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Bioactivity – Guided Isolation and Antibacterial Mechanistic Evaluation of Flavonoid Glycosides from Dried Apricot (prunus armeniaca L.) against Gram- Positive and Gram-Negative Bacteria

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ABSTRACT: This study aimed to investigate the bioactivity- guided isolation, antibacterial efficacy, and mechanistic actions of flavonoid glycosides extracted from dried apricots against representative gram-positive and gram-negative bacterial strains.

The ethanolic extract for Prunus armeniaca fruit was used in this analysis for testing two new glycosidic flavones in the extract , they were (4',5,7-trihydroxy flavone-7-O- [β-D-mannopyranosyl (1'''→2'')]-β-D-allopyranoside (1) and 3,4',5,7-tetrahydroxy-3',5'-di-methoxy flavone 3-O- [α-Lrhamnopyranosyl (1''''6'')]-β-D-galactopyranoside (2),) from the butanolic fraction.

Flavones used to research the antibacterial behavior of gram-positive and negative bacteria and showed the inhibition region, found that the ethanol extract inhibited B. subtilis and E. coli while pseudomonas aeroginosa and staphylococcus aureus did not show any behavior that allows us to discover new compounds which had inhibition activity for two bacteria pseudomonas aeruginosa, staphylococcus aureus thus removing the disease caused by those bacteria.

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INTRODUCTION

Prunus armeniaca fruit is referred to as apricot. It is one of the Rosaceae family's essential fruits. It is a hard tree bearing stone fruit closely related to peach (Martínez-Gómez P. *et al.*,2026). It has an actual benefit which eaten as a fresh fruit, dried fruit, fruit juice and a fruit gum (Opwoko DJ, *et al.* 2023). *Prunus armeniaca* (apricot) fruits are rich with flavonoid glycosides, polyphenols and related phytochemicals, and extracts from fruits, kernels and leaves show antibacterial and antioxidant activities (Miguel GR, *et al.*,2008).

Research indicates that the primary bioactive components in Prunus armeniaca include amygdalin, various glycosides, and fatty acid esters like 2(3H)-Furanone (Tang *et al.*, 2024; Basyony *et al.,* 2023).

apricots have antibacterial properties, specifically against skin illnesses like acne vulgaris, as well as antidandruff properties (Ahmed SZ, *et al.,* 2023).

The apricot was used as a remedy for various diseases in the folk medicine. A plant bark decocnition has worked as an astringent to sooth the irritated skin (Akhone MA. *et al.*,2022).

Many applications of folk medicine for apricots include hemorrhage therapy, infertility, inflammation of the eye and spasms. Paste to the apricot kernel can cure vaginal infections (Çömlekçioğlu N, *et al.*, 2025). The kernel oil has been used in cosmetics and as a pharmaceutical agent (laxative and expectorant) (Kiralan M. *et al.*,2019).

apricot fruits and kernels have been studied extensively in the past for their phytochemical richness—including carotenoids, fatty acids, sterols, glycosides, and polyphenols such as catechins, chlorogenic acid, and quercetin

derivatives—making the health benefits of these components well documented (Anna M. Grigoryan1, *et al.*, 2025).

Apricot leaves contain abundant polyphenols, flavonoids, phenolic acids, tannins, essential oils, and vital macro- and microelements with promising antioxidant, anti-inflammatory, and antimicrobial properties (Zagórska-Dziok M. *et al.*,2025, Grigoryan A, *et al.*, 2025). The apricot leaves were used for a wide variety of illnesses in traditional medicine (Akhone MA. *et al.*,2022).

Specifically, new apricot leaf juice has been used in traditional Chinese medicine with beneficial results in skin diseases such as scabies, eczema, sun burns and itching of the skin due to cold exposure. So, the apricot leaves using in folk medicine in clearing teeth at toothaches, stomatitis and at such diseases as diarrhea, diphtheria, etc. (Karapetyan, *et al.*, 2011).

Flavonoid is the common name of compounds that have fifteen-carbon skeleton, which consists of two phenyl rings (A- and B- rings) connected by a three- carbon bridge (C-ring). Flavonoids are hydroxylated phenolic substances and are synthesized by plants in response to microbial infection (Karak P., 2019).

There are several classes of flavonoids, i.e. anthocyanins, flavones, flavonols, flavonones, dihydro-flavonols, chalcones,aurons, flavan and proanthocyanidins isoflavonoids etc. Numerous types of flavonoids are also found in plants with additional patterns of hydroxyl, methoxyl, methyl and/or glycosyl substitution. In addition, flavonoid nuclei and their glycosides are also bound to aromatic andaliphatic acids, sulfates, prenyl or methylenedioxyl groups (Iwashina, 2003).

Flavonoids are a subdivision of a large group of polyphenolic compounds having a benzo-γ-pyrone structure, a versatile class of natural compounds that represent secondary metabolites. Over the past ten years, flavonoids have generated a great deal of interest due to their numerous health benefits for both humans and health of animals and pervasiveness in the kingdom of plants. They have been important components of both ancient and modern successful medical treatments, and in recent literature, they are referred to as "functional ingredients" and "health promoting biomolecules" because of their potential to promote health and prevent chronic degenerative diseases (Nijveldt RJ *et al.*, 2001). Overall, Vascular plants and bryophytes alone possess the flavonoid biosynthetic ability, except for green algae, Nitella hookeri (Characeae) (Kulshrestha S, *et al.*, 2022).

Flavonoids, a major class of plant polyphenols, show broad antibacterial activity against both Gram positive and Gram-negative bacteria through mechanisms including membrane disruption, inhibition of DNA gyrase, and interference with protein and cell wall synthesis (do Nascimento JB and da Costa JG, 2025).

One of the most important roles of flavonoids may be to serve as ultraviolet filters in land plants (Samanta A, *et al.*,2011, Ferreyra ML, *et al.,*2021). Some plants have been surveyed to show that the fIavonoids act as UV shield (Jungblut *et al.*, 1995).

Antimicrobial resistance has reduced the effectiveness of many conventional antibiotics, creating an urgent need for new antibacterial agents from plant sources (Abdallah EM, *et al.*,2023).

Ethanolic and aqueous extracts *of prunus armeniaca* showed the antibacterial activity against many types of bacteria like *Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomanas aeruginosa* (Zagórska-Dziok M., *et al.*,2025)

MATERIAL AND METHODS

A-materials

Ethyl acetate, Butanol, and ferric chloride reagent were obtained from fluka (Germany), Normal hexane from Gainland chemical company factory road (Gcc), (U.k), chloroform and methanol purchased from hayman (England). Nutrient broth and muller hinton agar obtained from fluka (Germany).

B-Methods

1-Plant material extraction and isolation

(Rashid, F. *et al.* 2007)

Dried fruits (2 kilograms) were soaked in ethanol 95% at room temperature for 15 days (3L× 3 batches) and then obtained (2723) ml of material that was concentrated by rotary evaporator with the resulting brown gummy substance (250) ml, then n-hexane, chloroform, ethyl acetate and butanol fractionation. The butanolic fraction of the ethanol extract has been collected in a dark brown sticky mass. This sticky mass was chromatographically subjected to silica gel column using chloroform-methanol (70:30).

The similar composition fraction was combined, obtaining two main fractions; F (30 mg) and G (10 mg).

2-Plant material separation

Two fractions were separated with BuOH: AcOH: H2O (12:3:7) and flavonoid method using thin layer chromatography (TLC) (vorin, 1983).

Antibacterial EtOAc: CHCl3: H2O (17:3:1) as the mobile phases.

Two fractions indicated with distinct Rf that gave prominent colored spots with ferric chloride reagent. Further separation was achieved through ion exchange chromatography at wavelength (200-600) nm for measuring the two fractions G and F.

Isolation and preparation of bacteria

In this study gram-positive and gram-negative bacteria were used, these bacteria were *Staphylococcus aureus, Bacillus subtilis, pseudomonas auroginosa, and Escherichia coli*, and they obtained from (Research & Technology center of Environment, Water &Renewable Energy) in scientific research commission / Baghdad. Iraq.

All bacteria isolated from polluted food and cultured it in nutrient agar media at 37° C for 24 hours. Pure colonies of bacteria were isolated from petri dishes, then activation was performed in nutrient broth at 37° C for 24 hours (Abdulrazaq, R. A.*et al.*,2026, Saeed MK *et al.*,2021)

Antibacterial activity

This stage was tested by agar well diffusion method and agar spot method against four type of gram negative and positive bacteria which clarified above (Rashid, F. *et al.*,2007). The inoculum was prepared for each bacterial isolate by matching the turbidity to 0.5 McFarland standard and spreading on Mueller–Hinton agar (MHA) plate (Rajput K. *et al.*, 2023). At the first bacteria activated by cultivating it for 24 hours in 37 ° C in nutrient broth, then using Inocula and spreading it on the muller hinton agar media surface.

Hollow cylinder (0.6 cm in diameter) was placed on the agar seeded with specific test microorganisms. A butanolic extract stock solution (10 mg / ml was prepared by 100% DMSO dissolving).

The stock solution was filtered and sterilized through a syringe filter with a pore size of 0.22µm.

Test extract (0.1) ml was inserted in petri dishes in hollow cylinders and 24 hours incubated at 37 ° C.

Spot method applied to the surface of Muller Hinton agar media by placing spots of particular microorganism. Measuring the inhibition zone of bacteria has established the antimicrobial activities of the extracts.

RESULTS AND DISCUSSION

Compound 1 was isolated as a light-yellow powder from the butanol soluble fraction of the ethanolic fruit extract via repeated column chromatography and thin layer chromatography (TLC). The UV spectrum showed λ max at 272 nm and 333 nm giving a very strong indication of the presence of a flavonoid (vorin, 1983).

Compound 2 was obtained via column and TLC as yellow powder from the butanolic fraction. The UV spectrum showed λ max at 253 nm, which indicates strongly the presence of flavonoid system (Vorin, 1983).

The UV–Visible spectroscopic analysis of the isolated compounds provides important preliminary evidence regarding the structural characteristics of the identified flavonoid glycosides. Flavonoids generally exhibit two characteristic absorption bands in the UV region: Band I (300–380 nm) associated with the cinnamoyl system of ring B, and Band II (240–280 nm) corresponding to the benzoyl system of ring A. These two diagnostic bands are widely used to identify flavonoid subclasses and evaluate substitution patterns within the aromatic rings.

The UV-Vis spectrum of compound (1), identified as 4',5,7-trihydroxy flavone-7-O- [β-D-mannopyranosyl (1'''→2'')]-β-D-allopyranoside, exhibited a major absorption maximum at λmax = 272 nm along with an additional band at 333 nm.

The peak at 272 nm corresponds to Band II, which is typical for flavone derivatives and arises from the benzoyl chromophore located in ring A. Meanwhile, the absorption at 333 nm corresponds to Band I, indicating the presence of a conjugated cinnamoyl system in the B ring. This spectral pattern is consistent with the UV behavior reported for flavone-type compounds, which usually show Band II in the range 250–280 nm and Band I between 310–350 nm.

The presence of hydroxyl substituents at positions 5, 7, and 4' likely contributes to the bathochromic shift observed in Band I, due to increased conjugation and electron-donating effects of phenolic hydroxyl groups. Such hydroxylation patterns are known to modify the electronic transitions within flavonoid molecules and shift absorption maxima toward longer wavelengths.

Furthermore, the occurrence of glycosylation at position C-7 may slightly alter the intensity of the absorption bands without significantly affecting their positions, which is commonly reported for flavonoid glycosides. Similar UV absorption maxima around 270 nm and 330 nm have been described for several naturally occurring flavone glycosides such as luteolin and apigenin derivatives.

Therefore, the spectral data of compound (1) strongly support its classification as a flavone glycoside, consistent with the proposed chemical structure.

Compound (2), identified as 3,4',5,7-tetrahydroxy-3',5'-dimethoxy flavone 3-O- [α-L-rhamnopyranosyl (1→6)]-β-D-galactopyranoside, displayed a maximum absorption at λmax = 253 nm.

This absorption falls within the typical Band II region (240–280 nm) associated with the benzoyl system of the flavonoid A-ring. The dominance of this band suggests strong π→π* electronic transitions in the aromatic system. Similar absorption maxima around 250–270 nm have been reported for several flavonoid glycosides and polyhydroxylated flavones.

The structure of compound (2) contains multiple hydroxyl groups as well as methoxy substituents at positions 3' and 5', which can influence the electron distribution across the aromatic rings and affect the UV absorption profile. Methoxy groups are known electron-donating substituents that can cause slight bathochromic or hyperchromic shifts depending on their position in the flavonoid skeleton.

Additionally, glycosylation at C-3 with a rhamnose-galactose disaccharide may reduce conjugation in the flavonoid backbone relative to aglycones, often resulting in a stronger Band II absorption compared with Band I. This phenomenon has been

reported in several flavonoid glycosides where sugar substitution modifies electronic transitions but retains the fundamental spectral pattern characteristic of flavonoids.

A comparison of the two compounds reveals that both exhibit absorption maxima consistent with flavone-type chromophores, confirming the presence of a conjugated C6–C3–C6 flavonoid backbone. However, notable differences in their spectral behavior can be attributed to variations in substitution patterns and glycosylation sites.

Compound (1) shows two characteristic bands (272 and 333 nm), which are typical of flavones with an unsubstituted or weakly substituted C-3 position as shown in figure 1,2&3 and table 1&2.

In contrast, compound (2) shows a dominant absorption at 253 nm, suggesting a stronger influence of the benzoyl system and possible electronic effects caused by methoxy substituents and disaccharide glycosylation at C-3.

These observations are consistent with previous spectroscopic studies indicating that hydroxylation, methoxylation, and glycosylation significantly influence UV spectral properties of flavonoids, particularly through shifts in Band I and changes in band intensity.

Overall, the UV-Vis spectral data obtained in this study agree well with previously reported spectral characteristics of flavone and flavone glycoside derivatives and provide supportive evidence for the structural assignment of the isolated compounds .

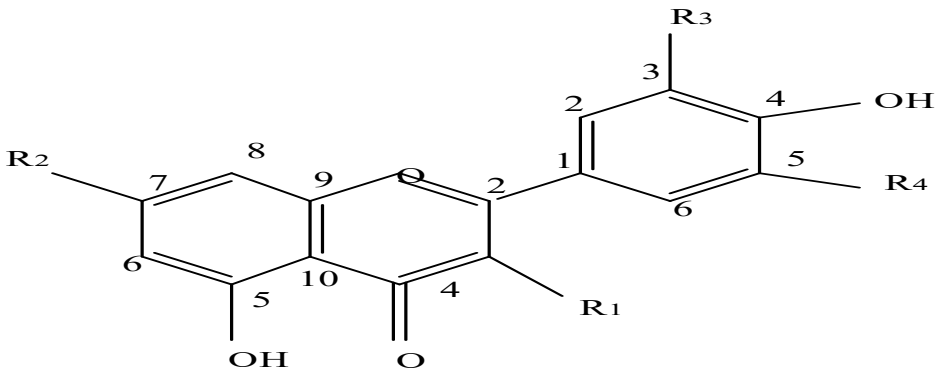


Fig (1) chemical structure of Compounds 1

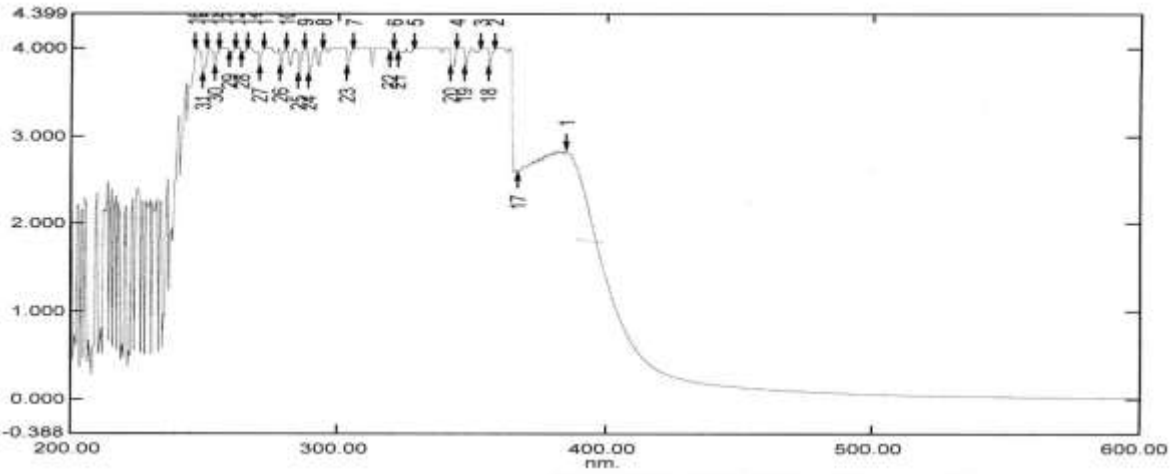


Figure (2): UV-Vis spectrum of compound 1at (200-600) nanometer, compound (1) in λ max at 272

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Table (1): UV-Vis Spectrum of compound 1at (200-600) nanometercompound (1) in λ max at 272

Number	P\ V	Wavelength	Absorbance
1	↑	385.5	2.853
2	↑	358	4
3	↑	352.5	4
4	↑	343.5	4
5	↑	327.5	4
6	↑	320.5	4
7	↑	305	4
8	↑	294	4
9	↑	287	4
10	↑	280	4
11	↑	272	4
12	↑	265.5	4
13	↑	261	4
14	↑	255.5	4
15	↑	251	4
16	↑	246.5	4
17	↓	367	367
18	↓	356	356
19	↓	347	347
20	↓	341.5	341.5
21	↓	322	322
22	↓	318.5	318.5
23	↓	303	303
24	↓	288.5	288

***Compound (1): 4',5,7-trihydroxy flavone-7-O- [β-D-mannopyranosyl (1'''→2'')]-β-D-allopyranoside**

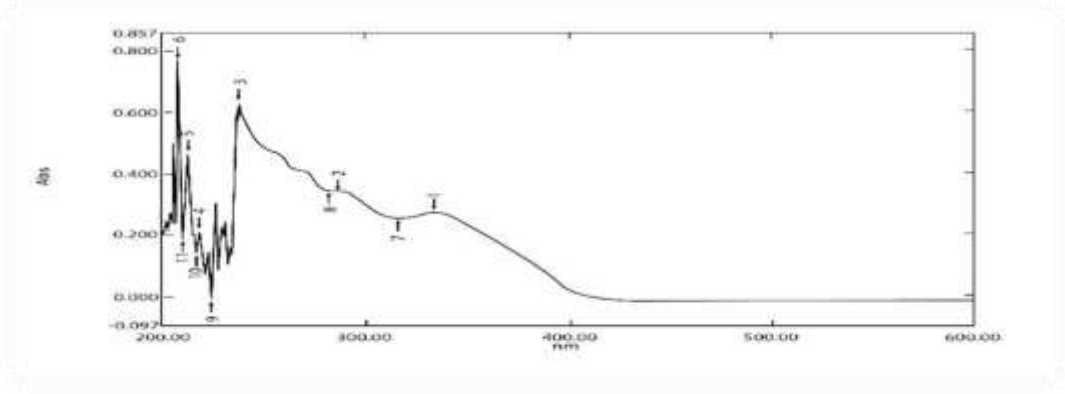


Figure (3): UV-Vis spectrum of compound 1at (200-600) nanometer compound (1) in λ max at 333

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Table (2): UV-Vis Spectrum of compound 1 at (200-600) nanometer compound (1) in λ max at 333

Number	P\V	Wavelength	absorbance
1	↑	333.00	0.270
2	↑	286.00	0.344
3	↑	238.50	0.639
4	↑	218.50	0.218
5	↑	213.00	0.473
6	↑	208.00	0.778
7	↓	316.00	0.250
8	↓	281.50	0.343
9	↓	224.00	-0.015
10	↓	216.50	0.128
11	↓	210.00	0.178

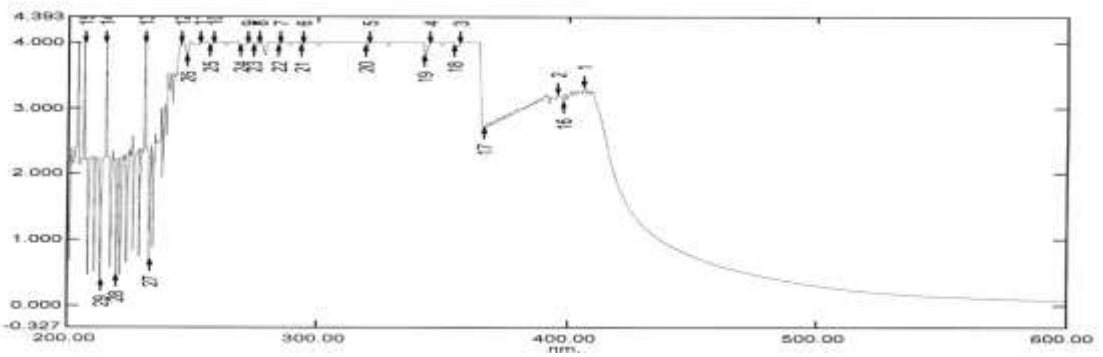


Figure (4): UV-Vis spectrum of compound 2at (200-600) nanometer compound (2) in λ max at 253

Table (3) UV-Vis spectrum of compound (2) at (200-600) nanometer compound (2) in λ max at 253

Number	P\V	Wave length	Absorbance
1	↑	406.50	3.325
2	↑	396.00	3.215
3	↑	356.50	4.000
4	↑	344.50	4.000
5	↑	320.00	4.000
6	↑	294.00	4.000

7	↑	285.00	4.000
8	↑	276.00	4.000
9	↑	271.50	4.000
10	↑	258.50	4.000
11	↑	253.00	4.000
12	↑	245.00	4.000
13	↑	231.00	4.000
14	↑	215.50	4.000
15	↑	206.50	4.000
16	↓	398.00	3.101
17	↓	366.50	2.698
18	↓	354.50	3.957
19	↓	342.50	3.806
20	↓	319.00	3.957
21	↓	293.00	3.957
22	↓	284.00	3.957
23	↓	274.00	3.957
24	↓	268.50	3.957

***Compound (2): 3,4',5,7-tetrahydroxy-3',5'-di-methoxy flavone 3-O- [α-Lrhamnopyranosyl (1""6")]-β-D-galactopyranoside**

The activity of ethanolic extract of *prunus armeniaca* (L.) was showed inhibition activity against the growth of *B. subtilis* was (15mm) in diameter on the media by well method while the extract had lower activity against the *E. coli* bacteria in about (8 mm) in a diameter. The observed inhibition zones align with contemporary research suggesting that *Prunus* species contain secondary metabolites with varying degrees of efficacy across different bacterial strains (Tang *et al*., 2024).

The extract did not improve any influence against *staphylococcus aureus* and *pseudomonas auroginosa*.

The resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in these results is noteworthy. While some studies on *Prunus africana* or *Prunus scoparia* show activity against *S. aureus*, the specific profile of *Prunus armeniaca* ethanolic extracts can vary significantly based on the plant part used (leaves vs. seeds) and the geographical origin (Basyony *et al*., 2023).

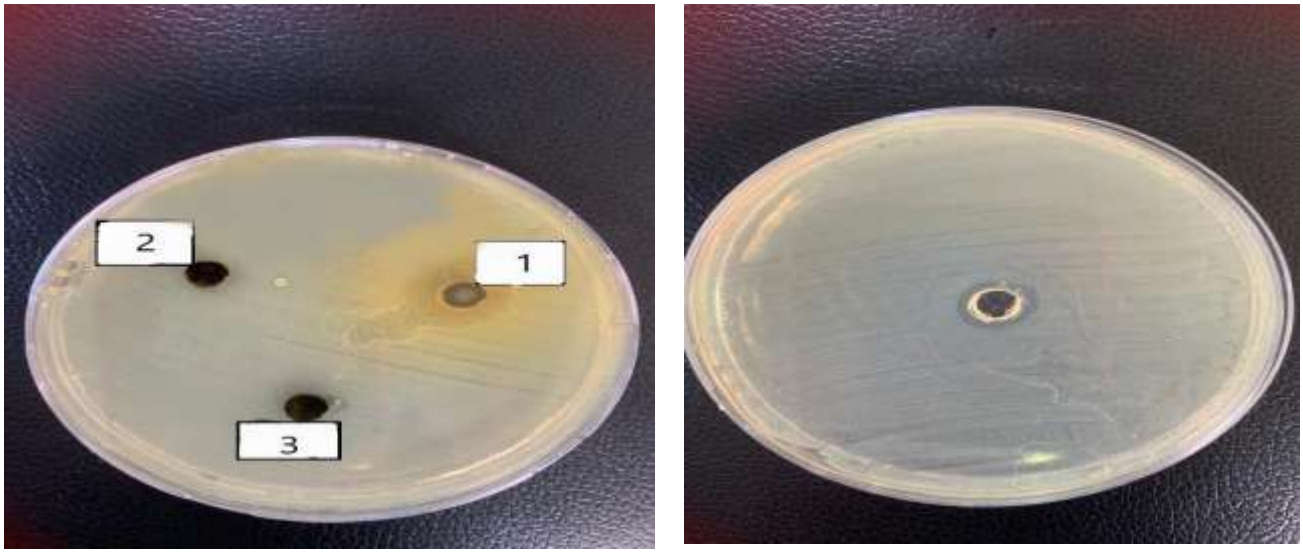
Pseudomonas aeruginosa is notoriously resistant to many antimicrobial agents due to its highly impermeable outer membrane and efficient efflux pumps.

staphylococcus aureus resistance in this specific extract might be due to the absence of specific alkaloids or terpenoids typically required to inhibit this strain (Al-Deen *et al*., 2019).

Thin layer chromatography confirms the presence of two specific compounds in the *prunus armeniaca* extract that it activates against two bacterial types *Bacillus subtilis* and *Escherichia coli* where other bacteria are not.

As showen in figure 5

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(A) **(B)**

Figure (5): Growth inhibition of(A)*Bacillus subtilis ,staphylococcus aureus, Pseudomonas auroginosa .(B)Escherichia coli*

CONCLUSION

The UV-Visible spectroscopic analysis confirms that both isolated compounds are flavonoid glycosides, as evidenced by their characteristic absorption bands corresponding to the benzoyl (Ring A) and cinnamoyl (Ring B) systems.

Feature	Compound 1	Compound 2
Identification	Trihydroxy flavone-7-O-glycoside	Tetrahydroxy-dimethoxy flavone 3-O-glycoside
Band II (Benzoyl)	272 nm — Typical of flavone derivatives	253 nm — Strong π→π* transitions in Ring A
Band I (Cinnamoyl)	333 nm — Indicates a conjugated B-ring system	Dominant Band II; Band I influenced by C-3 glycosylation
Key Substituents	5, 7, and 4' hydroxyl groups	3', 5' methoxy groups and C-3 disaccharide

The differences in their absorption maxima reflect specific variations in their molecular architecture, such as hydroxylation, methoxylation, and the site of glycosylation.Comparison of Spectral Characteristics Structural Implications Confirmation of Flavonoid Backbone: Both compounds exhibit the classic C6–C3–C6 skeleton, with UV peaks falling within the diagnostic ranges for flavones (Band II: 250–280 nm; Band I: 310–350 nm). Influence of Glycosylation: In Compound 1, glycosylation at position C-7 maintains the standard dual-band pattern. In Compound 2, the disaccharide substitution at C-3 reduces backbone conjugation, making the Band II absorption more prominent relative to Band I. Electronic Effects: The presence of electron-donating groups, such as the phenolic hydroxyls in Compound 1 and the methoxy groups in Compound 2, causes shifts in the absorption maxima (bathochromic shifts), providing a "spectral fingerprint" that supports their proposed chemical structures. Final Assessment The spectroscopic data provides robust preliminary evidence that these compounds are flavone-type chromophores. The results align with established literature for naturally occurring glycosides like luteolin or apigenin, reinforcing the structural assignments of the molecules isolated from the Prunus armeniaca fruit extract.

Research shows that we can use many types of plants such as prunus armeniaca to get rid of many types of bacteria caused diseases like E. coli and B. subtilis in human due to its chemical compounds that have a relationship to kill microorganisms such as bacteria, fungi, and others.

Furthermore, these results revealed that apricot fruit has a good source of components of antibacterial activity as well as in other experiments researchers used other parts of plant like leaves, barks, flowers, stems, and seeds to improve the activity of these plants against microorganisms.

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