026

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝

Կ.գ.դ. պրոֆ. Կարեն Արմենի Թռչունյան

Պաշտոնական ընդդիմախոսներ՝

Կ.գ.դ. Աստղիկ Զավենի Փեփոյան

Կ.գ.թ., Աննա Հովհաննեսի Թադևոսյան

Առաջատար կազմակերպություն՝

ՀՀ ԳԱԱ Գ.Ս. Դավթյանի անվան

հիդրոպոնիկայի պրոբլեմների ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2026թ. օգոստոսի 31-ին ժամը 15⁰⁰-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 2026թ. հուլիսի 31-ին:

051 մասնագիտական խորհրդի

Գիտական քարտուղար, Կ.գ.դ., դոցենտ



Մ.Ա. Փարսադանյան

The theme of dissertation has been approved at Yerevan State University

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

The defense of the dissertation will be held on 31th August, 2026, at 15⁰⁰, 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 of the dissertation has been sent on 31st of July, 2026.

Scientific Secretary of 051 Specialized Council,

D.Sc., Associate Professor



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INTRODUCTION

Topic's significance. The global spread of antibiotic resistance and the critical shortage of effective antimicrobial agents represent one of the most pressing challenges of contemporary medicine. The search for biologically active compounds of natural origin capable of complementing or replacing synthetic antimicrobials is therefore a scientific priority of the highest order. Essential oils (EOs) derived from medicinal plants have attracted growing attention as potential alternatives to synthetic antimicrobial agents, primarily because pathogenic microorganisms develop resistance to them far more slowly, given the complex multi-component nature of essential oils.

Medicinal plants constitute an important component of natural flora and represent valuable biological resources. The use of plant-derived essential oils is expanding year by year in the food, perfumery, cosmetics, and pharmaceutical industries. According to the World Health Organization, more than 80% of the world's population relies on plant-based medicines to manage various health conditions.

Even in the Middle Ages, medicinal plants were widely employed as analgesics, sedatives, hemostatic agents, and antiseptics, and this accumulated experience has been preserved and developed to the present day. Approximately 250,000–500,000 plant species are currently known worldwide, yet only a comparatively small fraction has been studied for its antimicrobial and other biological properties. In many countries, including Armenia, medicinal plants continue to hold an important place in traditional medicine.

A vivid example of Armenia's rich tradition of applying the healing properties of plants is the Matenadaran (Mesrop Mashtots Institute of Ancient Manuscripts), where distillates obtained from medicinal plants of the Armenian Highlands have been used for years to disinfect manuscripts and old printed books. This method is ecologically safe, causes no harm to either humans or cultural artefacts, and constitutes a sustainable alternative to synthetic chemical disinfectants. The approximately 30,000 manuscripts preserved in the Matenadaran encompass diverse fields of Armenian and world scientific and cultural heritage — history, mathematics, medicine, astronomy, and more.

Despite Armenia's rich botanical resources and centuries-old traditions of their application, comparatively few studies have been conducted in recent years on the properties and mechanisms of action of Armenian flora representatives and the bioactive compounds isolated from them. This is of particular importance given the continuously growing scientific and practical interest worldwide in ethnopharmacological preparations and bioactive substances of natural origin.

Antibiotics generally exert their bactericidal or bacteriostatic effects by targeting key cellular structures and processes, including the bacterial cell membrane, ion transport mechanisms, peptidoglycan biosynthesis enzymes, and the molecular machinery involved in DNA, RNA, and protein synthesis (Zhivich A., 2017). A critical determinant of antibiotic resistance, particularly in Gram-negative bacteria such as *Escherichia coli*, is the presence of highly efficient and structurally diverse efflux pump systems. While both Gram-positive and Gram-negative bacteria possess efflux mechanisms, the latter often exhibit a broader repertoire and greater efficacy in actively exporting a wide range of antibiotics and toxic compounds, thereby contributing to multidrug resistance phenotypes (Li, X.-Z. & Nikaido, 2009).

Plant-derived antimicrobial agents are emerging as promising alternatives or adjuncts in the fight against antibiotic resistance, owing to their structural diversity and multifaceted

mechanisms of action. These compounds frequently exhibit synergistic interactions with conventional antibiotics, enhancing their efficacy while disrupting bacterial viability through distinct biochemical pathways, including inhibition of ATPase activity (Qassadi, 2025). Furthermore, EOs derived from medicinal plants have been shown to modulate ion fluxes across bacterial membranes and interfere with key resistance mechanisms, thereby attenuating the adaptive capacity of pathogenic microorganisms (Nazzaro, 2013).

While several mechanisms underlying the antimicrobial properties of plant-derived compounds have been characterized, many remain incompletely understood. In this study, we investigate the chemical composition of *T. argyrophyllum* EO and its antibacterial potential against a range of microorganisms, including yeasts, Gram-positive, and Gram-negative bacteria, with a particular emphasis on antibiotic-resistant strains. Special attention is given to the peculiarities of the chemical composition of EO extracted from *T. argyrophyllum* harvested from Armenian highlands and its antibacterial activity against kanamycin-resistant *E. coli* while providing mechanistic insight into EO effects on bacterial membrane-associated properties.

The aim of this thesis was to investigate the antimicrobial and antioxidant activity of EO-s from *Achillea filipendulina* Lam. (Asteraceae), *Pelargonium graveolens* L'Hér. (Geraniaceae), *Prangos ferulacea* (L.) Lindl. (Apiaceae) and *Tanacetum argyrophyllum* (C. Koch) Tzvel. (Asteraceae) growing in Armenia, as well as to elucidate the biochemical mechanisms of their action against both antibiotic-sensitive and antibiotic-resistant *Escherichia coli* strains.

Research goals and tasks. To achieve this aim, the following specific objectives were formulated:

- To assess the antimicrobial activity of the EOs from all four species against a panel of microorganisms using screening and quantitative microbiological methods.
- To evaluate the effects of the EOs on the viability and growth of antibiotic-sensitive and antibiotic-resistant (kanamycin- and ampicillin-resistant) *Escherichia coli* strains.
- To characterize the chemical composition of the EOs and identify their major and minor components.
- To select the EOs with the most pronounced antimicrobial activity and assess their spectrum of action against Gram-positive, Gram-negative bacteria, and yeasts.
- To investigate the antibiotic-modulating properties of *T. argyrophyllum* EO by determining changes in the MIC of relevant antibiotics.
- To elucidate the effect of *T. argyrophyllum* EO on bacterial energy homeostasis by studying proton fluxes, ATPase activity, and cellular energetic processes.
- To evaluate the EOs effect on bacterial redox homeostasis by examining changes in ORP, pH, lipid peroxidation, and oxidative stress markers.
- To study the responses of enzymatic antioxidant defense systems (SOD, catalase) under EO exposure, and to propose possible cellular and molecular mechanisms of action.

Scientific novelty and practical value of the study

For the first time, a comprehensive investigation of the antimicrobial activity of EOs isolated from *A. filipendulina* Lam., *P. graveolens* L'Hér., *P. ferulacea* (L.) Lindl. and *T. argyrophyllum* (K. Koch) Tzvelev — plants growing wild and under cultivation in the Republic of Armenia — has been carried out.

For the first time, the biochemical composition of the EO of *T. argyrophyllum* grown in Armenia was fully characterized. GC-MS analysis identified 16 chemical constituents accounting for approximately 98.5% of the total oil composition, establishing an eucalyptol–camphor chemotype with 1,8-cineole (35.0%), camphor (24.0%), and camphene (17.0%) as the dominant components. This chemotype had not previously been reported for *T. argyrophyllum* populations growing in Armenian conditions. The EO yield of *P. ferulacea* (~4%) was found to exceed all previously reported values for this species in the scientific literature.

For the first time, the antibiotic-modulating activity of *T. argyrophyllum* EO was evaluated in combination with conventional antibiotics. The EO demonstrated significant synergism with kanamycin against the kanamycin-resistant *E. coli* pARG-25 strain, reducing the minimum inhibitory concentration of kanamycin four-fold (FIC index = 0.54).

For the first time, the effect of *T. argyrophyllum* EO on the total and DCCD-sensitive FoF₁-ATPase activity of both antibiotic-sensitive (*E. coli* K-12) and kanamycin-resistant (*E. coli* pARG-25) strains was demonstrated. The EO was shown to suppress total ATPase activity by approximately 1.6-fold in the sensitive strain and 1.5-fold in the resistant strain, while also reducing DCCD-sensitive ATPase activity, indicating disruption of the FoF₁ ATP synthase complex — a key component of bacterial energy metabolism.

For the first time, the influence of *T. argyrophyllum* EO on proton transport processes across bacterial membranes was investigated. The EO was found to attenuate DCCD-sensitive H⁺ flux by approximately 2.5-fold in *E. coli* K-12 under treatment conditions, mirroring the action of the known ATPase inhibitor DCCD. These results reveal that bioactive constituents of the EO may impair transmembrane proton translocation and disrupt the proton motive force, thereby compromising cellular bioenergetics.

The study also revealed, for the first time, the effect of *T. argyrophyllum* EO on bacterial redox homeostasis. Treatment induced a significant elevation of malondialdehyde (MDA) levels (by ~50%) in the resistant strain, indicating activation of lipid peroxidation and membrane oxidative damage. Corresponding changes in superoxide dismutase (SOD) and catalase activity were documented in both strains:

Dynamic monitoring of the oxidation-reduction potential (ORP) and extracellular pH during bacterial growth further confirmed that *T. argyrophyllum* EO delays and dampens the typical redox shifts associated with exponential growth, consistent with inhibition of respiratory chain activity and metabolic suppression.

Collectively, the findings of this dissertation establish *T. argyrophyllum* and *P. graveolens* EOs as promising sources of multi-target bioactive compounds of natural origin with demonstrated broad-spectrum antimicrobial activity, antibiotic-modulating properties, and the ability to disrupt bacterial energy and redox homeostasis. These results provide a biochemical basis for considering the studied EOs as potential adjuvants to conventional antibiotics and as natural alternatives in the context of growing antimicrobial resistance.

Main points to present at the defense

- EOs from *A. filipendulina*, *P. graveolens*, *P. ferulacea* and *T. argyrophyllum* grown in Armenia exhibit pronounced antimicrobial activity against various microorganisms, dependent on concentration and the biological characteristics of the strains tested.
- *T. argyrophyllum* and *P. graveolens* EOs are distinguished by a broad-spectrum antimicrobial activity.
- *T. argyrophyllum* EO significantly reduces the viability of both antibiotic-sensitive and antibiotic-resistant *E. coli* (K-12 and pARG-25) strains.

- *T. argyrophyllum* EO disrupts bacterial energy systems by impairing FoF₁-ATPase activity and the regulation of proton fluxes.
- Under EO exposure, bacterial redox homeostasis is disturbed, manifested by changes in ORP, pH, and oxidative stress markers.
- *T. argyrophyllum* EO potentiates antibiotic efficacy, demonstrating pronounced antibiotic-modulating activity.
- The antibiotic-modulating effect is conditioned by disruption of bacterial membrane permeability and energy-dependent processes.
- The biological activity of the studied EOs is associated with their multi-component chemical composition and the predominance of monoterpenic compounds.

Work approbation. Main results of the dissertation were discussed at seminars at the Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology of Yerevan State University: International Conference: Biological Sciences and Environmental Solutions (Sept. 2025) by poster titled “Influence of *Tanacetum argyrophyllum* essential oil on ATPase activity and proton flux on *E.coli* K-12, kanamycin-resistant *E. coli* pARG-25”; at European Network for diagnosis and treatment of antibiotic-resistant bacterial infections (EURESTOP), (Pula, Croatia), June 25-27 2026 with thesis “Disruption of bacterial redox homeostasis by *Tanacetum argyrophyllum* essential oil in antibiotic-susceptible and resistant *Escherichia coli*”; 14th and 15th Annual International Scientific and Practical Seminar titled “Faces of Memory” in Research Institute of ancient Manuscripts (Matenadaran, Yerevan, Armenia)

Publications. On the basis of the experimental data observed in dissertation, 5 papers, including 3 articles in peer-reviewed journals and 2 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 100 papers and books). The dissertation consists of 109 pages, 2 tables and 21 figures.

MATERIALS AND METHODS

Plant material was harvested from various locations in the Republic of Armenia at different altitudes above sea level.

The main research was carried out at the Research Institute of Biology of the Faculty of Biology of Yerevan State University, at the Department of Biochemistry, Microbiology and Biotechnology (Yerevan, RA) and at the Department of Restoration and Conservation of the Institute of Ancient Manuscripts named after Mesrop Mashtots (Matenadaran) (Yerevan, RA).

Plant identification was performed at the Yerevan Botanical Garden of the National Academy of Sciences of Armenia. Plant specimens are stored in an above-mentioned herbarium, and each was assigned a unique voucher specimen number. Essential oil has been extracted by Senior Researcher Armen Sahakyan (Mesrop Mashtots Institute of Ancient Manuscripts, Matenadaran, Armenia) from the dried aerial parts of the plant by hydro-distillation approach using Clevenger-type apparatus. The extracted EO was stored in a dark and cool place (8-10°C). The number of independent extractions reached 4-5.

Collection sites and periods: *A. filipendulina* — Vayots Dzor region (Noravank Gorge), 1,770 m a.s.l., May–June; *P. graveolens* — Armavir region (village of Nor Kesaria), 900–950 m a.s.l., May; *P. ferulacea* — various RA locations, 850–1,000 m a.s.l., May–June; *T. argyrophyllum* — Jermuk town, Vayots Dzor region, 2,080 m a.s.l., May–June.

Chemical Composition — GC-MS Analysis: The chemical composition of the EOs was analyzed by gas chromatography–mass spectrometry (GC-MS) at the FDA Analytical Laboratory ('Tonus-Les' LLC, Armenia). Analysis was performed using a Bruker 450-GC gas chromatograph. Oven temperature ranged from 40°C to 250°C, scanning rate 3°C/min, injector temperature 220°C. Helium (purity 5.6) was used as carrier gas at a flow rate of 1 mL/min. The GC was coupled with a Hewlett–Packard 5972 mass spectrometric detector. Peaks were identified using the NIST-2013 database. The Relative Retention Index (RRI) was calculated using a homologous mixture of n-alkanes (C9–C18).

Microbial Strains and Antimicrobial Assays: Gram-positive strains: *Bacillus subtilis* WT-A, isolated from heavy metal-contaminated soils of Kajaran; *Staphylococcus aureus* WDC 5233, (Microb Deposit Center, (Armbiotechnology Scientific and Production Center, Armenia). Gram-negative strains: *E. coli* K-12, ampicillin-resistant *E. coli* DH5 α -pUC18, and kanamycin-resistant *E. coli* pARG-25 (Armbiotechnology Scientific and Production Center, Armenia). Yeast strains: *Debaryomyces hansenii* WDC M104 and *Candida guilliermondii* NP-4 (Armbiotechnology Scientific and Production Center, Armenia). The strains were grown in Nutrient Broth (NB) medium (20 g/L peptone, 5 g/L NaCl, 2 g/L K₂HPO₄, supplemented with 2 g/L glucose) at a temperature of 37°C and pH 7.5 (FAITHFUL INCUBATOR, China).

Antimicrobial activity was assessed by: (1) disk-diffusion assay; (2) broth microdilution for MIC/MBC determination; (3) bacterial growth kinetics (specific growth rate, lag phase, doubling time); (4) colony-forming unit (CFU) quantification; and (5) antibiotic-modulating activity (MF and FICI determination using checkerboard microdilution).

Disk-diffusion and broth dilution assays:

The antimicrobial activity of *T. argyrophyllum* essential oil (EO) was initially assessed by agar disk-diffusion assay (Moghrovyan, A. *et al.*, 2019).

The minimum inhibitory concentrations (MIC) were determined using broth microdilution method in 96-well plates following standard protocols (using the abovementioned concentrations of EO).

Determination of bacterial growth parameters:

The generation succeeding factor (G), the specific growth rate (μ) of bacteria, and the cell doubling time were determined by using the DEN-1 McFarland Densitometer (Biosan, Latvia).

The cell doubling time was determined by the $\ln 2/\mu$ equation (Monod J, 1949).

Determination of Bacterial Colony-Forming Units (CFU):

The antibacterial activity of *T. argyrophyllum* EO was assessed by quantifying colony-forming units (CFU) using the Neidhardt method (Neidhardt FC *et al.*, 1990).

Determination of antibiotic modulatory activity.

Antibiotic modulatory activity of *T. argyrophyllum* EO was explored by determining the minimal inhibitory concentrations (MIC) of antibiotics in the presence and absence of EO at non-inhibitory concentrations (Ginovyan *et al.*, 2023).

Determination of ATPase activity:

The impact of plant EOs on the H⁺ translocating FoF₁-ATPase activity was investigated in bacterial membrane vesicles, which were obtained by the Kaback method. FoF₁-ATPase activity was evaluated by quantifying the release of inorganic phosphate (Pi) in a reaction buffer containing 50 mM Tris–HCl (pH 7.5), 1 mM CaCl₂, and 2.5 mM MgSO₄ at 37 °C. The absorbance was measured spectrophotometrically (SP-LUV752P UV-VIS Spectrophotometer, Bioevopeak, PRC). Enzymatic activity was expressed as nmol P_i min^{−1} mL^{−1} μ g^{−1} protein. DCCD-sensitive ATPase activity was calculated according the above-described principle under identical incubation conditions.

Membrane vesicles were isolated from *E. coli* K12 and *E. coli* pARG-25 strains according to a previously established protocol (Vanyan L et al., 2022). Protein concentrations were determined using the Lowry method (LOWRY O et al., 1951).

Determination of proton flux

The H^+ fluxes were determined in whole bacterial cells by employing an appropriate selective electrode (HI1131B and HI5222 HANNA Instruments). Results were expressed in $mmol\ min^{-1}\ 10^8\ cells^{-1}$ in 1 unit of volume (ml) (Honhannisyan et al., 2022). *N,N*-dicyclohexylcarbodiimide (DCCD) served as an inhibitor of the FoF_1 -ATPase and the bacterial cultures were incubated with 0.2 mmol/L of DCCD for 10 min (Taussky & Shorr, 1953).

Determination of SOD activity

SOD activity was measured according to the Beauchamp and Fridorovich (1971).

Determination of catalase activity

The catalase activity was measured according to Cherkaoui-Malki et al., 1990) and was expressed as % of untreated control of units. One unit is defined as 1 μ mol of H_2O_2 consumed in 1 minute, and specific activity is one unit of enzyme corresponding to 1 milligram of protein.

TBARS assay

The determination of the antioxidant capacity of *T. argyrophyllum* EO was analyzed out by studying its ability to inhibit malonaldehyde synthesis in bacterial cells. α -tocopherol was used as positive standard (Babayan A, Sahakyan N, 2025).

Determination of oxidation–reduction potential ORP (mV) and extracellular pH

The ORP and extracellular pH (pH_{out}) was determined using the Milwaukee MW150 MAX 3-in-1 pH/OPR/Temp. Bench Meter (Szeged, Hungary) with a selective pH electrode (HI1131, Hanna Instruments, Portugal), and ORP was measured using an ORP electrode (HI3131B, Hanna Instruments, Portugal).

External or medium pH (pH_{out}) and redox potential (ORP, mV) were measured during aerobic growth for up to 24 hours).

Data processing.

During the research work, all experiments were conducted independently with a minimum of three repetitions. Experimental data represent the mean $\pm$ standard deviation (SD) from three biologically independent replicates. Statistical analyses were performed using a grouped two-way ANOVA by GraphPad Prism version 8.0.3 (GraphPad Software, San Diego, CA, USA). The software is available at: <https://www.graphpad.com>. Differences were considered statistically significant at $p < 0.05$, unless stated otherwise. Graphical data visualization was performed using GraphPad Prism and Microsoft Excel 2010.

RESULTS AND DISCUSSION

Chemical composition of *T. argyrophyllum* essential oil. The results from the quantitative and qualitative analysis of EO constituents are presented in the Table 1. Gas-mass spectrometric analysis of *T. argyrophyllum* EO led to the identification of 16 chemical components, which make up about 98.5% of the total composition of the EO. Environmental conditions and the time the plant is harvested also affect the chemical composition of the plant. The analysis of the EO composition of *T. argyrophyllum* plant showed that it belongs to the eucalyptol-camphor chemotype

Table 1. The chemical composition of the essential oil isolated from the aerial parts of *T. argyrophyllum* essential oil

Chemical components	Area Percentage (%)	RRI*
<i>Camphene</i>	<i>17.0</i>	943
Carene	0.50	947
α -Pinene	1.50	948
β -Pinene	0.85	970
β -ocimene	0.55	976
Santolina epoxide	7.50	991
γ -Terpinene	0.70	1047
<i>Eucalyptol</i>	<i>35.0</i>	1059
<i>Camphor</i>	<i>24.0</i>	1121
Trans-Chrysanthenyl acetate	2.90	1131
Verbinol	0.90	1136
Terpinen-4-ol	1.80	1137
Davanone	0.80	1138
Bornyl acetate	2.25	1277
β -eudesmol	1.20	1504
<i>Caryophyllene oxide</i>	1.50	1576
Total (%)	98.50	

¹for HP-5 capillary column

The amount of the main component, eucalyptol (1,8-cineole) reaches 35.0% of the total content, followed by camphor (24.0%) and camphene (17.0%). The detected eucalyptol-camphor chemotype is consistent with studies on several species of *Tanacetum* and other herbs rich in oxygenated monoterpenes. Plants rich in eucalyptol and camphor are known to exhibit both antimicrobial and antioxidant activity and, under certain conditions, prooxidant effects as well.

Additional constituents included santolina epoxide (7.5%) and trans-chrysanthenyl acetate (2.9%), along with terpinen-4-ol (1.8%), bornyl acetate (2.25%), and verbinol (0.9%). Trace amounts of α -pinene, β -pinene, and γ -terpinene were also detected. Furthermore, caryophyllene oxide (1.5%) and β -eudesmol (1.2%) were present, contributing to the overall sesquiterpene content. The compositional profile highlights the chemical complexity of *T. argyrophyllum* EO and confirms the predominance of oxygenated monoterpenes in this species.

The EO yield of *P. ferulacea* (~4%) exceeded all previously reported values in the literature for this species (maximum previously recorded: $3.0 \pm 0.16\%$ for seeds), suggesting a favorable effect of the local biogeographic conditions of Armenia on the accumulation of bioactive compounds. For *A. filipendulina*, the mean EO yield was 0.5%, within the range reported in the literature (0.11–0.78%).

The manifestations of antioxidant activity of the plants examined in our research are not unique, but rather reflect the general property of EOs rich in eucalyptol. Nevertheless, what is important here is the extent of the activity manifestation and the specific features of the mechanisms of action, by which the studied EO may differ. The possible synergistic interactions between the various components of the EO composition may also be of significant importance.

This underscores the fact that although the general nature of the effect corresponds to similar chemotypes, certain distinct biological effects are shaped by the complete chemical profile.

Antibacterial Activity of the Essential Oils.

To determine the minimum inhibitory concentration (MIC) of plant EOs, selected agar plugs taken from zones of no microbial growth during the MIC assay were transferred into sterile nutrient broth corresponding to each microorganism (without EO or ethanol extract). After 24 hours of incubation at 37°C, the tubes were examined. The effect was considered bactericidal if no microbial growth was observed. If microbial growth was present in the medium after subculturing, the effect was considered bacteriostatic.

All four EOs demonstrated antibacterial activity. Initial disk-diffusion screening revealed inhibition zones against almost all tested microorganisms. Quantitative analysis confirmed that all EOs possess MIC values in the range of 12.5–100 µg/mL.

T. argyrophyllum EO exhibited the broadest spectrum of antimicrobial activity, being effective against Gram-positive and Gram-negative bacteria as well as yeasts. *P. graveolens* and *T. argyrophyllum* EO were demonstrating significant antifungal activity against both yeast strains at 25 µg/mL. *A. filipendulina* and *P. graveolens* EOs were equally effective against *B. subtilis* (MIC = 25 µg/mL). The determined MIC values are considered effective, and the action of all tested EOs was assessed as bactericidal rather than merely bacteriostatic.

This makes *T. argyrophyllum* EO particularly promising for further investigation as a natural antimicrobial agent targeting antibiotic-resistant pathogens.

Table 2. MIC values (µg/mL) of the four EOs against selected microbial strains

Strain	<i>A. filipendulina</i>	<i>P. graveolens</i>	<i>P. ferulacea</i>	<i>T. argyrophyllum</i>
<i>E. coli</i> K-12	25	25	25	100
<i>E. coli</i> pARG-25	25	25	25	100
<i>E. coli</i> DH5α-pUC18	100	25	25	100
<i>S. aureus</i> WDC5233	25	100	—	50
<i>B. subtilis</i> WT-A17	25	25	50	50
<i>C. guilliermondii</i> NP-4	—	25	—	25
<i>D. hansenii</i> WDC M104	50	25	—	12.5

—: no inhibitory activity detected at tested concentrations.

Antibiotic modulatory activity of *T. argyrophyllum* EO.

Investigation results suggest that *T. argyrophyllum* exhibits the antibiotic-modulatory effect on the kanamycin-resistant *E. coli* (Fig. 1). The 31.25 and 62.5 $\mu\text{L/mL}$ non-inhibitory concentrations of EO were used for the investigation of antibiotic-modulatory properties.

According to the obtained data, the *T. argyrophyllum* EO at the concentration of 62.5 $\mu\text{L/mL}$ exhibited notable antibiotic-modulating effect with kanamycin, reducing the MIC value of antibiotic fourfold (MF = 4) (Fig. 1B), meanwhile the lower concentration did not show any influence (Fig. 1C).

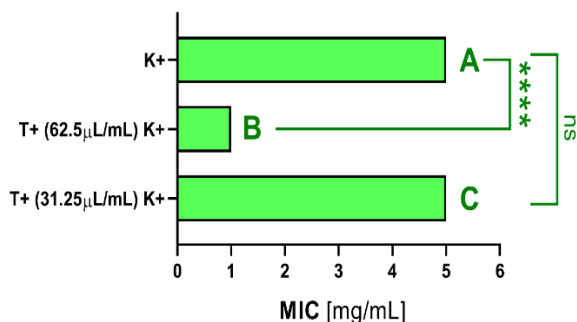


Figure 1. Modulatory effect of *T. argyrophyllum* essential oil at non-inhibitory concentrations on the antibiotic activity against kanamycin resistant *E. coli* pARG-25 strain. The abbreviations refer to: T+ – cells treated with *T. argyrophyllum* essential oil, K+ – cells treated with kanamycin. All experiments were independently repeated at least three times (**** $p < 0.0001$). The MIC values were identical across all replicates.

The interaction between the kanamycin and *T. argyrophyllum* EO could be described as synergistic, as FICI is calculated to be 0.54.

In case of ampicillin, there was not observed any modulation in the presence of *T. argyrophyllum* EO under the tested conditions.

These findings suggest that *T. argyrophyllum* EO may increase bacterial sensitivity to kanamycin by enhancing membrane permeability, impairing protective mechanisms, or facilitating intracellular antibiotic accumulation — making it a promising candidate as an adjuvant to aminoglycoside antibiotics in combating resistant infections.

The influence of *T. argyrophyllum* EO on Bacterial Colony-Forming ability and growth parameters.

To further evaluate the antimicrobial effect of EO, the colony-forming ability evaluation test was applied, quantitatively assessing the impact of EO treatment on bacterial viability.

The obtained results demonstrated that treatment with *T. argyrophyllum* EO led to a reduction of approximately 30% in the colony-forming ability of the non-resistant *E. coli* K-12 strain (Fig. 2). Similarly, in the kanamycin-resistant *E. coli* pARG-25 strain, colony formation was suppressed by 30%, a reduction comparable to that observed in the non-resistant control (Fig. 2).

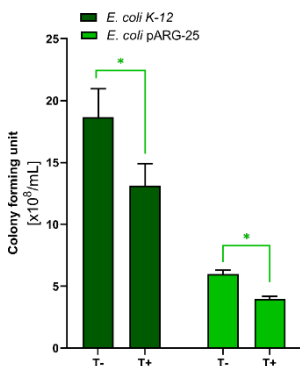
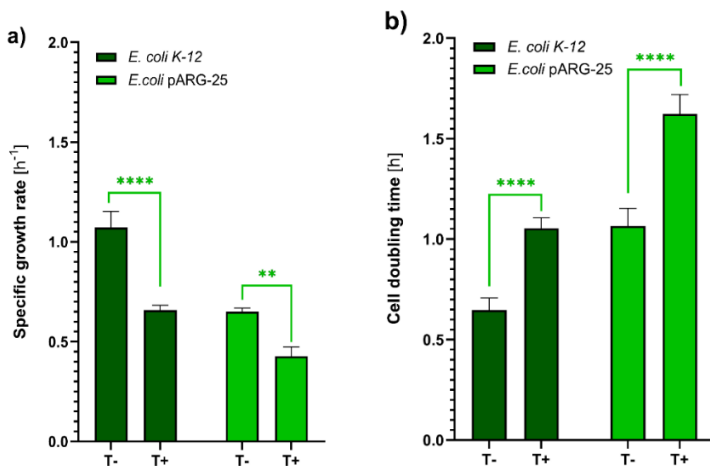


Figure 2. The colony-forming units of the *E. coli* K-12 and *E. coli* pARG-25 strains under the influence of *T. argyrophyllum* EO at minimal inhibitory concentration. (T- are the control cells without treatment with *T. argyrophyllum* EO, T+ -cells treated with *T. argyrophyllum* EO). The results are means $\pm$ SD of three independent experiments implemented in triplicate (* $p < 0.05$).

In addition, the same concentration of *T. argyrophyllum* EO exhibited a suppressive influence also on the specific growth rates of both tested bacterial strains. A measurable decrease in growth kinetics was observed following EO exposure, further confirming its bacteriostatic properties (Fig. 3).

The specific growth rates in case of *E. coli* K-12 and *E. coli* pARG-25 strains, reduced by 1.6-fold, and 1.5-fold, respectively (Fig. 3a). Additionally, the EO prolonged the lag phase in case of *E. coli* K-12 by 63% and by 52% in case of the antibiotic-resistant *E. coli* pARG25 (Fig. 3b), affecting the adaptation of bacteria to the growth conditions during the early stages.

Figure 3. The specific growth rate (a) and cell doubling time (b) of the *E. coli* K-12 and *E. coli* pARG-25 strains under the influence of *T. argyrophyllum* EO at minimal inhibitory concentration. (T- are the control cells without treatment with *T. argyrophyllum* EO, T+ - cells treated with *T. argyrophyllum* EO). The results are means $\pm$ SD of three independent experiments carried out in triplicate (** $p < 0.01$, **** $p < 0.0001$).



Colony-forming unit assays confirmed that EO treatment reduced the colony-forming ability by approximately 30% in both *E. coli* K-12 and *E. coli* pARG-25 strains. This indicates that EO action is not limited to growth retardation, but also significantly suppresses cellular reproductive viability. The comparable sensitivity of the antibiotic-resistant strain suggests that the EOs antibacterial mechanisms differ from those of kanamycin and may bypass certain resistance mechanisms.

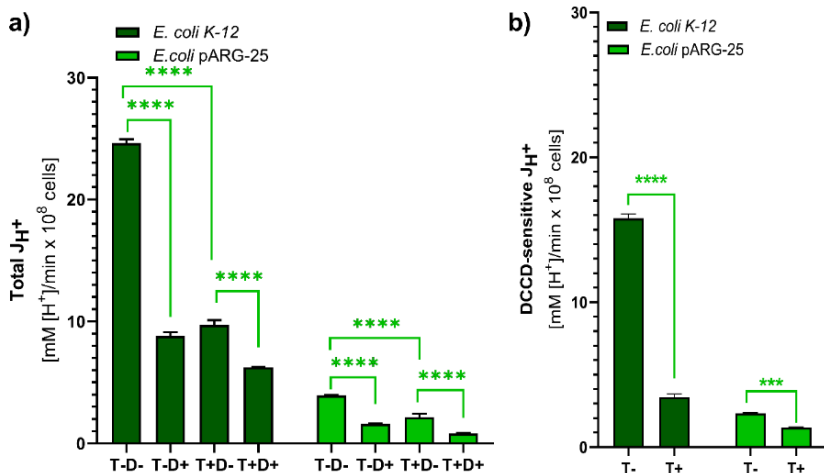
The influence of *T. argyrophyllum* EO on proton fluxes and ATPase activity.

The investigations of the effects of *T. argyrophyllum* EO on some membrane-associated functions in *E. coli* revealed significant alterations in proton flux dynamics (Fig. 4a). In the untreated *E. coli* K-12 control strain, the rate of DCCD-sensitive H^+ fluxes decreased by 2.8-fold (Fig. 4b, T-D+). In the presence of investigated EO alone (T+D-), this parameter was reduced by 2.5-fold compared to the untreated control. This suggests that the EO has similar effects with DCCD by targeting the FoF₁ domains in FoF₁ ATPase.

In case of the kanamycin-resistant *E. coli* pARG-25 strain, treatment with the EO resulted in a 1.8-fold reduction in J_{H^+} (Fig. 4a, T+D-). Under DCCD treatment, the control strain exhibited approximately a 4.5-fold decrease in DCCD-sensitive H^+ flux, while in the resistant strain, exposure to the EO under the same conditions led to a 1.7-fold decrease (Fig. 4b, T+).

These findings suggest that *T. argyrophyllum* EO affects proton translocation across bacterial membranes, with differential impacts observed between wild-type and antibiotic-resistant strain.

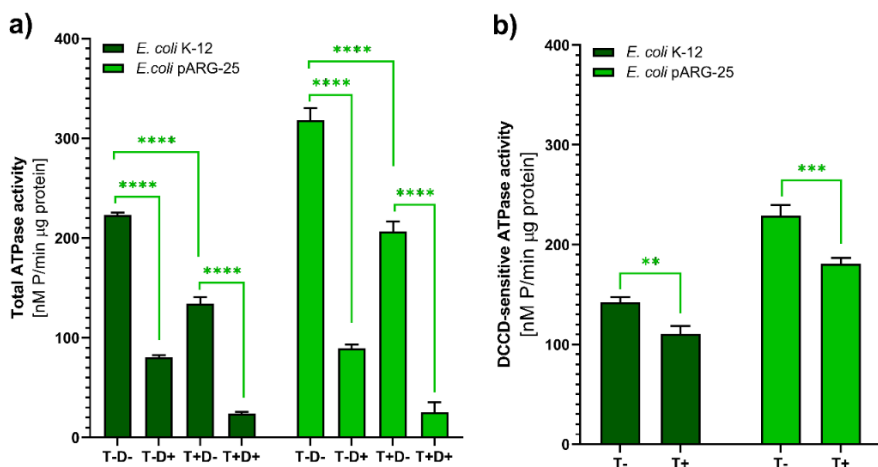
Figure 4. The effect of *T. argyrophyllum* EO on total (a) and DCCD-sensitive (b) H^+ -fluxes through the *E. coli* K-12 and kanamycin-resistant *E. coli* pARG-25 cell membranes. T-D- are the control cells without the treatment with EO and DCCD, T-D+ are the cells treated only with DCCD, T+D- are the cells treated only with EO and the T+D+ are the cells treated with both *T. argyrophyllum* EO and DCCD. The results are means $\pm$ SD of three independent experiments performed in triplicate (*) $p < 0.001$, ****) $p < 0.0001$.**



Further analysis of DCCD-sensitive ATPase activity revealed a 1.3-fold reduction in the *E. coli* K-12 strain and a 1.2-fold decrease in the *E. coli* pARG-25 strain upon EO treatment (Fig. 5b, T+).

Interestingly, in the control samples, the *E. coli* pARG-25 strain exhibited a 1.4-fold higher total ATPase activity compared to the *Wt* strain (Fig. 5a). Similarly, DCCD-sensitive ATPase activity was elevated by 1.6-fold in the resistant strain (Fig. 5b).

Figure 5. The effect of *T. argyrophyllum* essential oil on Total (a) and DCCD-sensitive (b) ATPase activity through the *E. coli* K-12 and *E. coli* pARG-25 strains' membranes. T-D- are the control cells without treatment with EO or DCCD, T-D+ are the cells treated only with DCCD, T+D- are the cells treated only with EO and T+D+ are the cells treated with both *T. argyrophyllum* EO and DCCD. The results are means $\pm$ SD of three independent experiments performed in triplicate (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).



These results suggest that *T. argyrophyllum* EO exerts a suppressive effect on both total and proton-translocating ATPase activities, with comparable magnitude across antibiotic-sensitive and -resistant strains.

Redox Homeostasis and Oxidative Stress Markers.

In order to study the ability of the EO of the *T. argyrophyllum* plant to disrupt bacterial redox homeostasis (as a possible mechanism of antibacterial effect), the fact that this EO is a reductive or oxidizing agent was first investigated in chemical tests, then its effect on some parameters of the antioxidant defense systems of bacteria was studied.

During our research, *T. argyrophyllum* EO showed pronounced free radical scavenging activity in chemical in vitro tests. $IC_{50} = 19.4 \pm 2.5$ μ l/ml value was obtained in the DPPH test, which indicates the high antioxidant potential of the examined sample. The relatively low value of IC_{50} suggests that the essential oil effectively participates in hydrogen atom or electron transfer reactions, stabilizing the DPPH free radical.

The indicated activity is most likely due to the combination of phenolic compounds and oxygenated mono- and sesquiterpenes, which are known for their redox-active properties and high ability to react with free radicals. In this context, the antioxidant activity of the essential oil can be considered as a redox-dependent property due to its chemical composition, the manifestation of which in biological systems is essentially determined by the redox balance, the characteristics of the membrane microenvironment and the intensity of intracellular metabolic processes. The DPPH test only reflects the radical scavenging potential in a cell-free, chemical system where the compounds act as direct H- or e⁻-donors.

The obtained data may indicate the pronounced prooxidative effect of bioactive metabolites of *T. argyrophyllum* EO in the kanamycin-resistant *E. coli* pARG-25 strain. In order to reliably evaluate such in effect, the effect of EO on the activity of the main enzyme components of the antioxidant protection of bacterial cells was studied.

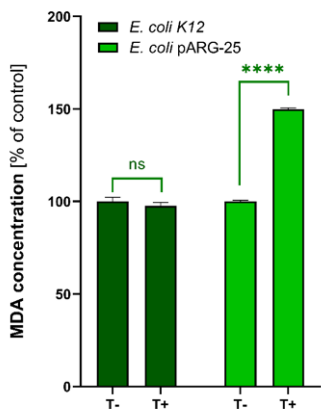


Figure 6. The synthesis of MDA in *E. coli* K12 and *E. coli* pARG-25 cells treated by *T. argyrophyllum* EO (T - are the control cells without treatment, T + -cells treated with *T. argyrophyllum* EO). The results are means $\pm$ SD of three independent experiments implemented in triplicate (**** $p < 0.0001$, ns – non significant).

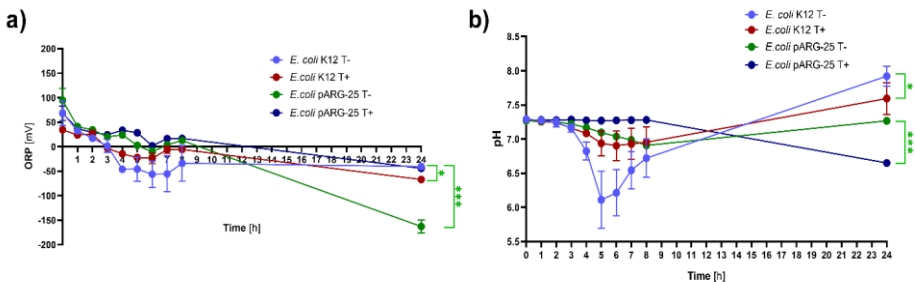
TBARS assay results revealed that MDA levels in kanamycin-resistant *E. coli* pARG-25 increased by 49.88% after EO treatment (AI = $50.22 \pm 0.71\%$), indicative of intensified lipid peroxidation in the antibiotic-resistant cells, likely reflecting ROS overproduction and structural disruption of the cell membrane. In the wild-type *E. coli* K-12 strain, no significant changes in MDA levels were observed, possibly reflecting differences in the redox buffering capacity or antioxidant defense mechanisms between the two strains (Fig.6).

Dynamic analysis of the oxidation-reduction potential (ORP) during 24-hour aerobic cultivation revealed that untreated control cultures of both strains showed substantial ORP decreases between hours 4–7, with minimum values of -55.3 mV (K-12) and -15.1 mV (pARG-25), corresponding to the period of maximum metabolic activity (Fig. 7a). In EO-treated cultures, ORP decrease was delayed (from approximately hour 6 onwards), and minimum values were significantly higher (less negative): -23.4 mV for K-12 and -1.7 mV for pARG-25. This delayed and attenuated ORP dynamic is consistent with EO-induced suppression of respiratory activity and bacterial metabolism, supporting a bacteriostatic mode of action.

The changes in extracellular pH strictly reflects the changes in ORP values in both tested bacterial cultures, indicating the accumulation tendency of organic acids in accordance to their metabolic activity level (Fig. 7b).

All of the obtained data create a platform for insisting about the redox-dependent mechanisms of influence of the investigated EO on bacterial cells, bringing to the reduction of viability in both tested *E. coli* strains.

Fig. 7. ORP (mV) (a) and pH values (b) changes during the growth of *E. coli* K12 and *E. coli* pARG-25 strains. T- are the control cells without treatment, T+ -cells treated with *T. argyrophyllum* EO. The results are means $\pm$ SD of three independent experiments implemented in triplicate.

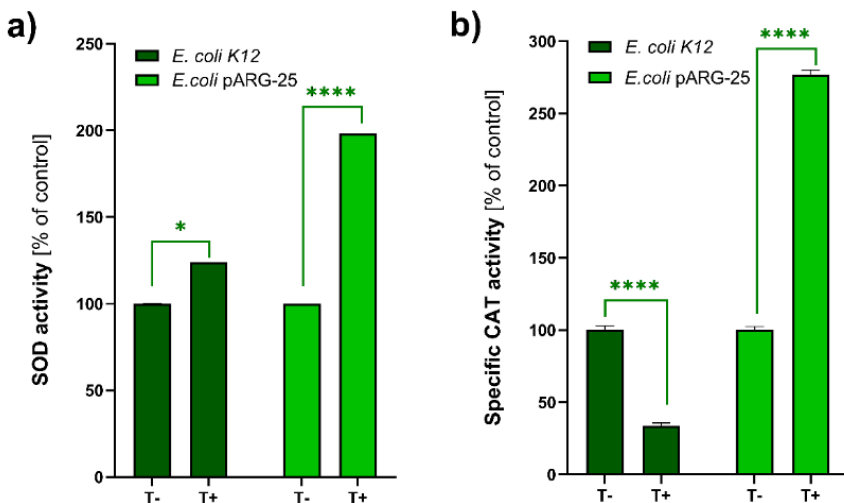


The combined elevation of SOD, catalase, and MDA levels in the antibiotic-resistant strain under EO treatment points to a more pronounced pro-oxidant effect in *E. coli* pARG-25 compared with the wild-type strain. These strain-specific enzymatic responses suggest distinct redox adaptation mechanisms, likely connected with the metabolic and membrane reorganisation that occurred during the acquisition of antibiotic resistance.

SOD activity increased by approximately 24% in *E. coli* K-12 and by ~98% in *E. coli* pARG-25 after EO treatment. The moderate increase in the wild-type strain may represent a compensatory response to elevated superoxide radical generation, while the markedly higher induction in the resistant strain indicates more intense oxidative stress, possibly reflecting the metabolic reprogramming and higher redox sensitivity of the plasmid-bearing cells (Fig. 8a).

Catalase activity showed strain-specific responses: a decrease of approximately 33.6% was observed in *E. coli* K-12, whereas an approximately 177% increase was recorded in *E. coli* pARG-25 under the same conditions. The pronounced catalase induction in the resistant strain is consistent with elevated hydrogen peroxide and ROS accumulation in those cells. The decrease in the wild-type strain may reflect either partial enzyme suppression or the activation of alternative stress-response strategies (Fig. 8b).

Fig. 8. The activity of SOD (a) and catalase (b) in *E. coli* K12 and *E. coli* pARG-25 under the treatment with *T. argyrophyllum* EO (10 μLmL^{-1}). T- are the control cells without treatment, T+ -cells treated with *T. argyrophyllum* EO. The results are means $\pm$ SD of three independent experiments implemented in triplicate (* $p < 0.05$; **** $p < 0.0001$).



These findings could support the notion that the EO exerts a more pronounced pro-oxidative effect in the kanamycin-resistant strain, leading to intensified activation of enzymatic antioxidant defenses.

CONCLUSIONS

Based on the results obtained in the present dissertation, the following conclusions are drawn:

1. All investigated EOs exhibit pronounced antibacterial and antifungal activity depending on concentration and strain characteristics.
2. The chemical composition of *T. argyrophyllum* EO is characterised by the predominance of monoterpenic compounds (eucalyptol-camphor chemotype: 1,8-cineole 35.0%, camphor 24.0%, camphene 17.0%).
3. *T. argyrophyllum* EO reduces the viability of both *E. coli* K-12 and *E. coli* pARG-25 strains, demonstrating high efficacy against both antibiotic-sensitive and resistant bacteria.
4. *T. argyrophyllum* EO disrupts bacterial energy systems by impairing F_0F_1 -ATPase activity and regulation of proton fluxes, resulting in disturbance of cellular energy homeostasis.
5. Under EO exposure, bacterial redox homeostasis is disturbed, manifested by changes in ORP, pH, and oxidative stress markers (MDA, SOD, catalase).

6. *T. argyrophyllum* EO exhibits antibiotic-modulating activity: in combination with kanamycin, it reduces the MIC of the antibiotic four-fold (FICI = 0.54, synergistic interaction) against the resistant *E. coli* pARG-25 strain.
7. The obtained results allow *T. argyrophyllum* and *P. graveolens* EOs to be considered as promising sources of multi-target natural antimicrobial and adjuvant agents, with potential application in strategies to combat antibiotic resistance.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. **Margaryan L.** (2026), Comparative evaluation of antimicrobial potential of *Achillea filipendulina*, *Prangos ferulacea*, and *Pelargonium graveolens* essential oils, ***Proceedings of the YSU B: Chemical and Biological Sciences***, 60(1): p 1-8.
2. **Margaryan L.**, Tadevosyan S., Trchounian K., Sahakyan N (2026), Disruption of bacterial redox homeostasis by *Tanacetum argyrophyllum* essential oil in antibiotic-sensitive and resistant *Escherichia coli*, *AIMS Biophysics*, 13(2): p 189-203.
3. **Margaryan L.**, Tadevosyan S, Sahakyan A, Trchounian K, Sahakyann N, (2026) The antibacterial effect of *Tanacetum argyrophyllum* essential oil on kanamycin- resistant *Escherichia coli* by disruption of energy metabolism and proton fluxes, *Scientific reports*, 16 (1).
4. **Margaryan L.**, Tadevosyan S, Trchounian K, Sahakyann N, (2025), Influence of *Tanacetum argyrophyllum* Essential Oil on ATPase Activity and Proton Flux of *E. coli* K-12, Kanamycin-Resistant *E. coli* pARG-25, *Journal of Innovative Solutions for eco-environmental Sustainability*.
5. **Margaryan L.**, Tadevosyan S., Sargsyan S, Trchounian K., Sahakyan N. June 27th 2026, Disruption of bacterial redox homeostasis by *Tanacetum argyrophyllum* essential oil in antibiotic-susceptible resistant *Escherichia coli*, European Network for diagnosis and treatment of antibiotic-resistant bacterial infections (EURESTOP), Pula, Croatia

ՄԱՐԳԱՐՑԱՆ ԼՈՒՄԻՆԵ ՅՈՒՐՈՒՄ

ՀԱՅԿԱԿԱՆ ՄԻՋՆԱԴԱՐՑԱՆ ՄԱՏՑԱՆՆԵՐԻ ԲԱՐԱՆԴԱՏՈՄԵՐՈՒՄ ՀԻՇԱՏԱԿՎԱԾ ՈՐՈՇ ԴԵՂԱԲՈՒՑՄԵՐԻՑ ԱՆՁԱՏՎԱԾ ԵԹԵՐԱՑՈՒՂԵՐԻ ԱԶԴԵՑՈՒԹՅԱՆ ԿԵՆՍԱՔԻՄԻԱԿԱՆ ՄԵՌԱՆԻՋՄՆԵՐԸ

Ամփոփագիր

Բանալի բառեր՝ եթերայուղ, *Tanacetum argyrophyllum*, հակամանրէային ակտիվություն, FoFi-ԱԵՖ-ազ, հակաբիոտիկ-մոդուլացնող ակտիվություն, օքսիդային սթրես, *Escherichia coli*, հակաբիոտիկ-կայունություն:

Բակտերիաների հակաբիոտիկ-կայունության զրոյալ տարածումը և արդյունավետ հակամանրէային միջոցների սղությունը պայմանավորում են բնական ծագման կենսաակտիվ նյութերի ուսումնասիրման արդիականությունը: Հայաստանի Հանրապետության տարածքը, ներկայացնելով ավելի քան 3600 բուսատեսակ, հանդիսանում է կենսաբանորեն ակտիվ միացությունների արժեքավոր, բայց քիչ ուսումնասիրված աղբյուր:

Սույն ատենախոսական աշխատանքի նպատակն էր ուսումնասիրել Հայաստանում աճող *Achillea filipendulina* Lam. (Asteraceae), *Pelargonium graveolens* L'Hér. (Geraniaceae), *Prangos ferulacea* (L.) Lindl. (Apiaceae) և *Tanacetum argyrophyllum* (C. Koch) Tzvelev (Asteraceae) բույսերի եթերայուղերի (ԵՅ) հակամանրէային ու հակաօքսիդանտային ակտիվությունը, ինչպես նաև ազդեցության կենսաքիմիական մեխանիզմները՝ ինչպես հակաբիոտիկ-զգայուն, այնպես էլ կայուն *Escherichia coli* շտամների նկատմամբ:

Եթերայուղերի քիմիական բաղադրությունը ուսումնասիրվել է ԳՔ-ԶՍ մեթոդով: Հակամանրէային ակտիվությունը գնահատվել է սկավառակ-դիֆուզիոն, արգանակային միկրոնոսրացման, ՆԱԿ որոշման, ինչպես նաև բակտերիաների աճի կինետիկայի և գաղութառաջացման ունակության (ԳԱՄ) մեթոդներով: Հակաբիոտիկ-մոդուլացնող ակտիվությունն ուսումնասիրվել է ՄԳ և ԿԱԿԳ (FICI) ցուցանիշների հիման վրա: Կենսաքիմիական մեխանիզմներն ուսումնասիրվել են՝ որոշելով բակտերիաների ցիտոպլազմային թաղանթի H⁺-հոսքերը, FoFi-ԱԵՖ-ազային ակտիվությունը, ՍՕԴ-ի և կատալազի ակտիվությունը, ՄԵԱ-ի մակարդակը (TBARS թեստ), ԴՖՊՀ (DPPH) ազատ ռադիկալների չեզոքացման ակտիվությունը, ինչպես նաև արտաբջջային pH-ի և օքսիդա-վերականգնողական պոտենցիալի (ՕՎՊ) փոփոխությունները:

T. argyrophyllum-ի եթերայուղի ԳՔ-ԶՍ վերլուծությունը ցույց տվեց, որ այն պատկանում է էվկալիպտոլ-կամֆորային քեմոտիպին, որտեղ 1,8-ցինեոլը (35.0%), կամֆորը (24.0%) և կամֆենը (17.0%) կազմում են ընդհանուր կազմի շուրջ 76%-ը: *P. ferulacea*-ի ԵՅ էլքը (~4%) գերազանցեց զրականությամբ հայտնի բոլոր ցուցանիշները, ինչը վկայում է Հայաստանի տվյալ բուսաաշխարհագրական շրջանի կլիմայական պայմանների բարենպաստ ազդեցության մասին:

Բոլոր ուսումնասիրված եթերայուղերն էլ ցուցաբերել են հակամանրէային ակտիվություն՝ 12.5–100 մկգ/մլ ՆԱԽ արժեքներով: Ամենալայն ազդեցության սպեկտրն արձանագրվել է *T. argyrophyllum*-ի և *P. graveolens*-ի ԵՅ-ի դեպքում, ներառյալ Գրամ-դրական, Գրամ-բացասական բակտերիաներ և խմորասնկեր: *T. argyrophyllum*-ի ԵՅ-ն ճնշել է *E. coli* K-12 և *E. coli* pARG-25 շտամների աճի տեսակարար արագությունը համապատասխանաբար 1.6 և 1.5 անգամ, երկարացրել է լազ-փուլը 63% և 52%-ով, ինչպես նաև նվազեցրել է գաղութառաջացման ունակությունը ~30%-ով երկու շտամներում էլ: *T. argyrophyllum*-ի ԵՅ-ն ցուցաբերել է արտահայտված հակաբիոտիկ-մոդուլացնող ակտիվություն կանամիցինի կայուն *E. coli* pARG-25 շտամի հանդեպ՝ 4 անգամ նվազեցնելով հակաբիոտիկի ՆԱԿ-ն (ՄԳ (MF)=4, ԿԱԿԳ (FICI)=0.54, սիներգիստական փոխազդեցություն): Ամպիցիլինի նկատմամբ մոդուլացնող ազդեցություն չի արձանագրվել, ինչը ցույց է տալիս ամփնոզլիկոզիդային հակաբիոտիկների էներգա-կախյալ ներթափանցման մեխանիզմի կարևոր դերը: Կենսաքիմիական ուսումնասիրությունները պարզել են, որ *T. argyrophyllum*-ի ԵՅ-ն ճնշում է FoF₁-ԱԵՖ-ազային ակտիվությունն *E. coli* K-12-ում ~1.6 անգամ, *E. coli* pARG-25-ում ~1.5 անգամ, ինչպես նաև ԴՅԿԴ-զգայուն H⁺-հոսքերի ինտենսիվությունն՝ ի ցույց դնելով Fo-ենթամիավորի ֆունկցիոնալ ճնշում: ԴՖՊՀ (DPPH) թեստում IC₅₀ կազմեց 19.4±2.5 մկլ/մլ, ինչը վկայում է բարձր հակաօքսիդանտային ակտիվության մասին: TBARS թեստում *E. coli* pARG-25 կայուն շտամում ՄԵԱ-ի (MDA) մակարդակն աճել էր 49.88%-ով (AI=50.22±0.71%), ինչը ցույց է տալիս լիպիդային գերօքսիդացման ինտենսիվացում հակաբիոտիկ-կայուն բջիջներում: ՍՕԴ-ի (SOD) ակտիվությունն աճել է *E. coli* K-12-ում ~24%-ով, *E. coli* pARG-25-ում ~98%-ով, կատալազինը համապատասխանաբար նվազել էր 33.6%-ով և աճել 177%-ով, ինչը վկայում է շտամ-սպեցիֆիկ ռեդոքս-ադապտացիոն մեխանիզմների մասին: ՕԿՊ-ի (ORP) դինամիկ վերլուծությունն ապացուցեց, որ ԵՅ-ն հետաձգում է ռեդոքս-ակտիվ փուլի ձևավորումն ու ճնշում բակտերիալ նյութափոխանակությունը՝ դրսևորելով բակտերիաստատիկ բնույթ:

Ստացված արդյունքները ցույց են տալիս, որ *T. argyrophyllum*-ի ԵՅ-ը գործում է որպես բազմամեխանիզմ հակամանրէային գործոն՝ համատեղ խաթարելով բջջաթաղանթի ֆունկցիոնալ ամբողջականությունը, FoF₁-ԱԵՖ-ազային համակարգը, պրոտոնային հոսքերը և ռեդոքս-հոմեոստազը, ինչն ապահովում է ոչ միայն ուղղակի հակամանրէային ազդեցություն, այլ նաև ամփնոզլիկոզիդային հակաբիոտիկների արդյունավետության բարձրացում: Ստացված արդյունքները ենթադրում են *T. argyrophyllum*-ի և *P. graveolens*-ի ԵՅ-երի կիրառական ներուժ՝ հակաբիոտիկ-կայունության դեմ ուղղված բնական ծագման թերապևտիկ միջոցների մշակման ոլորտում:

РЕЗЮМЕ

**БИОХИМИЧЕСКИЕ МЕХАНИЗМЫ ДЕЙСТВИЯ ЭФИРНЫХ МАСЕЛ
НЕКОТОРЫХ ЛЕКАРСТВЕННЫХ РАСТЕНИЙ, УПОМЯНУТЫХ В
АРМЯНСКИХ СРЕДНЕВЕКОВЫХ РУКОПИСЯХ**

Ключевые слова: эфирные масла, *Tanacetum argyrophyllum*, антимикробная активность, F₀F₁-АТФаза, антибиотик-модулирующая активность, окислительный стресс, *Escherichia coli*, антибиотикорезистентность.

Глобальное распространение антибиотикорезистентности бактерий и дефицит эффективных антимикробных средств определяют актуальность изучения биологически активных веществ природного происхождения. Территория Республики Армения, насчитывающая более 3600 видов растений, является ценным, но малоизученным источником биологически активных соединений. Целью данной диссертационной работы было изучение биологически активных свойств *Achillea filipendulina* Lam. (Asteraceae), *Pelargonium graveolens* L'Hér. (Geraniaceae), *Prangos ferulacea* (L.) Lindl. (Apiacea) и *Tanacetum argyrophyllum* (C. Koch) Tzvel. (Asteraceae), произрастающих в Армении, антимикробная и антиоксидантная активность эфирных масел (ЭМ) этих растений, а также биохимические механизмы их действия на чувствительные и резистентные к антибиотикам штаммы *E. coli*.

Химический состав ЭМ был изучен методом ГХ-МС. Антимикробная активность оценивалась методами дисковой диффузии, микроразведения, определения МИК, а также методами кинетики роста бактерий и колониеобразующей способности (КОЕ). Антибиотико-модулирующая активность была изучена на основании показателей (МФ) МФ и ИФИК (FICI), биохимические механизмы - путем определения потоков H⁺ цитоплазматической мембраны бактерий, активности F₀F₁-АТФ-азы, активности СОД и каталазы, уровня МДА (тест TBARS), свободнорадикальной нейтрализующей активности ДФПГ, а также изменений внеклеточного pH и окислительно-восстановительного потенциала (ОВП).

Анализ ЭМ *T. argyrophyllum* методом ГХ-МС показал, что оно относится к эвкалипто-камфорному хемотипу, где 1,8-цинеол (35,0%), камфора (24,0%) и камфен (17,0%) составляют около 76% от общего состава. Выход ЭМ *P. ferulacea* (~4%) превысил все известные в литературе показатели, что свидетельствует о благоприятном влиянии климатических условий этого фитогеографического региона Армении.

Все исследованные ЭМ проявили антимикробную активность со значениями МИК 12,5–100 мкг/мл. Самый широкий спектр был отмечен для ЭМ *T. argyrophyllum*

и *P. graveolens*, включая грамположительные, грамотрицательные бактерии и дрожжи. ЭМ *T. argyrophyllum* подавило удельную скорость роста штаммов *E. coli* K-12 и *E. coli* pARG-25 в 1,6 и 1,5 раза соответственно, удлинит лаг-фазу на 63 и 52 %, а также снизило колониеобразующую способность на ~30 % у обоих штаммов. ЭМ *T. argyrophyllum* проявило выраженную антибиотикомодулирующую активность в отношении канамицин-резистентного штамма *E. coli* pARG-25, снижая МИК антибиотика в 4 раза ($MФ = 4$, $(ИФИК) = 0,54$, синергическое взаимодействие). Модулирующего действия в отношении ампициллина не зафиксировано, что указывает на важную роль энергозависимого механизма проникновения аминогликозидных антибиотиков.

Биохимические исследования показали, что ЭМ *T. argyrophyllum* подавляет активность FoF₁-АТФазы у штамма *E. coli* K-12 ~1,6 раза, а у штамма *E. coli* pARG-25 ~1,5 раза, а также интенсивность ДЦКД-чувствительных H⁺-потоков, что указывает на функциональное подавление Fo-субъединицы. В ДФПГ-тесте IC₅₀ составила 19,4 ± 2,5 мкл/мл, что свидетельствует о высокой антиоксидантной активности. В тесте TBARS уровень МДА в резистентном штамме *E. coli* pARG-25 увеличился на 49,88% ($AI = 50,22 \pm 0,71\%$), что свидетельствует об усилении перекисного окисления липидов в антибиотик-резистентных клетках. Активность СОД увеличилась в штамме *E. coli* K-12 на ~24%, в штамме *E. coli* pARG-25 на ~98%, каталаза снизилась на 33,6% и увеличилась на 177% соответственно, что указывает на штамм-специфичные механизмы редокс-адаптации. Динамический анализ ОВП показал, что ЭМ задерживает образование редокс-активной фазы и подавляет бактериальный метаболизм, проявляя бактериостатический характер.

Полученные результаты показывают, что ЭМ *T. argyrophyllum* действует как комбинированный антимикробный фактор, совместно нарушая функциональную целостность клеточной мембраны, систему FoF₁-АТФ-азы, протонные потоки и окислительно-восстановительный гомеостаз, что обеспечивает не только прямое антимикробное действие, но и повышение эффективности аминогликозидных антибиотиков. Полученные результаты позволяют предположить потенциал применения ЭМ *T. argyrophyllum* и *P. graveolens* при разработке природных вспомогательных терапевтических средств при антибиотикорезистентности.

