**Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 558-569**

# Impact of Flavonoids and Nano Flavonoids Extraction from *Bacopa monnieri* on *fimA* Gene Expression in *Serratia marcescens*

**[1,2]Maysam Ihsan Ali Al-Hassany , [1]Bushra Mohammed Jabir Alwash**

[1] Department of biology, College of Science for women, University of Baghdad, Baghdad, Iraq
[2] Department of biology, College of Science, Mustansiriyah University, Baghdad, Iraq

**Received: February 20, 2025 / Accepted: April 30, 2025 / Published: November 16, 2025**

**Abstract :** *Serratia marcescens* is multidrug-resistant human pathogen bacteria. The pathogenicity of S. *marcescens* mainly depends on biofilms and quorum sensing gene are major virulence factors to resist antibiotics. Hence, targeting genes in S. *marcescens* will pave the way to combat its pathogenicity. This study aimed to assess the effect of Flavonoids and Nano Flavonoids on expression of *fimA* gene in S. *marcescens* . Flavonoid extraction from the *Bacopa monnieri* L. belong to family Plantaginaceae and identification by using High Performance Liquid Chromatographic (HPLC), then casein was used to synthesis flavonoid-casein nanoparticles by using the ultrasound probe method. The chemical and physical properties of the nanoparticles were characterized using Fourier Transfor Infrared Spectroscopy (FT-IR) and Transmission Electron (TEM) techniques. S. *marcescens* were selected for this study due to their resistance to multiple antibiotics. The bacteria were first identified using the VITEK 2 system. The polymerase chain reaction (PCR) was performed to amplify the *fimA* gene. RT-PCR was carried out to determine the effect of flavonoids and nano-flavonoids on the *fimA* gene. Flavonoids and nano-flavonoids exhibited significant inhibitory effects on the expression *fimA* gene (the fold of gene expression: 0.00936 and 0.00052) respectively. The *fimA* gene, which encodes a major component of type I fimbriae involved in bacterial adhesion and virulence, was downregulated following treatment with both flavonoid and nano-flavonoid. The nano-flavonoids demonstrated a more potent effect due to enhanced cellular uptake and bioavailability. This results suggest the potential of flavonoids, especially in nano-form, as anti-virulence agents targeting *fimA* gene expression in S. *marcescens.*

**Keywords**: *Serratia marcescens* , Green nanoparticles , *fimA* gene , *Bacopa monnieri* , Real Time Polymerase Chain Reaction (RT-PCR) .

**Corresponding Author:** (Email : maysam.ihssan@uomustansiriyah.edu.iq)

## Introduction

*Bacopa monnieri* also called brahmi is an essential medicinal plant throughout the world (1). B. *monnieri* is used medicinally for the treatment of insanity, epilepsy and skin disease. (2). This plant is reported to have sedative, vasoconstrictor, antimicrobial, antifungal, anti-inflammatory, antiepileptic, and anthelmintic activities (3). B. *monnieri* exhibits important biological activities like anticonvulsant, memory enhancing and antistress (4). Explore antimicrobial compound in the plants is an important study (5). Pharmacological effects of B. *monnieri* are due to its active compounds of alkaloids, flavonoids, sterols, saponins, hersaponin and stigmasterol (6). Flavonoids are the most important class of phenolic compounds (7). Studies have shown that flavonoids can be used

to prevent some important diseases, such as hypertension (8), ameliorate intestinal health (9), and anti-cancer efficacy (10) and Antibacterial activity (11).

Green nanoparticles play an extremely imperative responsibility in various fields, including pharmaceuticals, electronics, and bioengineering. Biological processes by green synthesis tools are more suitable to develop nanoparticles ranging from 1 to 100 nm (12). Green synthesis of nanoparticles using living cells through biological pathways. Plants are the sources of several components and biochemicals that can role as stabilizing and reducing agents to synthesize green nanoparticles. The green synthesized methods are eco-friendly, non-toxic, cost-effective, and also more stable when compared to other biological, physical, and chemical methods (13) .

*Serratia marcescens* is a ubiquitous bacterium from order Enterobacterales. S. *marcescens* has provoked infections and outbreaks in debilitated individuals, particularly newborns and patients in intensive care units. S. *marcescens* isolates recovered from clinical settings are frequently described as multidrug resistant (14). A significant factor contributing to its pathogenicity and antibiotic resistance is its ability to form biofilms, *fimA* gene and virulence factor (15). S. *marcescens* exhibits resistance to penicillins and first- and second-generation cephalosporins due to the production of AmpC β-lactamase. S. *marcescens* show resistance to Macrolides, Tetracyclines and Colistin (16). Aother study noticed S. *marcescens* isolates from clinical and environmental samples were high resistance to ampicillin (87.05% in clinical samples) (17). Several study reported that Flavonoids exhibited significant antibacterial activity against S. *marcescens*. The study also noted that Flavonoid inhibited biofilm formation and reduced the production of virulence factors in S. *marcescens*.

## Materials and methods

### Collection and preparation *Bacopa monniere* L. for extraction :

The plant was collected from Basra city on 2023/3/19 . The plant was Prepared, dried at room temperature (25-30) °C and then grinding the aerial part of plant (leaf , stem , flower) using electric blender (18).

### Extraction and identification of flavonoids :

Extraction the aerial part of plant according to Harborne (19) Method. By HPLC, flavonoids were identified and measured its quantities. The conditions of HPLC were the column separation was C18-OSD (250 mm , 4.6mm) . The column temperature was 30ºC the gradient elution method, with eluent (methanol) and eluent (1% formic acid in water ( 70 : 30 v/v ) at flow-rate of 0.7 mL/min. The injected volume of samples 100 µL and standards was 100 µL and it was done automatically using autosampler. The spectra were acquired in the 280 nm (20) .

### Synthesis of Flavonoids- Casein nanoparticles

The nanoparticles were synthesized from flavonoid and casein by using an ultrasound probe, following Wu, *et al.* (21) method .

### Characterization of Flavonoids - Casein nanoparticles :

**Fourier Transfor Infrared Spectroscopy (FT-IR)** for determining chemical composition of flavonoids, casein and Flavonoids- Casein nanoparticles .

**Transmission Electron Microscopy (TEM)** to produce a highly detailed magnified image of the nanoparticles .

***Serratia marcescens* collection , Isolation and identification:**

Total of 30 clinical isolates were collected from patients whom visited as to Al- Kindi teaching hospital and teaching laboratories hospital at medical city, then identified and confirmed by VITEK 2 system.

**Determination of flavonoid and nano flavonoid Minimum Inhibitory Concentration (MIC)**

Double serial dilutions (1-1024 µg / ml) of flavonoid extract and nano flavonoid were prepared from a stock (10mg/1ml) in a micro titer plate using MuellerHinton broth as diluent, by using Ohikhena *et al.* (22) method.

MIC was measured for only 8 isolates that considered strong biofilm formations, which had been previously identified.

**Genotyping Detection**

**1- DNA Template Preparation by boiling method**

DNA template was prepared by a boiling method as described by Ali *et al.* (23).

**2- PCR Amplification**

The PCR amplification procedure for the genetic level to detecting S. *marcescens* local isolates table (1) by follows step : Final volume for PCR mixture was 25 µl (12.5 of Master Mix 2x, 5 µl template DNA, 1 µl primers for each forward and reverse primer, finally, 5.5 µl nuclease free water) in uniplex PCR Eppendorf tubes but amount changed in multiplex PCR, mixed briefly via vortex then been placed in thermocycler polymerase chain reaction. The program used for each multiplex PCR mixture was illustrated in the table (2).

**Table (1): Primers Oligonucleotide Sequences Used in This Study.**

| Gene | Sequences (5'_3') | Product size/bp | Reference |
|---|---|---|---|
| ***fimA*** | GAACAACAACCCGGCCATTC | 208 | (24) |
| | CTTTTGATAAGGCCGCCACG | | |

**Table (2): Amplification program of primers**

| Amplified gene | Initial denaturation | No. of cycle | Denaturation | Annealing | Elongation | Final extension |
|---|---|---|---|---|---|---|
| ***fimA*** | 94°C/ 5min | 35 | 94°C/ 30sec | 59°C/30sec | 72°C/45sec | 72°C/10min |

**3- Primer dilution**

The oligonucleotide primers which in lyophilization status were dissolved and diluted first in free nuclease D.S.D.W (amount according to recommended of manufactured company) to obtains 100 picomol/µl, then this stock was diluted in free nuclease D.D.W to obtain nearly 10 picomol/µl. This technique accorded on all primers in this study, as listed in table (2). The Specifications primers of all genes provided from Alpha DNA Company, USA.

**4- Agarose Gel Electrophoresis**

Briefly, gel electrophoresis was used for the detection of amplified PCR products, which visualized with the aid of Ethidium bromide dye and UV transilluminator documentation system. (25).

**Real Time Polymerase Chain Reaction (RT-PCR)**

To assess the effect of the flavonoid extract nano flavonoid at MIC on the gene expression of *fimA* gene dene qRT PCR Technique was used. The measurement of the gene expression of the genes was done before and after the treatment with sub MIC concentrations of the flavonoid extract and nano flavonoid. Treated isolates transferred into RT-PCR procedure depend on

manufacture's guidelines used kits as follow:

**1- RNA Purification**

RNA was isolated from sample according to the protocol of TRIzol™ Reagent as the following steps: Sample lysis, Three phase's separations (lower organic phase, interphase, and a colorless upper aqueous phase), RNA precipitation, RNA washing, RNA solubility .

**2- Estimation of DNA concentration and purity**

Concentration and purity of extracted RNA were determined by using nanodrop spectrophotometer. The concentration of extracted RNA was estimated by adding 1 µl of extracted RNA on the exact location in the device and then read the result. The concentration of extracted DNA between (20-100 ng/µl) is usually accepted. The purity ratio estimate according to the following formula:

**RNA purity = O.D260 / O.D280**

**3- Reaction Setup and Thermal Cycling Protocol**

**3.1- One Step RT-PCR**

**Table (3): RT-PCR Component Calculation**

| | | | | |
|---|---|---|---|---|
| **No. of Reaction** | 5 | Rxn | **Annealing temperature of primers** | 54°C,59°C |
| **Reaction Volume /run** | 20 | Ul | **No. of primers** | 2 |
| **Safety Margin** | 5 | % | **No. of PCR Cycles** | 40-45 |

For one sample applying test, components added within 10ul as total volume in consisting of: 10ul qPCR master mix, 0.4ul RT mix, 0.6ul Mgcl2, 1ul Forward primer, 1ul Reverse primer, 3ul Water free nuclease, 4 ul RNA.

**Table (4): RT-PCR Treated Persister MRSA Isolates Targeted Gene Detection**

| Gene/Target | Primer Name | Sequence (5' →3') | Annealing Temperature (°C) |
|---|---|---|---|
| ***16S rRNA*** | ***16S rRNA*** F<br>***16S rRNA*** R | ATGCAAGTCGAGCGAAC<br>TGTCTCAGTTCCAGTGTGGC | 54°C |
| ***fimA*** | ***fimA*** F<br>***fimA*** R | GAACAACAACCCGGCCATTC<br>CTTTTGATAAGGCCGCCACG | 59°C |

**3.2- Real Time PCR Program**

**Table (5): RT-PCR programing.**

| Steps | Temperature | Time M:S | Cycle |
|---|---|---|---|
| **RT. Enzyme Activation** | 37°C | 15:00 | 1 |
| **initial Denaturation** | 95°C | 10:00 | 1 |
| **Denaturation** | 95°C | 00:20 | 40 |
| **Annealing** | 54°C,59°C | 00:30 | 40 |
| **Extension** | 72°C | 00:30 | 40 |

**4- Calculation the fold of gene expression**

Levak equation was used to evaluate the fold expression against house keeping gene and control by the following steps

Ct Control – Ct house keeping control = ΔCt control

Ct sample – Ct house keeping sample =ΔCt sample

Δ Ct sample – Δ Ct control = ΔΔ Ct

Fold of gene expression = $(2^{-\Delta\Delta Ct})$

**Results and discussion**

**Quantification of Flavonoids by HPLC Analysis :**

Flavonoids extract were determine and identified, such as (Myricetin, Hesperidin, Rutin, Qurcetine and Kaempferol ) in Table (6) and Figure (1). Major flavonoids found in B. *monnieri* are Qurcetine 70.2 (µg/gm) followed by Rutin 69.8 (µg/gm) and Kaempferol 55.4 (µg/gm).

This is consistent with earlier research findings (26) showed the quercetin was present in B. *monnieri* at a concentration of 42.99 µg/g and found rutin. Flavonoid present in B. *monnieri*, has been shown to exhibit significant antimicrobial activity (27). Another study revealed the potential of natural compounds like flavonoid compound against *Serratia marcescens* by inhibition quorum sensing (28) .

**Table (6): Quantification of flavonoids by HPLC**

| No. | Name of flavonoids | Concentration | Retention Time min |
|---|---|---|---|
| **1** | **Myricetin ( µg / gm )** | 38.9 | 2.90 |
| **2** | **Hesperidin ( µg / gm )** | 41.0 | 4.01 |
| **3** | **Rutin ( µg / gm )** | 69.8 | 4.81 |
| **4** | **Qurcetine ( µg / gm )** | 70.2 | 6.05 |
| **5** | **Kaempferol ( µg / gm )** | 55.4 | 7.85 |

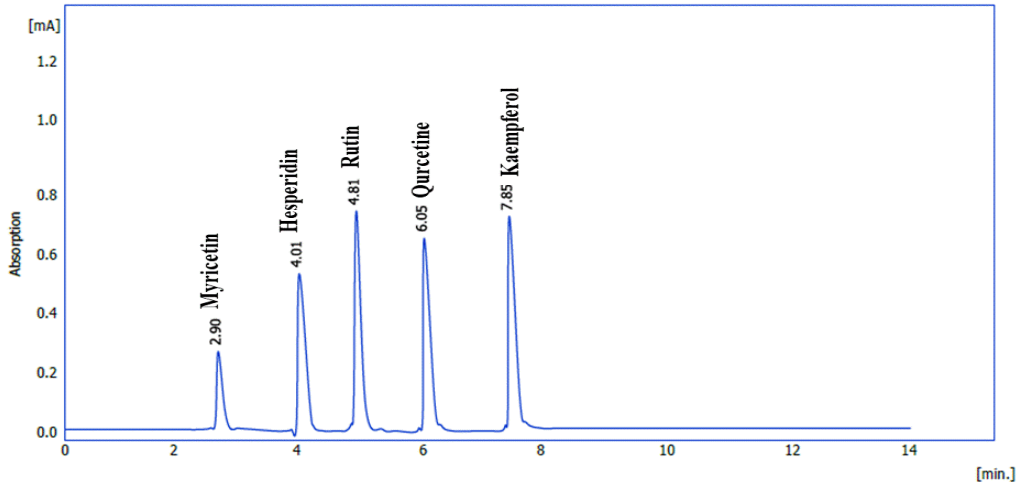


**Figure (1): HPLC analysis of flavonoids to *Bacopa monnieri* L.**

## Characterization of Flavonoid - Casein Nanoparticles

### FTIR

The FTIR measurement of flavonoid in Figure (2) showed the presence of a band starting at 3200 cm-1 and ending at 3750 cm-1 centered at 3392 cm-1, can be attributed to the O-H stretching vibration in the hydroxyl replaced by a carboxyl form, or it can be attributed to the presence of a substituted amine group, as it indicates stretching vibration of N-H as well. The measurement also showed the characteristic bands of flavinoids, which were at 3050, 2931, 1541, and 1080 cm-1 , which could be assigned to the stretching vibration of the aromatic =C-H (in the aromatic ring), the aliphatic C-H (in the pyran ring), the C=C of the benzene ring and the C-O (in the pyran ring), respectively. Moreover, the spectrum demonstrated the presence of C=O, which proves the presence of flavonones, as it gave a band with high intensity at 1662 cm-1. Finally, the bands below the frequency 1000 cm-1 indicate substitution on the aromatic rings, as it refers to the out-of-plane CH (bending vibration).

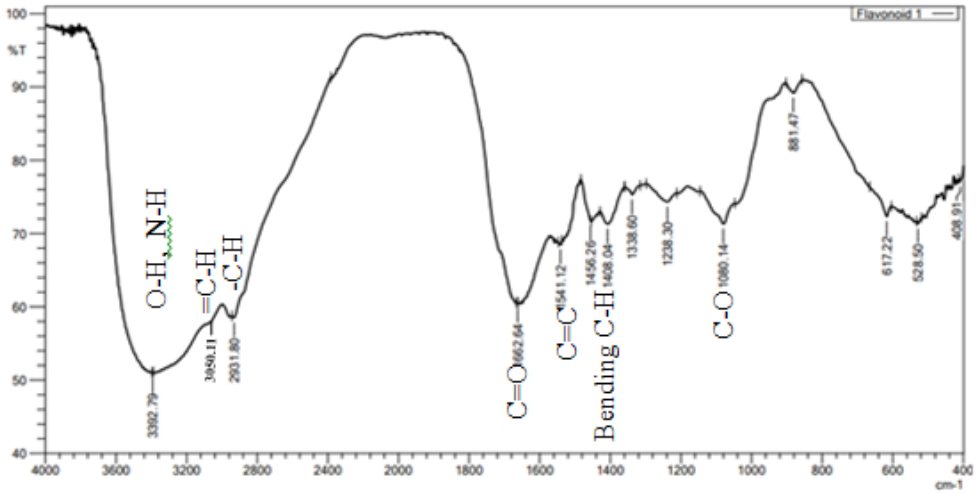


**Figure (2): FTIR of flavonoid**

FTIR of casein was used to determine the functional groups that make up the casein and its nano derivatives in order to determine the changes that will occur to the casein and then determine whether the bond is chemical or physical . The FTIR measurement for the casein in Figure (3) showed a band at 3282 cm-1, which is attributed to the stretching vibration of phenolic and carboxyl O-H, which is overlapped by the NH and NH2 band. Also, the spectrum showed a band at 3066 cm-1, attributed to the stretching vibration of aromatic =C-H, while the bands at 2960 and 2877 cm-1 could be assigned to stretching vibration of the aliphatic C-H Moreover, the spectrum showed bands at 1688 and 1521 cm-1, which represent the stretching vibration of amide I (C=O) and amide II (N-H), which are the characteristic bands of casein. The measurement also showed bands of asymmetric bending vibration for CH3 and C-CH at 1458 and 1396 cm-1, while the bands at 1338 and 1244 cm-1 due to the symmetrical bending vibration of these groups. Finally, the bands at 1080 and 929/669 cm-1 assigned to the bending vibration of phenolic C-O and the para-substituted on the aromatic ring. The presence of these bands is definitive evidence of the existence of casein . This result is consistent with (29).

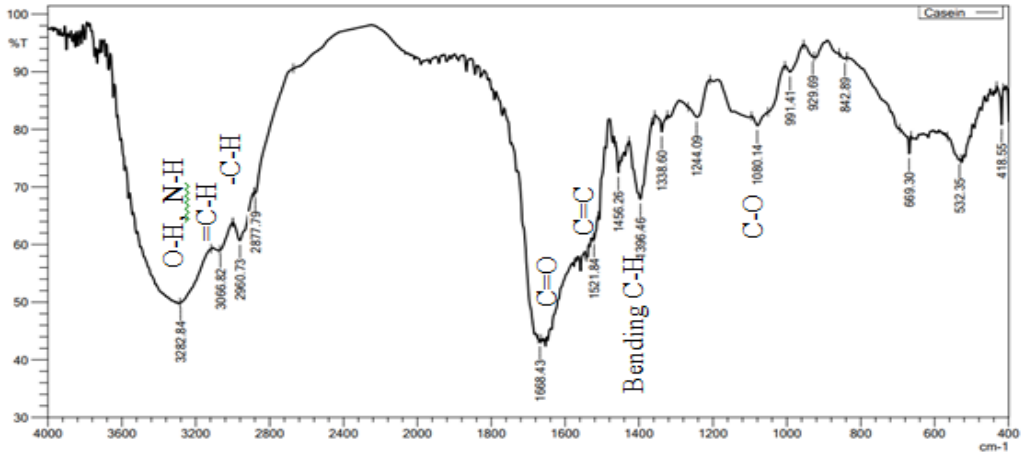


**Figure (3): FTIR of casein**

The FTIR measurement of flavonoid-casein nanoparticles in Figure (4) showed a band at 3404 cm-1, which is attributed to the stretching vibration of phenolic and carboxyl O-H, with which the NH and NH2 bands overlapped. Regarding, the band at 2931 cm-1, it is attributed to the stretching vibration of aliphatic C-H. Moreover, the spectrum showed bands at 1722 and 1512 cm-1, which represent the stretching vibration of amide I (C=O) and amide II (NH), which are the characteristic bands of casein. The measurement also showed an asymmetric bending band for CH3 and C-CH at 1404 cm-1, while the band at 1219 cm-1 is due to the symmetrical bending vibration of these groups. Finally, the band at 1076 cm-1 could be assigned to the bending vibration of phenolic C-O. When this spectrum compared with the spectrum of flavonoid, the spectrum also showed a band at 1635 cm-1, that is attributed to the C=O in the flavonone. Likewise, the spectrum showed a band at 1037 cm-1, of the stretching vibration of C-O in the pyran ring. The presence of these bands for flavonoid and casein is evidence that the desired nanocomposite (flavonoid-casein) was successfully formed.

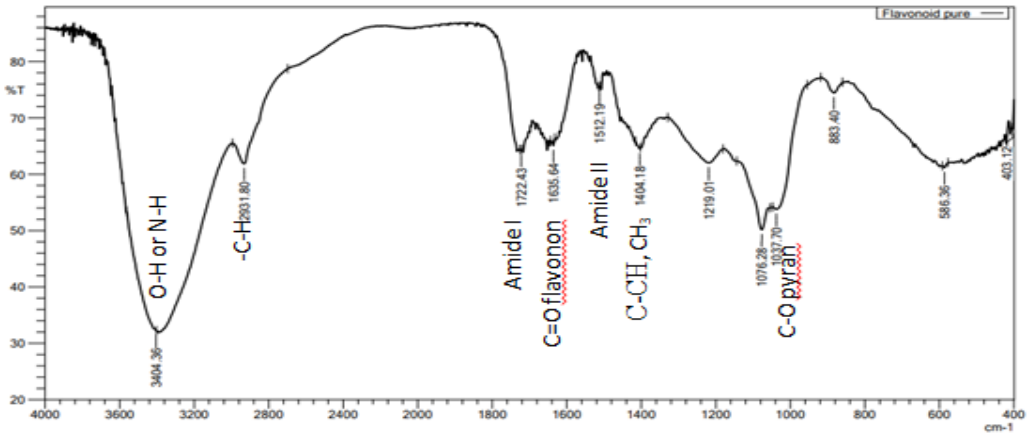


**Figure (4): FTIR of flavonoid-casein NPs**

## TEM

The TEM measurement showed the presence of dark-colored structures with a size extending from 10-20 nanometers in the form of irregular sphere-like structures, in addition to oval to rectangular shapes with a width not exceeding 100 nanometers, which are attributed to flavonoid particles, as they appear dark because they are organic materials that are often crystalline. The measurement also showed irregular structures to which the flavonoid particles are attached, which appear in a polymeric form, since casein is a polymeric protein structure, which appeared as light-colored areas with a thickness not exceeding 10 nm. See Figure (5).

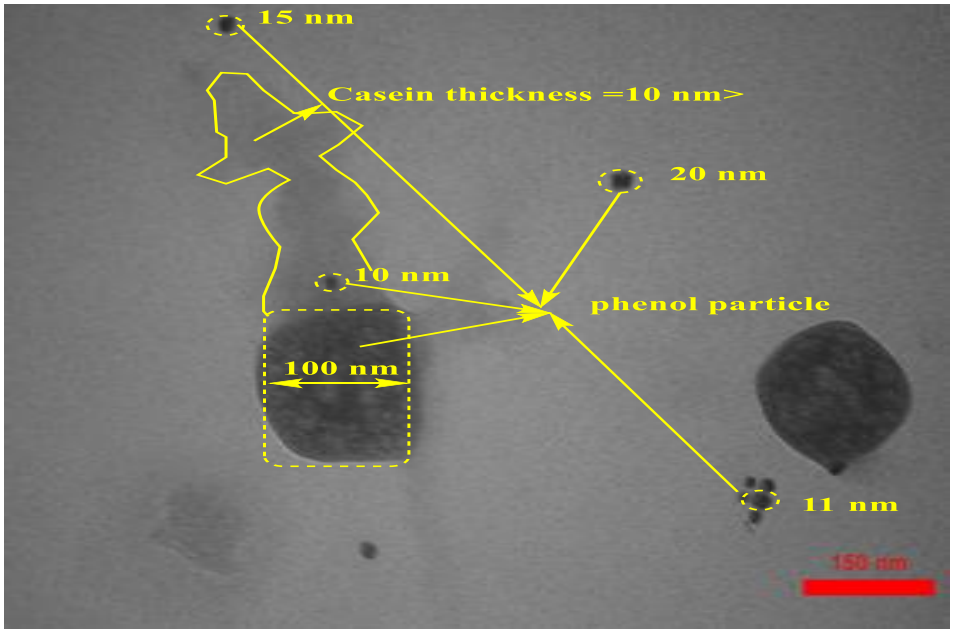


**Figure (5): TEM of flavonoid-casein**

**Determination of flavonoid extract and nano flavonoid MIC :**

The susceptibility of the S. *marcescens* with higher biofilm formation proberity against flavonoid and nano flavonoid to determine the MIC. The results showed that the MIC of flavonoid extract ranged from 64-512 µg/ml . Also the results showed that the MIC of nano flavonoid ranged from 8-64 µg/ml .The MIC of flavonoid extract and nano flavonoid which can inhibit bacteria growth shown in table (7).

The studies have explored the antibacterial activity of flavonoids against *Serratia* species, particularly focusing on S. *marcescens* (30).

In study showed the MIC of flavonoid against S. *marcescens* was determined to be 175 µg/mL ,these values indicate that flavonoid exhibits significant antibacterial activity against S. *marcescens*. The study also explored the potential mechanisms by which flavonoid exerts its antibacterial effects. Findings suggest that flavonoid may disrupt bacterial cell membranes, leading to increased permeability and cell death. Additionally, flavonoid was observed to reduce the production of extracellular polymeric substances (EPS), which are critical components of biofilms, and to inhibit the synthesis of prodigiosin, a red pigment associated with S. marcescens virulence (31).

**Table (7): The MIC of flavonoid extract and nano flavonoid against *Serratia marcescens* .**

| S. *marcescens* Isolates | Flavonoid extract (µg/ml) | Nano flavonoid (µg/ml) |
|---|---|---|
| **1** | 64 | 16 |
| **2** | 512 | 64 |
| **3** | 256 | 32 |
| **4** | 512 | 32 |
| **5** | 64 | 8 |
| **6** | 64 | 32 |
| **7** | 128 | 16 |
| **8** | 128 | 16 |

***fimA* Gene Amplification**

In this study, a rapid and easy duplex PCR assay was designated to identify S. *marcescens* isolates that carry quorum sensing genes. This study demonstrates the *fimA* gene is highly prevalent six out

of eight (75%) among S. *marcescens*. Figure (6) illustrated shine bands of positive results of gene as compared with DNA ladder .

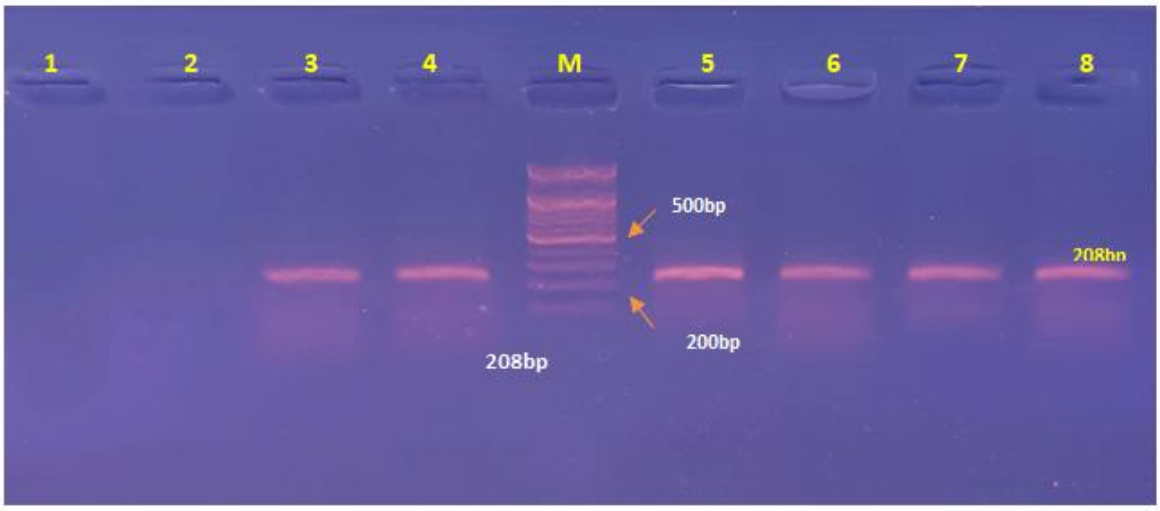


**Figure (6): Agarose gel electrophoresis of *Serratia marcescens* (1.5% agarose,7v/cm$^2$ for 60 min) *fimA* gene (208 bp amplicon) respectively, lane M represent M100bp DNA Ladder, lanes 1-2 represent negative results , 3-8 represent positive bands.**

In the study by deAssis *et al*. (32), out of 20 S. *marcescens* isolates 6 isolates tested positive for the *fimA* gene. The *fimA*-positive isolates demonstrated a higher capacity for biofilm formation compared to the negative ones. The *fimA* gene encodes the major structural subunit of type 1 fimbriae in S. *marcescens*, playing a crucial role in biofilm formation and surface adhesion (33). *fimA* is integral to S. *marcescens* biofilm formation, with its expression finely tuned by intracellular signaling pathways and environmental cues (34).

**The Effect of Flavonoids and Nano Flavonoids on *fimA* Gene Expression**

After treatment of *S. marcescens* with flavonoids and nano flavonoids at diffierent concentrations according to sub MIC of each bacterium. These isolates were subjected to *fimA* gene expression using the quantitative RT-PCR. The results of the gene expression are revealed in table (8). The *fimA* gene expression in isolate after flavonoids treatment decreased (the fold of gene expression :0.00936 ). Moreover, the *fimA* gene decreased more when S. *marcescens* were treated with nano flavonoids (the fold of gene expression: 0.00052) .The flavonoids and nano flavonoids inhibit the expression of *fimA* gene as a consequences decrease the bacterial communication leading to decrease the virulence, antibiotic resistance and biofilm formation in the developing biofilm.

Flavonoids play important role in altering bacterial cell membrane integrity, leading to increased permeability and cell death, also interfering with bacterial enzymes essential for DNA replication and metabolism (24).The ability of flavonoids to interfere with bacterial gene expression lead to downregulation of adhesion factors by disrupting quorum sensing. Flavonoids are reduce the expression of genes like *fimA* that are involved in adhesion and biofilm formation thereby impairing bacterial adhesion capabilities and decreased virulence factors (35) .

**Table (8): Estimation of gene expression of *fimA* gene by qRT-PCR after flavonoids and nano flavonoids treatment.**

| Sample | Ct *fimA* | Ct *16srRNA* | ΔCt | ΔΔCt | Fold Chang |
|---|---|---|---|---|---|
| **Control** | 27.49 | 12.43 | 15.06 | --------- | 1 |
| **Flavonoid** | 34.58 | 12.78 | 21.80 | 6.74 | 0.00936 |
| **Nano flavonoid** | 38.32 | 12.34 | 25.98 | 10.92 | 0.00052 |

## Conclusion:

In conclusion, flavonoids and nano-flavonoids have a significant inhibitory effect on the expression of the *fimA* gene in S. *marcescens*, which plays a key role in quorum sensing regulation. This inhibition leads to a reduction in virulence factor production and decreases the bacteria's ability to resist antibiotics. These findings suggest that flavonoids and nano-flavonoids could be potential anti-quorum sensing agents, offering a promising approach to combat antibiotic-resistant S. *marcescens* infection .

## References


1. Verma, M. (2014). Ethno-medicinal and antimicrobial screening of bacopa monnieri (l.) pennell. Journal of Phytology, 6: 1-6.
2. Gupta, P.; Khatoon, S.; Tandon, P.K. and Rai, V. (2014). Effect of cadmium on growth, bacoside A, and bacopaside I of Bacopa monnieri (L.), a memory enhancing herb. The Scientific World Journal, 2014:1-6.
3. Mkk, F.; Sp, D and Irfan, M. (2019). Antibacterial and antifungal activity of various extracts of Bacopa monnieri. International Journal of Pharmaceutical Research, 11:1-5.
4. Alwash, B. M. (2018). Triterpenoid saponins investigation and pharmacological (cytotoxic and antioxidant) properties of Bacopa monnieri L. cultivated in Iraq. Baghdad Science Journal, 15(2): 123-129.
5. Vijay, R.; Shukla, J. and Rajesh Saxena, R.( 2016). Propagation of Bacopa monnieri (BRAHMI): important medicinal plant. Cibtech Journal of Biotechnology, 5:17-23.
6. Zhou, Y.; Shen, Y.H.; Zhang, C. and Zhang, W.D. (2007). Chemical constituents of Bacopa monnieri. Chemistry of Natural Compounds, 43(3): 355-357.
7. Dias, M.C.; Pinto, D.C.G.A. and Silva, A.M.S. (2021). Plant Flavonoids: Chemical Characteristics and Biological Activity. Molecules, 26(17): 5377.
8. Maaliki, D.; Shaito, A.A.; Pintus, G.; El-Yazbi, A. and Eid, A.H. (2019). Flavonoids in hypertension : a brief review of the underlying mechanisms. Current Opinion in Pharmacology, 45(1471-4892): 57-65.
9. Landete, J.M.; Arques, J.; Medina, M.; Gaya, P.; de Las Rivas, B. and Munoz, R. (2016). Bioactivation of phytoestrogens: intestinal bacteria and health. Critical Reviews in Food Science and Nutrition, 56 (11):1826-1843.
10. Salman, Z.O.; Alwash, B.M. and Kadhim, E.J. (2024). Isolation and Identification of Flavonoid Compounds from Euphorbia Milii Plant Cultivated in Iraq and Evaluation of its Genetic Effects on Two Types of Cancer Cell Line. Baghdad Science Journal, 21(9): 2805-2805.
11. Farhadi, F.; Khameneh, B.; Iranshahi, M. and Iranshahy, M. (2019). Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytotherapy Research, 33(1): 13-40.
12. Vijayaram, S.; Razafindralambo, H.; Sun, Y.Z.; Vasantharaj, S.; Ghafarifarsani, H.; Hoseinifar, S.H. and Raeeszadeh, M. (2024). Applications of green synthesized metal nanoparticles—a review. Biological Trace Element Research, 202(1), 360-386.
13. Mustapha, T.; Misni. N.; Ithnin, N.R.; Daskum, A.M. and Unyah, N.Z. (2022) A review on plants and microorganisms mediated synthesis of silver nanoparticles, role of plants metabolites and applications. International Journal of Environmental Research and Public Health, 19(2):674.
14. Tavares-Carreon, F.; De Anda-Mora, K.; Rojas-Barrera, I.C. and Andrade, A. (2023). Serratia marcescens antibiotic resistance mechanisms of an opportunistic pathogen: a literature review. PeerJ, 11:e14399.
15. Labbate, M.; Zhu, H.; Thung, L.; Bandara, R.; Larsen, M.R.; Willcox, M.D.and Kjelleberg, S. (2007). Quorum-sensing regulation of adhesion in Serratia marcescens MG1 is surface dependent.

Journal of bacteriology, 189(7): 2702-2711.

16. Mahlen, S.D. (2011). Serratia infections: from military experiments to current practice. Clinical Microbiology Reviews, 24(4): 755–791.
17. Al-Mosawi, R.M. (2023). Detection of Serratia marcescens from clinical and environmental samples with antimicrobial sensitivity test in Basrah. International Journal of Research in Pharmaceutical Sciences, 9(4): 1384–1389.
18. Nichakool, B.; Jamphon, A.; Pootang-on, Y.; Techakriengkrai, W. and Techakriengkrai, T. (2021). A study about Brahmi (Bacopa monnieri) preparation steps on its saponin quantity. Trends in Sciences, 18(24): 1439-1439.
19. Harborne, J.B. (1984). Phytochemical methods . Champman and Hill, New York,USA second edition.
20. Radovanovic, B.; Mladenovic, J.; Radovanovic, A.; Pavlovic, R. and Nikolic, V. (2015). Phenolic composition, antioxidant, antimicrobial and cytotoxic activites of *Allium porrum* L. (serbia) extracts. Journal of Food and Nutrition Research, 3(9):564-569.
21. Wu, M.; Jiang, B.; Huang, P. and Wu, W. (2023). Ultrasound-assisted synthesis of PDA nanoparticles with controllable size regulation. Materials Letters, 351: 135073.
22. Ohikhena, F.U.; Wintola, O.A. and Afolayan, A.J. (2017). Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. The Scientific World Journal, 2017 (1): 1-8.
23. Ali, M.R.and Khudhair, A.M. (2018). Detection of colony adhesion factors and genetic background of adhesion genes among multidrug-resistant uropathogenic Escherichia coli Isolated in Iraq. Journal of Pure and Applied Microbiology, 12: 2017–2025.
24. Hezam, A.M. and Abd Zaid, A.M. (2023). Serratia marcescens isolated from newborn meningitis in the Iraqi city of Diwaniyah: Molecular characterization. Revis Bionatura, 8(2): 94.
25. Mishra, B.B.T.S.; Patel, B.B. and Tiwari, S. (2010). Colloidal nano carriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine: Nanotechnology, biology and medicine, 6(1) :9-24
26. Mohamed, M.F.; and Hamza, E.M. (2022). Mass propagation, Cultivation, Phytochemical and Fingerprint of Bacopa monnieri L. in Egypt. Egyptian Journal of Horticulture, 49(2): 199-213.
27. Emran, T.B.; Rahman, M.A.; Uddin, M.M.N.; Dash, R.; Hossen, M.F.; Mohiuddin, M.; and Alam, M.R. (2015). Molecular docking and inhibition studies on the interactions of Bacopa monnieri's potent phytochemicals against pathogenic Staphylococcus aureus. DARU Journal of Pharmaceutical Sciences, 23: 1-8.
28. Annapoorani, A.; Parameswari, R.; Pandian, S.K.; and Ravi, A.V. (2012). Methods to determine antipathogenic potential of phenolic and flavonoid compounds against urinary pathogen Serratia marcescens. Journal of microbiological methods, 91(1): 208-211.
29. Tang, H.; Dong, L.; Xia, X.; Chen, X.; Ren, M.; Shu, G.; Fu, H.; Lin, J.; Zhao, L.; Zhang, L.; Cheng, G.; Wang, X. and Zhang ,W. (2024). Preparation, optimization, and anti-pulmonary infection activity of casein-based chrysin nanoparticles. International Journal of Nanomedicine, 5511-5522.
30. Adamczak, A.; Ożarowski, M. and Karpiński, T.M. (2019). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. Journal of clinical medicine, 9(1): 109.
31. Kannan, S.; Balakrishnan, J.; Govindasamy, A. and Arunagiri, R. (2022). New insights into the antibacterial mode of action of quercetin against uropathogen Serratia marcescens in-vivo and in-vitro. Scientific Reports, 12(1): 21912.
32. de Assis, M.C.; da Cunha, A.L.M.; dos Santos,L.A.; Neves, A.P.P. and Sardi, P.E.O. (2013). Detection of fimA gene in Serratia marcescens and its role in biofilm formation. Brazilian Journal of Microbiology, 44(3):899–902.
33. Shanks, R.M.; Stella, N.A.; Kalivoda, E.J.; Doe, M.R.; O'Dee, D.M.; Lathrop, K.L.; Guo, F.L. and Nau, G.J. (2007). A Serratia marcescens OxyR homolog mediates surface attachment and biofilm formation. Journal of bacteriology, 189(20): 7262-7272.

34. Rice, S.A.; Koh, K.S.; Queck, S.Y.; Labbate, M.; Lam, K.W. and Kjelleberg, S. (2005). Biofilm formation and sloughing in Serratia marcescens are controlled by quorum sensing and nutrient cues. Journal of bacteriology, 187(10): 3477-3485.

35. Pandey, P.; Rao, L.; Shekhar, B. R.; Das, D. K.; and Vavilala, S.L. (2024). Molecular insights into flavone-mediated quorum sensing interference: A novel strategy against Serratia marcescens biofilm-induced antibiotic resistance. Chemico-Biological Interactions, 396: 111027.