Research Article Open Access

Phenolic compounds and antimicrobial activity of extracts of apricot leaves derived from the trees treated with pesticides

Anna M. Grigoryan[1], Varsik S. Mirzoyan[2], Ruzanna M. Hanisyan[3], Naira Zh. Sahakyan[4], Tamara D. Karapetyan[3]

[1]Russian-Armenian University (RAU), Yerevan, Armenia; [2]Scientific Center for Risks Assessment and Analysis in Food Safety Area, Yerevan, Armenia; [3]Yerevan State Medical University, Yerevan, Armenia; [4]Yerevan State University, Yerevan, Armenia.

***Corresponding author:** Varsik S. Mirzoyan, PhD, Researcher, Scientific Center for Risks Assessment and Analysis in Food Safety Area; 0071, Masisi St., 107/2 Building, Yerevan, Armenia

Submission Date: November 6th, 2024, **Acceptance Date:** December 8th, **Publication Date:** December 13th, 2024

Please cite this article as: Grigoryan A. M., Mirzoyan V. S., Hanisyan R. M., Sahakyan N. Z., Karapetyan T. D. Phenolic compounds and antimicrobial activity of extracts of apricot leaves derived from the trees treated with pesticides, *Functional Foods in Health and Disease* 2024; 14(12): 968-983. DOI: https://doi.org/10.31989/ffhd.v14i12.1510

ABSTRACT

Background: The apricot tree (*Armeniaca vulgaris* L.; family *Rosaceae*) is one of the most important fruit trees in Armenia. While apricots are widely studied for their alimentary uses (e.g., fresh and dried fruits, and oil production), the leaves are valued in traditional medicine due to their high concentration of bioactive compounds. These compounds make apricot leaves valuable for both medicinal and chemical applications. The healing properties of apricot leaves are primarily attributed to their antimicrobial and antioxidant activities, which are linked to chemical constituents such as flavonoids and phenolic compounds. However, the increasing use of agrochemicals may influence the content of these bioactive compounds and negatively affect their properties. Despite this concern, studies addressing these issues remain scarce in literature.

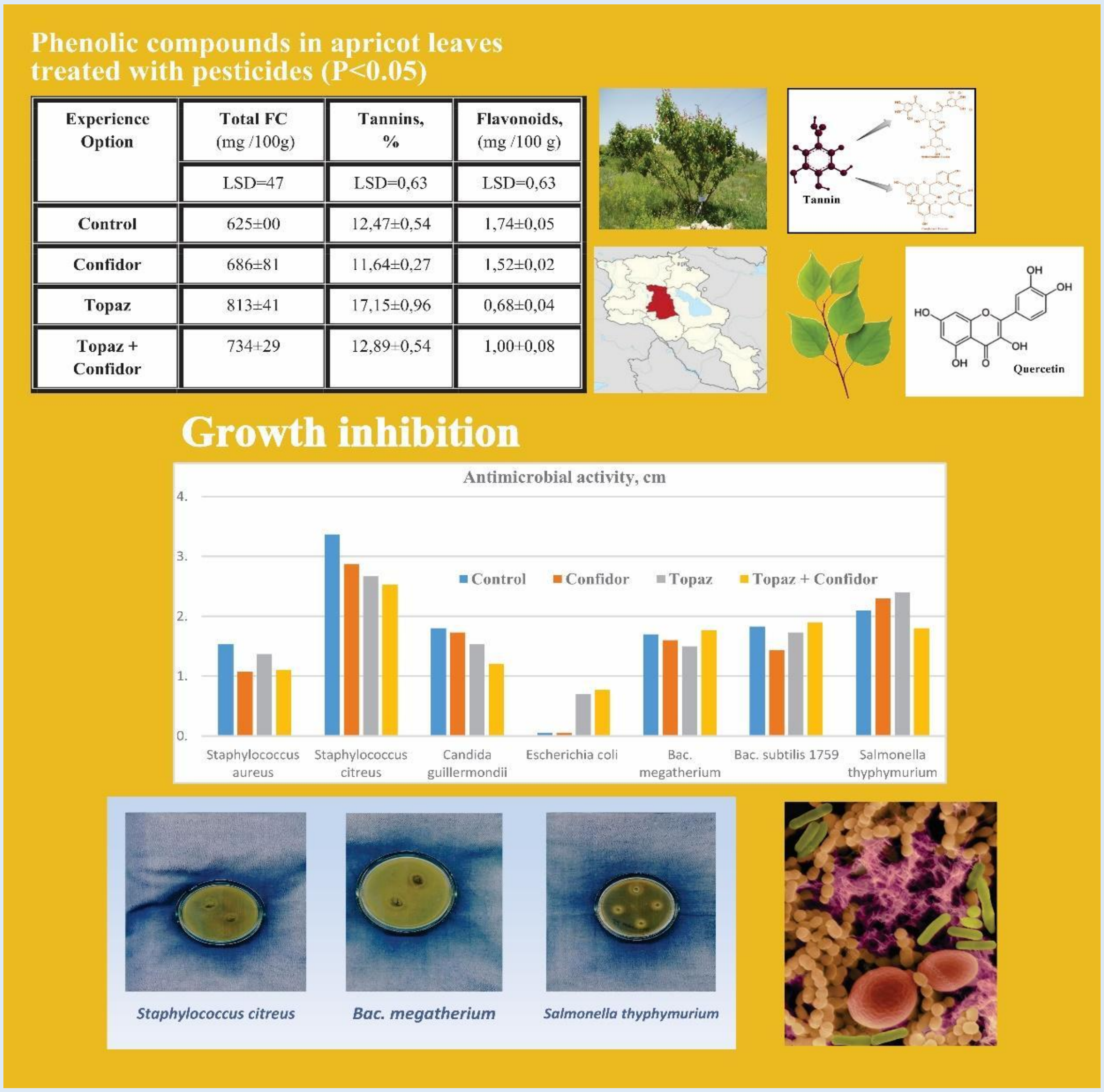
Objectives: To evaluate the impact of pesticides (Topaz and Confidor) on flavonoid and total phenolic content in apricot leaves through field surveys and biochemical analyses and to identify correlations between the phenolic content in the extracts and the antimicrobial activities of the examined samples.

Methods: The experiments were conducted in the Kotayk region of the Republic of Armenia (RA). The research material was the apricot leaves collected from the trees treated twice with the fungicide Topaz and the insecticide Confidor, after allowing for complete detoxification in the leaves. Trees sprayed with water served as a control group. Antimicrobial activity of the extracts was evaluated using the agar well diffusion assay (Method of Wells). Polyphenolic compounds were quantified using methods described by Yermakov.

Results: According to the results obtained, the inhibitory activity of the apricot leaf extracts against the tested microorganisms (Gram positive *(Staphylococcus citreus, Staphylococcus aureus, Bac. megatherium, B. subtilis*), Gram negative bacteria (*Escherichia coli* and *Salmonella thyphymurium*) and fungus (*Candida guillermondii*) is correlated with the concentration of total phenolic compounds, tannins and flavonoids in the leaves by polynomial 3 order equations.

Conclusions: Our data indicates a certain impact of pesticides on the content of the total PC, tannins and flavonoids in apricot leaves, which explains the different antimicrobial activity on the same microorganisms of the tested extracts coming from.

Keywords: Apricot leaves, phenolic compounds, antibacterial and antifungal activities, pesticides



Experience Option	Total FC (mg /100g)	Tannins, %	Flavonoids, (mg /100 g)
	LSD=47	LSD=0,63	LSD=0,63
Control	625±00	12,47±0,54	1,74±0,05
Confidor	686±81	11,64±0,27	1,52±0,02
Topaz	813±41	17,15±0,96	0,68±0,04
Topaz + Confidor	734±29	12,89±0,54	1,00±0,08

Graphical Abstract: Phenolic Compounds and Antimicrobial Activity of Extracts of Apricot Leaves Derived from The Trees Treated with Pesticides

INTRODUCTION

In recent decades, the importance of the health benefits of functional foods has attracted more and more attention, leading to an increase in their demand. This progress has been supported by advances in *Quantum* and *Tempus* theories within the field of functional food science. A key criterion for qualifying a food product as functional is the presence of biologically active substances or compounds that contribute to the prevention of specific diseases [1-2]. The apricot (*Armeniaca vulgaris* L.; family *Rosaceae*) is one of the most health-promoting fruits, predominantly cultivated in the Ararat Plain and the Kotayk region in the Republic of Armenia (RA).

Although apricot leaves have not been as extensively researched as fruit, they are rich in bioactive compounds that contribute to their use in traditional and herbal medicine. These leaves contain polyphenols (e.g., catechins, chlorogenic acid, rutin, naringin), vitamin C, carotenoids, and trace elements, all of which possess antioxidant and antimicrobial; properties [3-4].

The three predominant compounds in apricot leaves - quercetin-3-O-rutinoside, 5-O-caffeoylquinic acid, and 3-O-caffeoylquinic acid - have demonstrated significant anti-obesity effects by inhibiting pancreatic lipase and cyclooxygenase-1, as well as notable antioxidant capacity, particularly oxygen radical absorbance capacity, which strongly correlates with polyphenolic content [5].

Apricot leaf tea has been valued for its mild detoxifying and digestive benefits, providing a natural approach to promoting health. Fresh apricot leaf juice is used in both Armenian and Chinese traditional medicine to treat skin conditions such as scabies, eczema, sunburn, and cold-induced itching. Additionally, apricot leaves have been employed for oral health, including relieving toothaches and treating stomatitis, as well as addressing ailments like diarrhea and diphtheria [6-7].

However, the increasing use of agrochemicals in apricot orchards may alter the levels of these secondary metabolites, potentially affecting their health benefits [8–11]. Despite this concern, such studies remain underexplored in literature. Additionally, there is a lack of research on the antibacterial and antifungal properties of apricot leaf extracts in relation to their phenolic compounds.

This study aimed to (1) evaluate the antimicrobial activity of apricot leaf extracts from the *Erevani* variety following treatment of the trees with the insecticide Confidor (Bayer Co.) and the fungicide Topaz (Syngenta Co.), after complete detoxification of their residues in the leaves. (2) identify correlations between the antimicrobial activity of the examined samples and the phenolic content of the leaves.

MATERIALS AND METHODS

The research material comprised of leaves from the native Yerevani apricot cultivar, which were collected from orchards in the Kotayk region of the Republic of Armenia (RA)

Spraying: The study utilized the fungicide Topaz and the insecticide Confidor, both of which are commonly used in Armenia. Confidor (Bayer Co) is a contact and systemic insecticide that provides effective control of a wide range of insect pests. It is a neonicotinoid insecticide that works by disrupting the nervous system of insects. The active ingredient is imidacloprid: (2E)-1-[6-chloropyridin-3-yl]-N-nitroimidazolidin-2-imin).

Topaz (Syngenta Co) is a highly effective systemic fungicide for prophylactic and direct action against stone fruits (including apricot), wheat, sorghum, citrus and others. The active ingredient is penconazole: 1-(2,4-dichloro-β)-propylphenethyl)-1H-1,2,4-triazole. Forty young apricot trees (less than 8 years old) were selected for the study and treated twice with 0.1% Confidor and

0.1% Topaz, either singly or combined, in accordance with standard agricultural practices. The first application was made immediately after blooming, and the second, 20 days later. Trees treated with water served as the Control.

Sampling: Leaves were collected after the second spraying, once full detoxification of Confidor and Topaz was achieved in the leaves. The leaves were fixed in a steam bath for 10 minutes, dried at room temperature in the absence of direct sunlight, ground into powder using an electric blender, and sieved through a 1.0 mm mesh.

Antimicrobial Screening

Extraction: Ground, air-dried leaves were extracted with distilled water for 20 minutes. The extract was then centrifuged for 5 minutes at 8000 rpm, and the supernatant was used for further analysis. The sample was diluted to achieve a concentration of 10 mg/mL. Antimicrobial activity of the extracts was investigated by agar well diffusion assay (Method of Wells) [12]. Briefly, a hole with a diameter of 5,0 mm was made on the agar seeded with test microorganism. 0,2 mL of test extract was introduced into the hole in the Petri dishes. The dishes were put in a refrigerator at "10°C" for 20-24 hrs. and then incubated at "37°C" for 24 hrs. The antimicrobial activity of the extract was determined by measuring the size of a growth inhibition zone around the sample. The microbial strains used in the assay were obtained as pure cultures from the Museum of the Republican Center for the Deposition of Microorganisms and the culture collection of the Faculty of Microbiology and Biotechnology of Plants and Microorganisms (RA).

Quantitative Determination of Polyphenol Compounds

Reagents: ethanol, sodium phosphomolybdate and sodium tungstate, (+) catechin, quercetin, $KMnO_4$, H_2SO_4. All reagents were of analytical grade [13].

Tannins were determined by titration with a 0.02 N solution of $KMnO_4$, using indigo carmine as the indicator, to the endpoint of a golden yellow color. Total phenolic content was assessed using the Folin-Denis's reagent (sodium phosphomolybdate and sodium tungstate), which reacts with phenolic compounds to form colored complexes. Water control with all reagents was used. The optical density (OD) of the extracts was measured at 765 nm after a 30-minute incubation. The phenolic content was expressed as catechin equivalents (mg/100g). Total flavonoids were determined calorimetrically following extraction with solvent and reacting with $AlCl_3$. Flavonoids with hydroxyl groups in the C3 and C5 positions form yellow chelates, which can be measured. The optical density was measured at 440 nm, and total flavonoid content was expressed as quercetin or rutin equivalents per 100 g of dried leaves (mg/100 g). The total phenolic compounds, tannins, and flavonoids were calculated based on dry weight of the leaves. Determination of Dry Matter in fresh material was carried out by drying the samples at 100 -105°C in a thermostat until they reached a constant weight.

Statistical analysis: Data was collected in triplicate and reported as the mean ± standard deviation. Statistical analysis was performed using a Two-Factor Without Replication ANOVA and regression analysis. The Least Significant Difference (LSD) test was used, in which $P < 0.05$ was regarded statistically significant.

RESULTS

Antimicrobial activity: Apricot leaf aqueous extracts were tested for antimicrobial activity against both Gram-positive bacteria (*Staphylococcus citreus*, *Staphylococcus aureus*, *Bacillus megatherium*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*), and the fungus *Candida guillermondii*. The results are shown in Figure 1 and Table 1.

When interpreting the results, it was conventionally accepted that the diameter of the growth inhibition of a microorganism over 2,0 cm is high activity, 1,0-2,0 cm - moderate activity, less than 1,0 cm - low activity. Among the tested microorganisms, *Staphylococcus citreus* (with inhibition zones ranging from 2.53 to 3.37 cm) and *Salmonella typhimurium* (1.8–2.4 cm) were most affected by the apricot leaf extracts.

Extracts from untreated trees (Control) exhibited the highest activity against *Staphylococcus citreus* (3.37±0.02 cm), while extracts from trees treated with *Topaz + Confidor* showed the lowest activity (2.53±0.11 cm). The strongest inhibitory effect against the Gram-negative bacterium *Salmonella typhimurium* was observed in the extracts from *Topaz*-treated trees (2.4±0.43 cm). Notably, *Escherichia coli*, which was resistant to extracts from Control and *Confidor*-treated trees, showed sensitivity to extracts from *Topaz* (0.7±0.00 cm) and *Topaz+Confidor*-treated trees (0.77±0.14 cm).

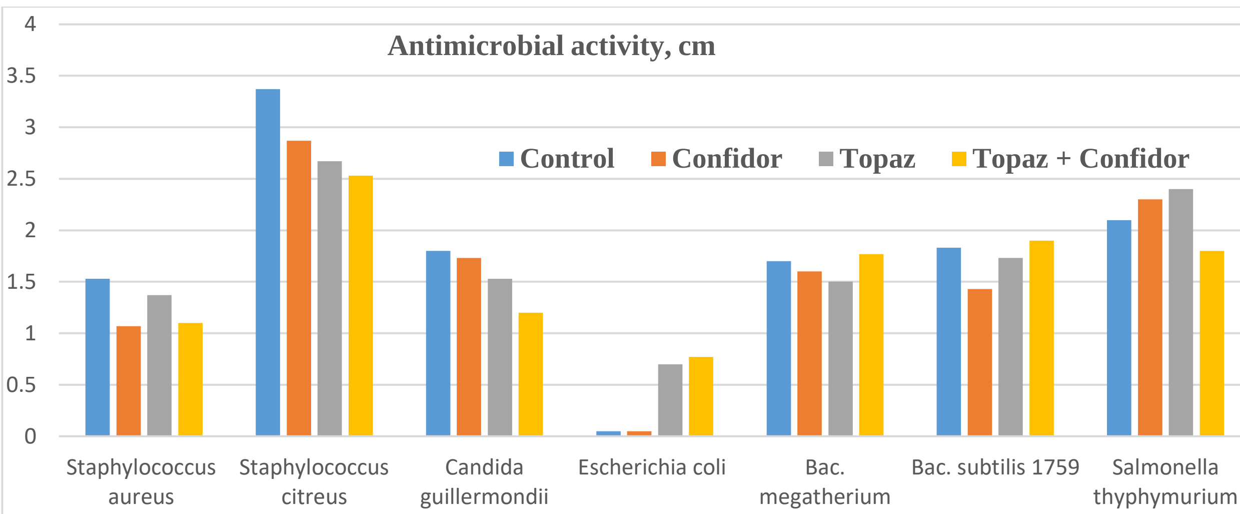


Figure 1. Antimicrobial Activity of Dried Apricot Leaf Extracts at the background of pesticide-treated trees.

Table 1. Antimicrobial Activity of Dried Apricot Leaf Extracts from trees treated with pesticides

Test-organism	Control	LSD	Topaz	Topaz + Confidor	LSD (P<0.05)
	Diameter of inhibitory zone, cm				
Bacteria					
Staphylococcus aureus st.205	1,53±0,04	1,07±0,02	1,37±0,02	1,1±0,08	0.37
Staphylococcus citreus	3,37±0,02	2,87±0,11	2,67±0,10	2,53±0,11	0.28
Bac. megatherium	1,7±0,01	1,6±0,05	1,5±0,06	1,77±0,04	0.20
Bac. subtilis 1759	1,83±0,10	1,43±0,04	1,73±0,07	1,9±0,01	0.30
Escherichia coli M 17	0	0	0,7±00	0,77±0,14	-
Salmonella thyphymurium	2,1±0,08	2,3±019	2,4±0.43	1,8±0.22	0.55
Fungi					
Candida guillermondii	1,8±0,05	1,73±0.11	1,53±0,04	1,2±0,09	0.42

Results of Antifungal Activity: The antifungal effect of apricot leaf extracts was tested against the common pathogenic fungus *Candida guillermondii*. The aqueous extract of apricot leaves demonstrated moderate antifungal activity against the fungus, with inhibition zones ranging from 1.2 to 1.8 cm. Extracts from untreated (Control) trees exhibited the highest antifungal activity, showing an inhibition zone of 1.8±0.14 cm against *Candida guillermondii.*

The results revealed variations in the antimicrobial activity of apricot leaf extracts, depending on the type of agricultural treatment the trees had undergone.

Phenolic Compounds: Our experiments (Table 2) revealed that pesticide treatment of apricot trees increased the accumulation of dry matter and total phenolic compounds in the leaves. The highest levels were observed with the fungicide *Topaz* (41.1% and 813±41 mg/100g, respectively), followed by the combination of *Topaz* and *Confidor* (37.6% and 734±29 mg/100g, respectively).

The tannin content varied significantly between treatments, ranging from 11.64% to 17.15%. Leaves from trees treated with *Topaz* had the highest tannin content (17.15±0.96%), while those treated with *Confidor* had the lowest (11.64±0.27%).

Flavonoids, however, showed a different trend. The highest flavonoid content was found in the Control leaves (1.74±0.05 mg/100g), while the lowest was in the leaves treated with *Topaz* (0.68±0.04 mg/100g). When *Topaz* and *Confidor* were applied together, flavonoid levels were lower than in the Control but higher than in the *Topaz*-only treatment (1.00±0.08 mg/100g).

Table 2. Dry matter and phenolic compounds in apricot leaves treated with pesticides (P<0.05)

Option	Dry matter, %	Total FC (mg /100g)	Tannins, %	Flavonoids, (mg /100 g)
		LSD=47	LSD=0,63	LSD=0,63
Control	34.7	625±00	12,47±0,54	1,74±0,05
Confidor	36,2	686±81	11,64±0,27	1,52±0,02
Topaz	41,1	813±41	17,15±0,96	0,68±0,04
Topaz + Confidor	37,6	734±29	12,89±0,54	1,00±0,08

The phenolic compound content in the studied leaves was shaped by the plant's natural metabolic processes, which were modified by the applied pesticides.

Regression analysis and Correlation Establishment: To generalize the results obtained, regression analysis was conducted using Microsoft Excel software. The dependent variable (y) represented the inhibition zone of the microorganisms (d, in cm), while the independent variable (x) corresponded to the concentration of the studied phenolic compound. The regression curves for total phenolic compounds (PC), tannins, flavonoids, and inhibitory zones of Gram-positive and Gram-negative bacteria, as well as fungi, are presented in Figure 2.

The analysis revealed that the inhibitory activity of apricot leaf extracts against the tested microorganisms' correlates either positively or negatively with the concentration of total phenolic compounds. The curves are modeled using third-order polynomial correlations with R^2=1, indicating a strong fit. However, this suggests that factors other than phenolic compounds may influence the inhibitory activity of the extracts.

For bacteria in the *Bacillus* family, including *Bacillus megatherium* and *Bacillus subtilis* 1759, the correlations were negative ($y = -5E-07x^3 + 0.0012x^2 - 0.8211x + 194.4$, $R^2=1$; $y = -1E-06x^3 + 0.0028x^2 - 2.0088x + 478.74$, $R^2=1$). This indicates that the inhibitory activity of the extracts may decrease at higher concentrations of total phenolic compounds in the extract (Fiure 2a).

Notably, at the peak of inhibitory activity against these bacteria, an opposite phenomenon is observed: reactivation of the extracts occurs against *Staphylococcus citreus*, *Salmonella typhimurium*, and the fungus *Candida guilliermondii.*

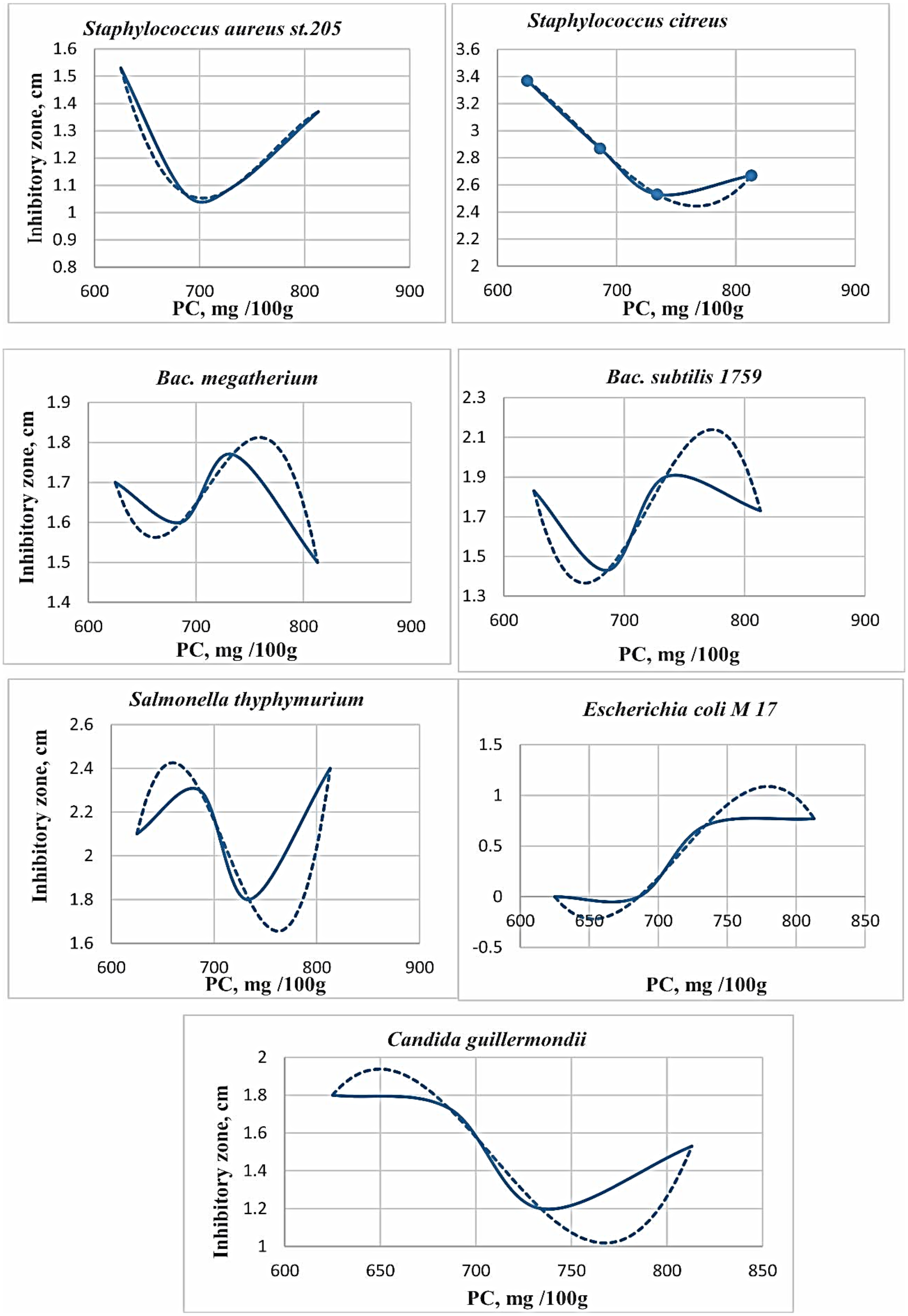
Another group of polyphenolic compounds found in apricot leaves in significant quantities are tannins. Extensive literature analyses have demonstrated that tannins exhibit antimicrobial activity against a wide range of microorganisms, including fungi, yeasts, and bacteria [14-16]. Our regression analysis results indicated a strong correlation between the antibacterial and antifungal activities of tannins in apricot leaf extracts and the growth inhibition of microorganisms across different groups (Figure 2b). The curves are described by positive third-order polynomial correlations: (*Staphylococcus aureus* st. 205: $y = 0.2713x^3 - 11.3x^2 + 154.67x - 696.18$, $R^2=1$; *Staphylococcus citreus*: $y = 0.4567x^3 - 18.979x^2 + 259.01x - 1160.8$, $R^2=1$; *Salmonella typhimurium*: $y = 0.1019x^3 - 4.1483x^2 + 55.34x - 240.48$, $R^2=1$; Fungus *Candida guilliermondii*: $y = 0.2781x^3 - 11.499x^2 + 156.04x - 695.19$, $R^2=1$).

These results suggest that higher concentrations of tannins (exceeding 13%) are unfavorable for the growth of these microorganisms. Moreover, the inhibitory activity of apricot leaf extracts could be enhanced as the tannin concentration increases.

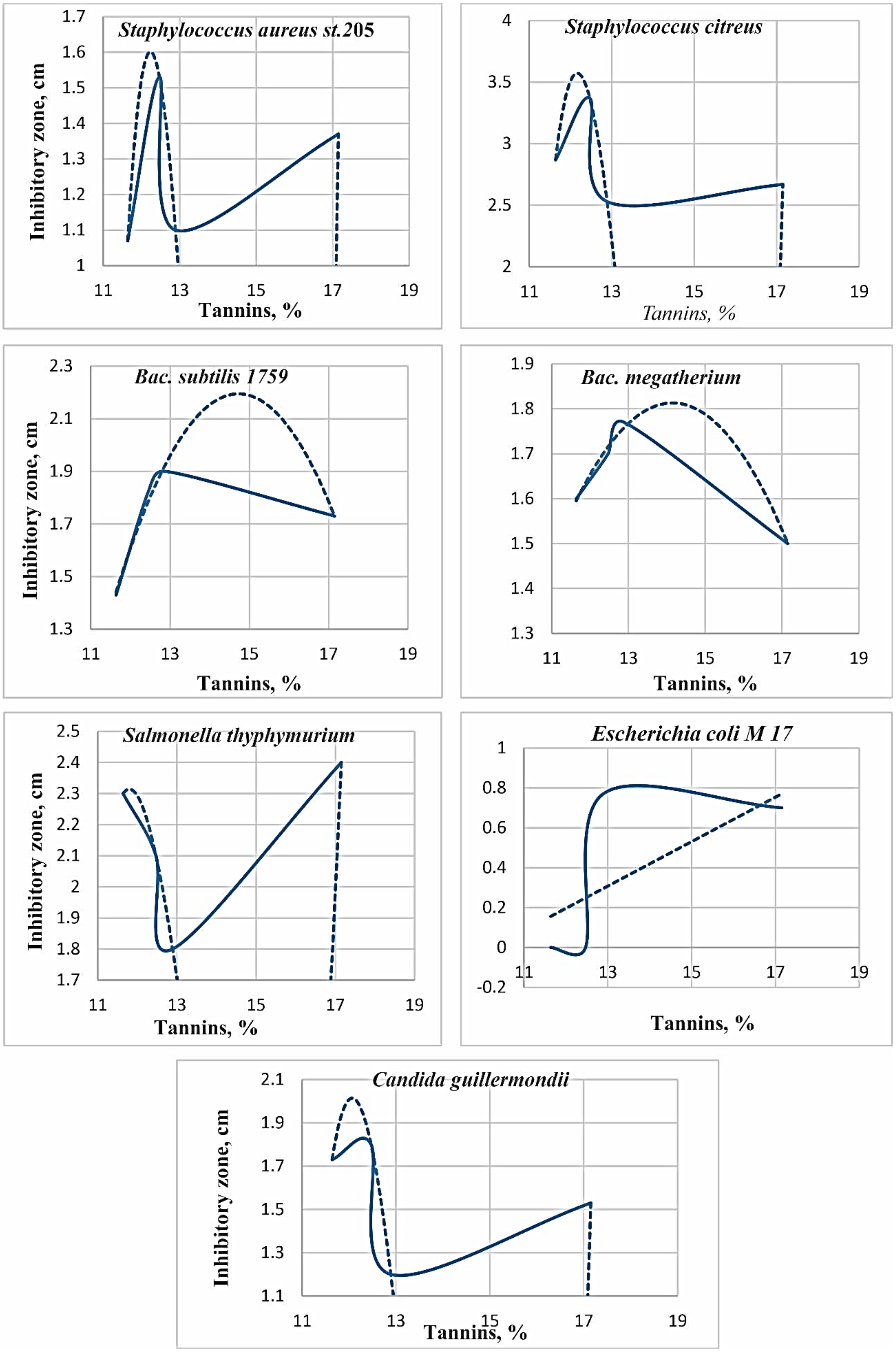
As to *Bac. megatherium* and *Bac. subtilis 1759*, the curves are polynomial 2 order negative correlations: $y = -0.0347x^2 + 0.9807x - 5.1234$ and $y = -0.0792x^2 + 2.3312x - 14.97$ with correlation coefficients 0.9903 and 0.9817, respectively. The inhibitory activity of apricot leaf extracts could be decreased alongside the concentration of tannins.

From these equations, could be roughly calculated the maximal and minimal inhibitory activities of the leaf extracts against tested microorganisms: the least growth for Gram-positive bacteria *Staphylococcus aureus st. 205*, *Staphylococcus citreus*, for Gram negative bacteria *Salmonella thyphymurium* and fungus *Candida guillermondii* is observed when the concentration of tannins in the extracts was achieved up to 12,8%.

For *Bacillus megatherium* and *Bacillus subtilis* 1759, the regression curves are represented by second-order negative polynomial correlations: $y = -0.0347x^2 + 0.9807x - 5.1234$ and $y = -0.0792x^2 + 2.3312x - 14.97$ with a correlation coefficient of $R^2 = 0.9903$ and 0.9817, respectively. These correlations suggest that the inhibitory activity of apricot leaf extracts decreases as the concentration of tannins increases. From these equations, could be roughly calculated the maximal and minimal inhibitory activities of the leaf extracts against tested microorganisms: the least growth for Gram-positive bacteria *Staphylococcus aureus st. 205*, *Staphylococcus citreus*, for Gram negative bacteria *Salmonella thyphymurium* and fungus *Candida guillermondii* is observed when the concentration of tannins in the extracts achieved up to 12.


Staphylococcus aureus st.205
Inhibitory zone, cm
PC, mg /100g
Staphylococcus citreus
PC, mg /100g
Bac. megatherium
Inhibitory zone, cm
PC, mg /100g
Bac. subtilis 1759
PC, mg /100g
Salmonella thyphymurium
Inhibitory zone, cm
PC, mg /100g
Escherichia coli M 17
PC, mg /100g
Candida guillermondii
Inhibitory zone, cm
PC, mg /100g

(2a)



Staphylococcus aureus st.205
Staphylococcus citreus
Bac. subtilis 1759
Bac. megatherium
Salmonella thyphymurium
Escherichia coli M 17
Candida guillermondii
Inhibitory zone, cm
Tannins, %

(2b)

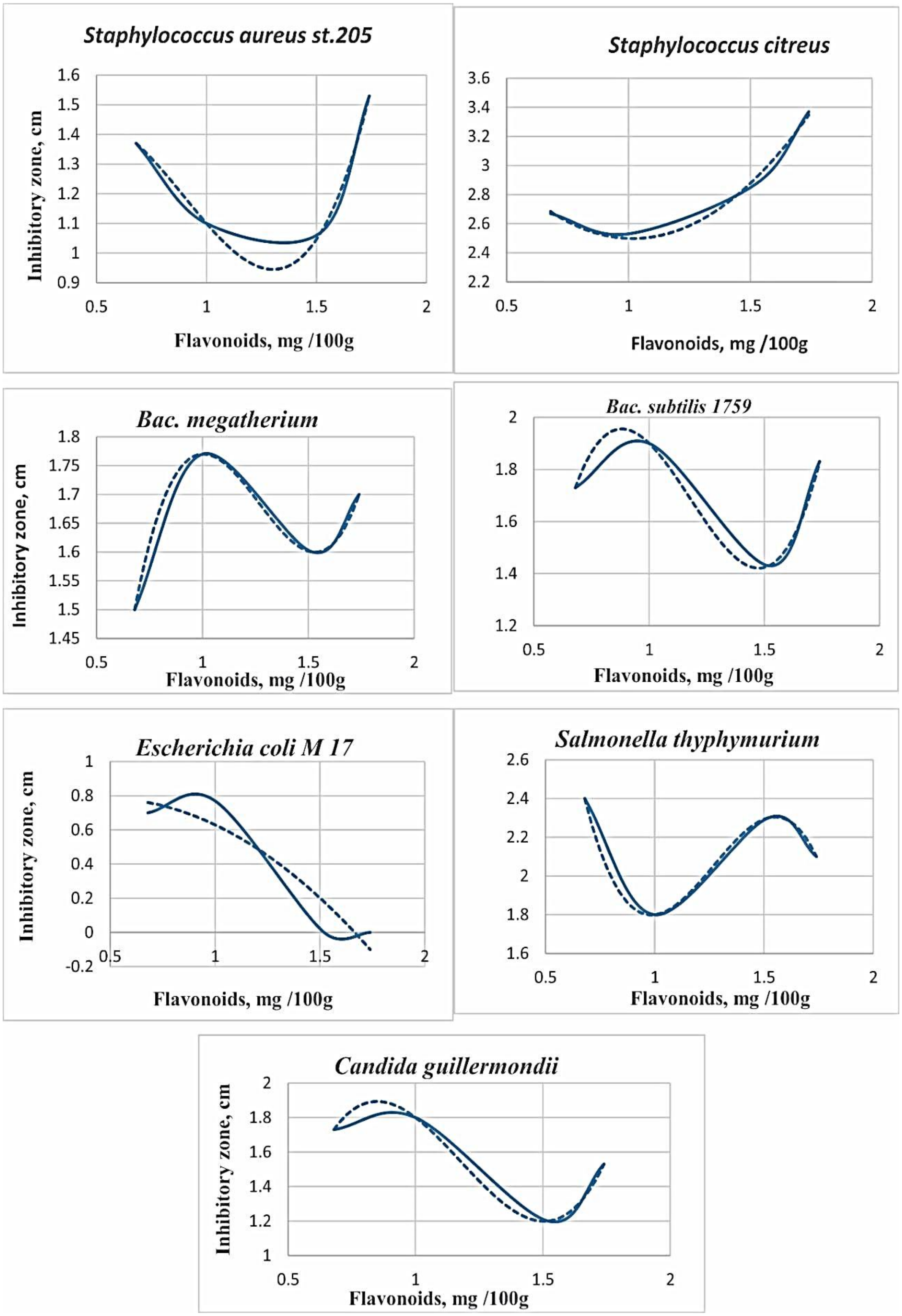


Figure 2. Correlation between the antimicrobial activity of total PC (a), tannins (b) and flavonoids (c) and antimicrobial activity of leaf extract.

Flavonoids are phenolic compounds well known for their antimicrobial, antiviral, and anti-inflammatory properties [17–19]. Their antibacterial activities have garnered increasing attention. When considering the role of flavonoids in the inhibitory activity of apricot leaf extracts against Gram-positive bacteria such as *Staphylococcus aureus* st. 205, *Staphylococcus citreus*, *Bacillus megatherium*, *Bacillus subtilis* 1759, and the fungus *Candida guilliermondii*, positive regressions are observed. This overall indicates that the inhibitory activity of the extracts against these microorganisms tends to increase, after some fluctuations, as the flavonoid content in the extracts rises (Figure 2c). In contrast, regarding the inhibitory activity of the extracts against Gram-negative bacteria such as *Salmonella typhymurium* and *Escherichia coli* M17, the regressions are negative ($y = -5.5705x^3 + 21.202x^2 - 25.561x + 11.729$, $R^2 = 1$; $y = -0.5455x^2 + 0.5084x + 0.6671$, $R^2 = 0.8794$, respectively). This indicates that the inhibitory activity of apricot leaf extracts decreases at higher concentrations of flavonoids in the extract.

DISCUSSION

The antibacterial and antifungal activities of the herbs are mainly attributed to the presence of various biologically active substances, usually related to secondary metabolic products. Among these secondary metabolites, phenolic compounds appear to be the most important due to their promising antioxidant and antimicrobial activities, as demonstrated in both *in vivo* and *vitro* studies [20-24]. Polyphenolic compounds have been widely used to combat pathogenic bacteria [25-26]. and show diverse mechanisms of action against different microbial strains [27-28].

Our previous investigations revealed that the phytochemical composition of apricot leaves includes a complex mix of phenolic compounds, primarily condensed tannins, flavones, flavanols, 5-hydroxy flavanols, 5-hydroxy flavones, chalcones, aurons, catechins, and coumarins. Tannins were found to be the most abundant, reaching up to 23.6% in the leaves of young trees when extracted with ethanol. The optical density of these extracts was measured using a self-registering UV-1700 spectrophotometer (Shimadzu, Japan), with a 1 mm cuvette at wavelengths ranging from 400 to 600 nm. The maximum optical density was observed at 465 nm, confirming the presence of flavonols, particularly quercetin, in apricot leaves [29].

Our analysis revealed that the accumulation and preservation of phenolic compounds in apricot leaves were largely affected by the type of pesticide used on the trees. The highest concentrations of dry matter, total phenolic compounds (PC), and tannins were found when the fungicide *Topaz* was used (41.1%, 813±41 mg/100g, 17.15±0.96%, respectively) or when *Topaz* and *Confidor* were applied together (37.6%, 734±29 mg/100g, 12.89±0.54%, respectively).

A different pattern emerged when we examined the effect of these pesticides on flavonoid content. The highest concentration of flavonoids was found in the Control leaves (1.74±0.05 mg/100g), while the lowest concentration was found in the *Topaz* variant (0.68±0.04 mg/100g). When *Topaz* and *Confidor* were used together, the reduction in flavonoid content was less pronounced (1.00±0.08 mg/100g).

It is noteworthy that the increase in the content of total phenolic compounds and tannins was accompanied by a reduction in flavonoid levels. These changes are likely linked to the increased activity of enzymes involved in the synthesis of plant phenolic compounds, such as shikimate dehydrogenase, phenylalanine ammonia lyase, and cinnamate dehydrogenase, in response to pesticide exposure. Additionally, the rise in phenolic compound levels may be a protective response by the plant cells [30-32]. Secondary metabolites such as flavonoids, phenolics, stilbenes, hydroxycinnamic acids, etc., are produced in extremely lower concentrations within plants under normal environmental conditions for signaling and metabolism. Phenolic compounds and flavonoids play crucial roles in regulating plant development and tolerance to various biotic and abiotic stresses, including light, drought, salinity, UV radiation, soil pH, temperature, heavy metals, agrochemicals, microbial infections, and climate changes. The accumulation of these compounds depends on the plant's adaptive capacity [30-33]. These compounds provide resistance to the plant and regulate multiple of its functions. Some polyphenolic compounds serve as a scavenger that removes the ROS molecules generated during oxidative bursts, and some of them function as protective in nature, such as flavonoids and anthocyanins [9-10]. Under biotic and abiotic stresses, plants enhance phenolic compounds to avoid any toxic effects.

Several reviews have discussed how agrochemicals can enhance the accumulation of phenolic compounds in plants. The accumulation of phenolic compounds results from upregulating key biosynthetic genes and activating the key enzymes of phenylpropanoid-branched chain reactions such as PAL and CHS [34-36]. In some species, pesticides can affect flavonoid accumulation. For instance, the application of pesticides and fertilizers in the field led to increased flavonoid levels in leek but had variable effects in parsley and significantly reduced their levels in celery and lettuce [11].

Phenolic compounds serve various ecological functions in plants and, on the other hand, also have significant inhibitory effects on the bacteria and yeasts. Our analysis showed that the inhibitory activity of apricot leaf extracts against the tested microorganisms correlated with the concentration of total phenolic compounds, tannins, and flavonoids, as determined by polynomial regression equations of the third order.

The Gram-positive bacteria *Bacillus subtilis* 175 and *Bacillus megatherium* showed less susceptibility to higher concentrations of total phenolic compounds and tannins compared to other tested microorganisms. These findings were not entirely unexpected, as these bacteria form resting spores, making them more resistant to environmental conditions and oxidizing agents than other bacteria [37]. Literature suggests that some plant-derived metabolites can act as protectants for microbial cells against oxidative stress and may even promote their growth. Notably, *Bacillus subtilis* has developed mechanisms for tannin degradation in its natural habitat, which is an interesting ecological observation [38].

Our experiments also revealed differences in susceptibility between Gram-positive and Gram-negative bacteria to apricot leaf extracts. Extracts rich in flavonoids exhibited higher inhibitory activity against Gram-positive bacteria, such as *Staphylococcus aureus* st.205 (1.53±0.63 cm) and *Staphylococcus citreus* (3.37±0.14 cm). In contrast, higher flavonoid concentrations resulted in reduced activity against Gram-negative bacteria like *Salmonella thyphymurium* and *Escherichia coli*. Interestingly, the flavonoid-rich leaf extracts from untreated (*Control*) trees and trees treated with *Confidor* showed no inhibitory effect on *E. coli*. This discrepancy between Gram-positive and Gram-negative bacteria in their response to flavonoids has been noted as well by Osonga F.J. [39]. The difference in flavonoid (quercetin) susceptibility may be partly due to differences in the composition of the cell membranes of Gram-positive and Gram-negative bacteria. Lipophilic flavonoids are particularly effective against Gram-positive bacteria because they can pass through the bacterial cell wall, interact with the internal membrane, interfere with microbial metabolism, and ultimately cause cell destruction [40].

The antifungal activity of apricot leaf extracts was assessed against the common pathogenic fungus *Candida guillermondii*. The aqueous extracts exhibited moderate antifungal activity, with the highest inhibition observed in flavonoid-rich leaf extracts from young untreated (*Control*) trees (1.8±0.14 cm).

These findings further support the notion that herbal extracts, even from plants of the same genus, can exhibit varying effects on different microbial species due to differences in chemical composition and the interactions of these components with each other and with the metabolism of the test organisms.

Thus, apricot leaf extracts, rich in flavonoids, demonstrate significant antibacterial activity, particularly against the *Staphylococcus* genus. This group includes strains that are leading causes of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. In addition to their efficacy against *Staphylococcus* species, apricot leaf extracts, which are rich in tannins and total phenolic compounds, have also shown high activity against the Gram-negative bacterium *Salmonella typhymurium*. This pathogen, while primarily associated with enteric diseases, can also cause a range of conditions, notably gastroenteritis and septicemic diseases.

Given these properties, apricot leaves hold promises for incorporation into functional food applications (as product for tea) and oral or skincare products. This dual utility in combating harmful bacteria and supporting traditional wellness practices suggests potential for apricot leaf extracts to bridge modern scientific innovation and time-tested herbal remedies.

Apricot leaves deserve further investigation as a promising medicinal herb, particularly since our previous studies demonstrated that apricot leaf extracts show no cytotoxicity *in vitro* [41].

CONCLUSION

Summarizing the results of our experiments, we can state that the total phenolic compounds (PC), tannins, and flavonoids present in apricot leaf extracts exhibit varying inhibitory properties against the tested microorganisms. The mode of action appears to depend on the specific microorganism species.

The inhibitory activity of these extracts against the tested microorganisms - including Gram-positive bacteria (*Staphylococcus citreus, Staphylococcus aureus, Bacillus megatherium, Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli, Salmonella typhymurium*), and fungi (*Candida guilliermondii*) - correlated with the concentration of total PCs, tannins, and flavonoids in the leaves. This relationship can be mathematically described using third-order polynomial equations.

Our data also indicate that the use of pesticides (*Topaz* and *Confidor*) affects the content of total phenolic compounds, tannins, and flavonoids in the leaves of treated trees. This variation in chemical composition explains the differences in antimicrobial activities of the tested extracts on the same microorganisms.

Abbreviations: PC, phenolic compounds, LSD, the least significant difference, phenylalanine ammonia lyase, PAL, chalcone synthase, CHS.

Authors' contribution: VSM, RMH, and TDK conceived and designed the work. VSM and RMH determined phenolic compounds, while NZS performed antibacterial activity assays. VSM and AMG analyzed and interpreted the results. VSM and RMH drafted the manuscript, with AMG advising, contributing to writing, and editing. All authors read and approved the final manuscript.

Novelty: The novelty of the presented research lies in the investigation of the antimicrobial activity of apricot leaf aqueous extracts in relation to the content of phenolic compounds. The extracts were prepared in a way that maximally preserved the natural composition of the plant. Changes in the content of phenolic compounds, tannins, and flavonoids occurred because of the plant's natural metabolic processes, which were influenced by pesticide treatments. To the best of our knowledge, no similar studies have been conducted previously.

Acknowledgements: The authors would like to acknowledge the Head of the Department of Microbiology and Biotechnology of Plants and Microorganisms, as well as the Head of the Museum of the Republican Center of Deposition of Microorganisms of Armenia, for their cooperation and support in obtaining pure cultures of the microbial strains. The authors are also deeply grateful to the local apricot growers for their permission to conduct experiments and collect samples during the field surveys.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES:

1. Martirosyan D.M., Lampert T., Ekblad M. Classification and regulation of functional food proposed by the functional food Center. Functional Food Science 2022, 2(2), 25-46. DOI: https://doi.org/10.31989/ffs.v2i2.890
2. Martirosyan DM, Stratton S. Quantum and tempus theories of function food science in practice. Functional Food Science 2023, 3(5), 55-62. DOI: https://www.doi.org/10.31989/ffs.v3i5.1122
3. Keçe Murat Y., Yaman M., Tunç Y., Yilmaz K U., et al. Characterization of apricot cultivars; nutrient content, biochemical content andantioxidant activity in leaves. Genet Resour Crop Evol, 2024. DOI: https://doi.org/10.1007/s10722-024-02193-y
4. Zargar SA, Wani AA, Saggoo MIS, Kumar N, Mir JI, Jan S, Sabbou S. Chemical quality attributes, phenolic compounds, and antioxidant properties of wild and cultivated apricot (Prunus armeniaca L.) accessions of North-Western Himalayas. Erwerbs-Obstbau 2023, 65, 2325-2336. DOI: https://doi.org/10.1007/s10341-023-00937-1
5. Wojdyło A., Nowicka P. Profile of Phenolic Compounds of Prunus armeniaca L. Leaf Extract Determined by LCESI-QTOF-MS/MS and Their Antioxidant, Anti-Diabetic, Anti-Cholinesterase, and Anti-Inflammatory Potency. Antioxidants 2021, 10, 1869. DOI: https://doi.org/10.3390/antiox10121869
6. Amasiaci A. Useless for Ignoramus. (In Russian) M. Science, 1990, 880(294).
7. Yeung. Him-Che. Handbook of Chinese Herbs and Formulas. Institute of Chinese Medicine, Los Angeles 1995.
8. Sharma A., V. Kumar B., Shahzad M. Tanveer G.P.S. Sidhu, N. Handa, S.K. Kohli, P. Yadav, A.S. Bali, R.D. Parihar, et al. Worldwide pesticide usage and its impacts on ecosystem, SN Appl. Sci., 2019, 1, 1446. DOI: https://doi.org//10.1007/s42452-019-1485-1
9. Sharma A., Kumar V., Thukral A.K., Bhardwaj R. Epibrassinolide-imidacloprid interaction enhances non-enzymatic antioxidants in Brassica juncea L. Ind. J. Plant Physiol. 2016, 21, 70–75. DOI: https://doi.org/10.1007/s40502-016-0203-x
10. Sharma A.,Thakur S., Kumar V., Kanwar M.K., et al. Pre-sowing Seed Treatment with 24-Epibrassinolide Ameliorates Pesticide Stress in Brassica juncea L. through the Modulation of Stress Markers. Front. Plant Sci. 2016, 7, 1569. DOI: https://doi.org/10.3389/fpls.2016.01569
11. Akono EN, Faustin PTM, Jean S, et al. Effect of Agrochemicals Use on Total Phenolic Compounds and Flavonoid Content in Aromatic Fresh Herbs from Santa (Cameroon). Academia Journal of Agricultural Research 2017, 5(2), 018-027. DOI: https://doi.org/10.15413/ajar.2017.0207
12. Zolotarev P.N. Evaluation of antibacterial activity of medicinal layers containing II fraction of carbohydrate extract of the flowers Caryophyllus aromaticus L. Series of Natural Science 2006, 9(49), 145-154.
13. Yermakov A.I., Arasimovich V.V., Yarosh N.P. Methods of biochemical research of plants. Agropromizdat, 1987, 430.
14. Czerkas K, Olchowik-Grabarek E, Lomanowska M, Abdulladjanova N, Sekowski S. Antibacterial activity of plant

polyphenols belonging to the tannins against streptococcus mutans—potential against dental caries. Molecules. 2024; 29(4):879.DOI: https://doi.org/10.3390/molecules29040879

15. Wang W, Cao J, Yang J, Niu X, Liu X, Zhai Y. Antimicrobial Activity of Tannic Acid In Vitro and Its Protective Effect on Mice against Clostridioides difficile. Microbiol Spectr 2022, 11 (1), e02618-22. DOI: https://doi.org/10.1128/spectrum.02618-22
16. Chevallier P., Wiggers HJ, Copes F. et al. Prolonged Antibacterial Activity in Tannic Acid–Iron Complexed Chitosan Films for Medical Device Applications. Nanomaterials 2023, 13(3), 484. DOI: https://doi.org/10.3390/nano13030484
17. Aghababaei F., Hadidi M. Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals 2023, 16, 1020. DOI: https://doi.org/10.3390/ph16071020
18. Kim S., Shin Y., Choi Y., Lim K.-M., Jeong Y., Dayem A.A., et al. Improved wound healing and skin regeneration ability of 3, 2-dihydroxyflavone-treated mesenchymal stem cell-derived extracellular vesicles. Int J Mol Sci, 2023, 8:6964 DOI: https://doi.org/10.3390/ijms24086964
19. Lee JH, Kim YG, Park I, Lee J. Antifungal and antibiofilm activities of flavonoids against Candida albicans: Focus on 3,2'-dihydroxyflavone as a potential therapeutic agent. Biofilm 2024, 8, 100218. DOI: https://doi.org/10.1016/j.bioflm.2024.100218
20. Iriti M., Varoni E. M., Vitalini S. Bioactive Compounds in Health and Disease – Focus on Rutin. Bioactive Compounds in Health and Disease 2023, 6(10), 35-242. DOI: https://www.doi.org/10.31989/bchd.v6i10.1145
21. Abdulqahar F. W., Mahdi Z. I., Al-kubaisy S.H. M., Hussein F. F., Kurbonova M., El-Said M. M., El Messery T. M. Computational study of antiviral, anti-bacterial, and anticancer activity of green-extracted Sidr (Ziziphus spina-Christi) fruit phenolics. Bioactive Compounds in Health and Disease 2023, 6(10), 271-291. DOI: https://www.doi.org/10.31989/bchd.v6i10.1192
22. Sun W., Shahrajabian M.H. Therapeutic potential of phenolic compounds in medicinal plants-Natural health products for human health. Molecules 2023, 28, 1845. DOI: https://doi.org/10.3390/molecules28041845
23. Ispiryan A., Atkociuniene V., Makstutiene N., et al. Correlation between Antimicrobial Activity Values and Total Phenolic Content/Antioxidant Activity in Rubus idaeus L. Plants 2024, 13(4), 504. DOI: https://doi.org/10.3390/plants13040504
24. Gomez-Molina M., Albaladejo-Marico L., Yepes-Molina L., et al. Exploring Phenolic Compounds in Crop By-Products for Cosmetic Efficacy Int. J. Mol. Sci. 2024, 25(11), 5884. DOI: https://doi.org/10.3390/ijms25115884
25. Zhang L., Yan, Y., Zhu J., Xia X., Yuan G., Li, S., Deng B., Luo X. Quinone pool, a key target of plant flavonoids inhibiting gram-positive bacteria. Molecules 2023, 28, 4972. DOI: https://doi.org/10.3390/molecules28134972
26. Kováč J., Slobodníková L., Trajčíková E.,et al.. Therapeutic Potential of Flavonoids and Tannins in Management of Oral Infectious Diseases - A Review. Molecules 2022, 28(1), 158. DOI: https://doi: 10.3390/molecules28010158
27. Yu Yan, Xuexue Xia, Aiman Fatima, Li Zhang, Ganjun Yuan, Fengxian Lian, Yu Wang Antibacterial Activity and Mechanisms of Plant Flavonoids against Gram-Negative Bacteria Based on the Antibacterial Statistical Model. Pharmaceuticals 2024, 17(3), 292. DOI: https://doi.org/10.3390/ph17030292
28. Faleye OS, Lee JH, Lee J. Selected flavonoids exhibit antibiofilm and antibacterial effects against Vibrio by disrupting membrane integrity, virulence and metabolic activities. Biofilm 2023, 6, 100165. DOI: https://doi.org/10.1016/j.bioflm.2023.100165
29. Karapetyan T.D., Mirzoyan V.S., Hanisyan R.M. Qualitative and quantitative determination of flavonoids in apricot leaves (*Armeniaca Vulgaris L*) (Rosacea) leaves. Biological Journal of Armenia 2022, 74 (2), 53-58. DOI: https://doi.org/10.54503/0366-5119-2022.74.2-53
30. Zagoskina N.V., Zubova M.Y., Nechaeva T. L., et al. Polyphenols in Plants: Structure, Biosynthesis, Abiotic Stress Regulation, and Practical Applications (Review) Int. J. Mol. Sci. 2023, 24(18), 13874. D DOI: https://doi.org/10.3390/ijms241813874
31. Salam U., Ullah S., Tang Z.H., Elateeq A.A., Khan Y., Khan J., Khan A., Ali S. Plant metabolomics: An overview of the role of primary and secondary metabolites against different environmental stress factors. Life 2023, 13,706. DOI: https://doi.org/10.3390/life13030706
32. Zhan X., Chen Z., Chen R., Shen C. Environmental and genetic factors involved in plant protection-associated secondary metabolite biosynthesis pathways. Front. Plant Sci. 2022, 13 877304. DOI: https://doi.org/10.3389/fpls.2022.877304
33. Zakaryan N., Gevorgyan V., Kartashyan N., Poghosyan A., Adamyan R., Eloyan I., Shahazizyan I., et al. A comprehensive

analysis of morphological and biochemical parameters of Medicago sativa during biotic stress. Bioactive Compounds in Health and Disease 2024, 7(11), 581-593. DOI: https://www.doi.org/10.31989/bchd.v7i11.1498

34. Jańczak-Pieniążek M., Cichoński J., Michalik P., Chrzanowski G. Effect of Heavy Metal Stress on Phenolic Compounds Accumulation in Winter Wheat Plants Molecules 2023, 28(1), 241. DOI: https://doi.org/10.3390/molecules28010241
35. Panigrahy N., Priyadarshini A., Madhusmita Sahoo M., Kumar Verma A., Achlesh Daverey A., Kumar Sahoo N. A comprehensive review on eco-toxicity and biodegradation of phenolics: Recent progress and future outlook. Environmental Technology and Innovation 2022, 27, 102423. DOI: https://doi.org/10.1016/j.eti.2022.102423
36. Kumar K., Debnath P., Singh S., Kumar N. An Overview of Plant Phenolics and Their Involvement in Abiotic Stress Tolerance. Stresses 2023, 3, 570–585. DOI: https://doi.org/10.3390/stresses3030040
37. Mahapatra S., Radheshyam Y., Wusirika R. Bacillus subtilis impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. J Appl Microbiol. 2022, 132, 3543–3562. DOI: https://doi.org/10.1111/jam.15480
38. Bhat Tej K, Singh B., Sharma Om P. Microbial degradation of tannins. Biodegradation, 1998, 9(5), 343-57. DOI: https://doi.org/10.1023/a:1008397506963
39. Osonga F.J., Akgul A., Miller R.M., et al. Antimicrobial Activity of a New Class of Phosphorylated and Modified Flavonoids. ACS Omega 2019, 4, 12865-12871. DOI: https://doi.org/10.1021/acsomega.9b00077
40. Nat´alia Bueno Leite, Danubia Batista Martins, Dayane S. Alvares, Marcia Perez dos Santos Cabrera. Quercetin induces lipid domain-dependent permeability. Chemistry and Physics of Lipids, 2022, 242, 105160. DOI: https://doi.org/10.1016/j.chemphyslip.2021.100
41. Karapetyan T.D., Hanisyan R.M., Grigoryan R.M., Sarkisyan N.K. Armeniaca Vulgaris l. (Rosacea) apricot leaves extract cytotoxicity in vitro. Medicine Science and Education Scientific and Informational journal, 2013, 15, 136-139.