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Evaluation of the Antibiofilm Activity of *Laurus nobilis* Leaves Extract and Assessment of Its Effect on *fimA* and *papC* genes in *Escherichia coli* isolates

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ABSTRACT

Background: The beneficial gut bacterium *E. coli* can cause blood poisoning, diarrhoea, and other gastrointestinal and systemic disorders.

Objective: This study amid to examines the antibiofilm activity of Laurus nobilis leaves extract on *E. coli* isolates and compares pre- and post-treatment gene expression of fimA and papC genes.

Subjects and Methods: Ten isolates of *E. coli* were obtained from the Genetic Engineering and Biotechnology Institute, University of Baghdad, which was previously collected from Baghdad city hospitals and diagnosed by chemical tests, the diagnosis was confirmed using VITEK-2 System. The preparation of the aqueous and methanolic *Laurus nobilis* leaves extracts was done by using the maceration method and Soxhlet apparatus respectively. HPLC were conducted to determine the active compounds in the extracts. Moreover, molecular detection of *fimA* and *papC* genes and analysis of the gene expression by comparing the isolates treated with sub MIC of methanolic *L. nobilis* leaves extract with the untreated isolates.

Results: Methanolic and aqueous extracts contained alkaloids, tannins, phenols, saponins, flavonoids, and glycosides. Seven polyphenolic compounds, four flavonoids derivatives (Apigenin, Luteolin, Rutin, and kaempferol) and three phenolic acids (Caffeic acid, Gallic acid, and Syringic acid), were identified by matching retention time with the standards. Laurus nobilis methanolic leaf extract inhibited 90% and 100% of *E. coli* biofilm development at 32 and 64 mg/ml.

Conclusion: The result of the gene expression revealed that there is a decrease in the expression of the *fimA* and *papC* genes. The present study concluded that the *Laurus nobilis* leaves extract have rich phytochemical contents, so the methanolic extract had an excellent reduction effect on biofilm formation and showed remarkable down-regulation on the *papC* and *fimA* genes, which are responsible for the biofilm formation in *E. coli*.

Keywords: *L. nobilis*, *E. coli*, Antibiofilm activity, Gene expression of *fimA* and *papC* genes.

INTRODUCTION

Although most strains of *E. coli* are innocuous and the bacteria are a regular resident of the large intestine of humans, it is nevertheless important to pinpoint the source of E. coli and other gram-negative bacteria pathogens that cause newborn illnesses. These dangerous strains are the cause of diarrheal diseases all around the world, in addition to newborn meningitis, septicemia, and urinary tract infections (UTIs). Those in the hospital's nursery or other nearby locations may be a source of the gram-negative bacteria that can spread throughout the facility [(1)].

Biofilms are communities of bacteria that have banded together on a surface and been coated in a polysaccharide and protein matrix in response to stressful situations such as irradiation or treatment. Most biofilms on water surfaces are produced by polysaccharide/polymeric matrices, and these matrices are resistant to antimicrobial treatments [(2)].

In order to ensure the safety of medicinal plant material used in alternative and complementary medicine, it is routinely subjected to microbial and bacterial testing. Humans have been using plants to treat their ailments for as long as we've been around, based on instinct, taste, and experience [(3)].

Among these healing plants is one of the most popular culinary spices in all Western and Asian nations as well as being grown as an ornamental plant throughout Europe and America is laurel *(Laurus nobilis L.)*, an aromatic plant and evergreen tree that belongs to the *Lauraceae* family [(4)].

Plants' therapeutic properties have been well-documented for thousands of years, and traditional medicines continue to play a significant role in the routine treatment of many illnesses in various regions of the world.

Alternative therapies and the therapeutic use of natural goods, particularly those produced from plants, have gained popularity in recent years. One of the primary sources of biologically active materials is thought to be plants [(5)].

The leaves and berries of the plant are frequently used to flavor and improve meals, particularly meats, sauces, and soups. In addition to its distinctive perfume, it is used to treat illnesses all over the world. Beyond rotting bacteria, various components from this plant, including its essential oils and organic acids, have demonstrated potent antibacterial activity against a variety of foodborne disease microorganisms [(6, 7)].

MATERIALS AND METHODS

Chemical reagents: The chemical. reagent absolute methanol was purchased from BDH (England), while

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Resazurin dye, Eosin-Methylene Blue Agar, tryptic soy broth (Himedia, India) and crystal violet (Pro-Lab, Canada).

Bacterial. Isolates:

Ten isolates of *E. coli* were gained from the Institute of Genetic Engineering and Biotechnology, University of Baghdad, which was previously collected from patients urine from Baghdad City Hospitals and was diagnosed by the chemical and molecular tests, and the diagnosis was confirmed by culturing the isolates on Eosin-Methylene Blue (EMB) agar for 18-24 h at 38°C and using VITEK-2 System. After re-culturing the isolates on nutrient agar and putting them through an aerobic incubation, the results were positive at 37°C. for 24. hours.

Assessment of biofilm formation

Quantification of biofilm formation was evaluated as described by **Patel *et al.*** [8]. After overnight growth in Brain Heart Infusion Broth at 37°C, all *E. coli* isolates were transferred to tryptic soy broth (TSB) containing 1% glucose and pipetted to thoroughly combine. There was a suspension of the bacterium that was brought to the McFarland No. 0.5 turbidity threshold .

A volume (200 µl) of each isolates culture was added in triplicate, to a sterile 96 wells microtiter plate with a flat bottom. The plate was covered with their lids and incubated under aerobic conditions at 37°C for 24h. After the incubation period, the planktonic cells were rinsed twice with distilled water to remove the unattached bacteria.

The adhering bacterial cells in each well were fixed with 200 µl of absolute methanol for 20 min at room temperature, after that 200 µl of 0.1% crystal violet were poured into each well and left there for 15 minutes to stain the adherent cells. After the staining process was finished, additional stain was removed by repeatedly washing with distilled water (two to three times).

The plate was dried by leaving them at room temperature for approximately 30 min to ensure they were completely dry, the stain was remedied by adding acetic acid 33%. Optical density (OD) Readings were determined by using an ELISA auto reader with a 630 nm wavelength. Average of OD calculated sterile medium values were added to all test results, then subtracted. Cut off value (ODc) a calculation was made that can classify isolates as biofilm producers or not [8].

ODc: Average OD of negative control + (3 × standard deviation (SD) of Negative control),

OD isolate: Average OD of isolate – ODc.

By the estimation of cutoff value (ODc), the result of biofilm was detected as shown in table (1).

Table (1): Classification of bacterial adherence

Mean OD630	Biofilm intensity
OD ≤ ODc*	Non Biofilm
ODc < OD ≤ 2ODc	Weak
2ODc < OD ≤ 4ODc	Moderate
OD > 4ODc	Strong

Collection of plant: *Laurus nobilis* were collected from the local Iraqi markets, identified as (*Laurus nobilis L*) by the specialist, Department of Biology, College of Science, University of Baghdad. The leaves were washed in water, air-dried, ground into a powder, and then stored at 4 °C for subsequent study.

Preparation of aqueous extract

In accordance with the instructions given by **Kirmusaoglu** [9], an aqueous extract was made by macerating 250 grams of *Laurus nobilis* leaves in 2000 milliliters of distilled water for three days. The mixture was filtered using Whatman No. 1 paper following extraction. The filtrate was dried out using a rotary evaporator heated to 50 degrees Celsius and working under vacuum so that the water could be extracted. The completed extract was stored in amber glass vials at a temperature of 4 °C until examination.

Preparation of methanolic extract

The Soxhlet equipment was used to make the methanolic extract [10]. 250 gm of Laurus nobilis leaves were put in a thimble, to which 1700 ml of 70% methanol and 300 ml of distilled water were added over the course of six hours at a temperature between 40 and 60 °C. The methanol was removed from the solution by filtering it through Whatman No. 1 filter paper and evaporating it to dryness in a rotary evaporator set to 40 °C in a vacuum. The extract was then stored in amber glass vials at a temperature of four degrees Celsius until it was analyzed.

Phytochemical tests of *L. nobilis* extracts

The phytochemical (**alkaloids, phenols, flavonoids, glycosides, saponins and tannins)** study of the aqueous and methanolic leaves extracts has been done according to [10-12].

High-Performance Liquid Chromatography (HPLC)

Methanolic and aqueous extracts of *Laurus nobilis* leaves extract were identified by HPLC (Shimadzu, Japan) according to [13].

Study the antibiofilm activity of *L. nobilis* extracts

The 96 - well microtiter plate was utilized to determine the antibiofilm activity of *L. nobilis* methanolic and aqueous extract. The working solution of the plant extracts was prepared at 50 ppm for the nano-methanolic and nano-aqueous extract to make the concentrations (128-1) mg/ml. Each sample was added

200 µl at a time to the first wells in row A. Columns had 100 µl of the broth in just rows B through H. Micropipette serial dilutions twice were performed methodically down the columns (from rows A-H). The procedure was carried out to the very last row (H), after which the final 100 l were thrown away. After removing 100 l from the starting concentrations in row A, they were combined in the correct manner with the 100 l of broth in the following row. This process was repeated all the way up until the final row (H), at which point the final 100 µl were thrown away. One hundred microliters of the bacterial inoculum containing 106 CFU per milliliter was put into each well, with the exception of the well serving as the negative control. The process was carried out exactly as described in the previous paragraph (Assessment of biofilm formation).

Identification of virulence genes at the molecular level using PCR

Extraction of genomic DNA:

The Genomic DNA extraction was done by a commercial purification method, the genomic DNA Extraction Mini Kit (iNtron®, Korea), which was used to extract DNA from *E. coli* bacteria. This kit was made to isolate DNA from both Gram-positive and Gram-negative bacteria. This kit used the bacterial technique to extract DNA (for Gram-negative bacteria).

DNA concentration and purity estimation:

The Nanodrop is used to determine the DNA concentration. Nanodrop is used to measure the optical density (O.D.) at 260 nm and 280 nm by introducing 1 micro liter of the isolated DNA [14]. Using this approach, estimating of the DNA purity ratio using this formula:

- **DNA purity ratio** = O.D 260 nm / O.D 280 nm
- **Molecular Detection of *fimA* and *papC* genes:**

The reaction was carried out by adding 12.5 µl from One Taq (NEB®) master mix, 3 µl of DNA sample, 1 µl of 10 pmol / µl from each primer, and 7.5 µl of free-nuclease water. The reaction was carried out under the best PCR conditions of each gene as indicated in table (2).

Table (2): PCR conditions of *fimA* and *papC* genes

Cycle No.	Step	Temperature	Time
1	Initial Denaturation	94 ºC	5 min.
38x	Denaturation	94 ºC	30 sec.
	Annealing	57 ºC	45 sec.
	Extension	72 ºC	45 sec.
1	Final Extension	72 ºC	7 min.

Gene expression Analysis using qRT PCR Technique:

It was possible to assess how the *L. nobilis* extract altered the genes *fimA* and *papC,* which are involved in the creation of biofilms, by measuring the expression of the two genes in the isolates before and after treatment with the methanolic extract.

The methanolic extract was used at a concentration that was below the minimum inhibitory concentration (MIC) in order to permit bacterial growth. TRIzolTM Reagent was used to extract RNA in accordance with the manufacturer's recommended technique. In order to evaluate the *fimA* and *papC* gene expression, Newly designed primer through geneious prime software and the primer specifications have boon checked by oligo analyzer tool and were listed in table (3). The reaction mixture was summarized in table (4). Also, the thermal cycler protocol was improved after numerous tests, and the procedure is described in table (5).

Table (3): Primers utilized in this research

Primer names		Sequence (5´-3´)	Production	Reference
FimA	F	CAGGTTGTCAC ACTCGGTGA	110 bp	Newly designed
	R	GCAACAACAGG ATCGCAGTC		
PapC	F	GGTTTGTGCGG TGGTTTGAA	134 bp	
	R	CCCACGGAGTT GAAGAACGA		
House Keeping gene *16S rRNA*	F	GGATCAGAATG CCACGGTGA	170 bp	
	R	GCAGGTTCCCC TACGGTTAC		

Table (4): Volumes and concentrations of qPCR reaction mix

Component	Volume (µl)
Luna Universal qPCR Master Mix	10
Forward primer (10 µM)	1
Reverse primer (10 µM)	1
Template DNA	5
Nuclease-free Water	3
Total	**20**

Table (5): RT-PCR Cycling Program

Cycle Step	Temperature	Time	Cycles No.
Initial Denaturation	95 °C	60 seconds	1
Denaturation	95 °C	15 seconds	40
Anneling	60 °C	30 seconds	
Melt Curve	60-95 °C	40 minutes	1

The results of qRT-PCR were determined by comparing the Ct values of target and reference (housekeeping) genes. The ΔΔCt technique was used to evaluate the genes by relative measurement of gene expression levels (fold change) as described by **Stephenson** [15,16].

Ethical approval: The research was approved by Iraqi Ministry of Health's Ethics Committee/ Medical city, National Center Teaching Laboratories, and Genetic Engineering and Biotechnology Institute for post graduate studies, University of Baghdad. A consent document was signed by all those involved. The World Medical Association's Declaration of Helsinki was strictly adhered to in all human subjects' studies.

RESULTS

Detection of biofilm formation

Quantification of biofilm production in plastic microtiter plates was performed as previously described, and as shown in table (6) the result was strong biofilm production.

Table (6): Biofilm development of *E. coli* isolates using the Microtiter plate method

isolate	Source of isolate	4ODC	OD	Biofilm production
E_1	Urine	0.8	1.124	Strong
E_2	Urine	0.8	2.837	Strong
E_3	Urine	0.8	2.772	Strong
E_4	Urine	0.8	1.873	Strong
E_5	Urine	0.8	1.658	Strong
E_6	Urine	0.8	2.837	Strong
E_7	Urine	0.8	1.385	Strong
E_8	Urine	0.8	2.622	Strong
E_9	Urine	0.8	1.396	Strong
E_{10}	Urine	0.8	1.181	Strong

E: *E. coli*

Phytochemical screening of *L. nobilis* leaves extracts.

Phytochemical characterizations of both *L. nobilis* extracts were presented in table (7). Phenols, flavonoids, alkaloids, tannins, glycosides, and saponins were all present in the methanolic and aqueous extract.

Table (7): Phytochemical screening of *L. nobilis* leaves extracts

Phytochemical compound		Aqueous Extract	Methanolic Extract
Alkaloids	Meyer's test	+	+
	Wagner's reagent	+	+
Tannins	Lead acetate	+	+
	Ferric chloride	+	+
phenols	Lead acetate	+	+
	Ferric chloride	+	+
Saponins		+	+
Flavonoids		+	+
Glycosides		+	+

High-performance liquid chromatography (HPLC)

In this study, seven polyphenolic compounds consisted of four flavonoids derivatives (Apigenin, Luteolin, Rutin, and kaempferol) and three phenolic acids. (Caffeic acid, Gallic acid, and Syringic acid) were discovered in methanolic and aqueous extracts (Figure 1), when compared with standard compounds as shown in figure (2). A quantitative analysis of *L. nobilis* extracts showed different quantities in the aqueous and methanolic extract, as shown in table (8).

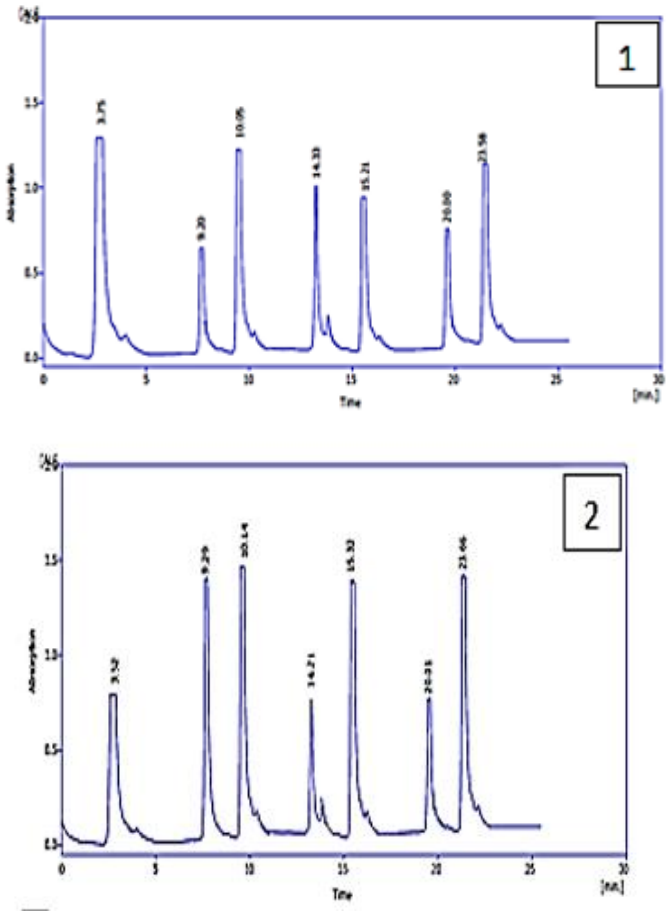

Figure 1: HPLC chromatogram of phenolic compound in (1) methanolic *Laurus nobilis* leaves extract, (2) aqueous extract

Table (8): Quantitative analysis of *L. nobilis* leaves extracts

No.	Active Compounds	Methanolic extract (ppm)	Aqueous extract (ppm)
1	Apigenin	36.8	14.78
2	Caffeic acid	10.9	6.33
3	Gallic acid	44.8	31.9
4	Luteolin	20.56	12.33
5	Rutin	41.2	29.88
6	Syringic acid	28.9	16.55
7	kaempferol	28.9	20.8

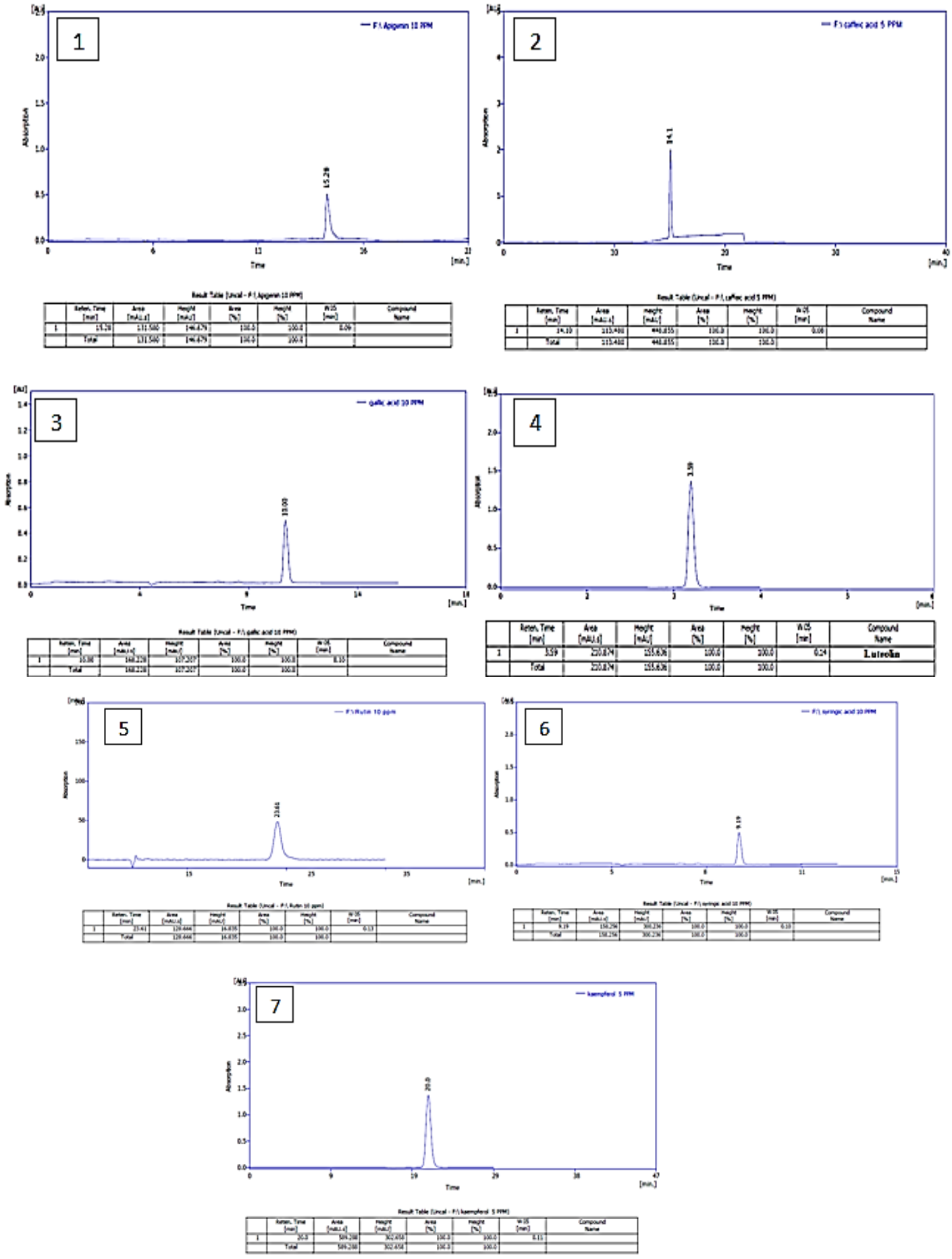

Figure 2: HPLC chromatogram of phenolic compound standard: (1) Apigenin, (2) Caffeic acid, (3) Gallic acid, (4) Luteolin, (5) Rutin. (6) Syringic acid, (7) Kaempfero.

Anti-Biofilm activity of *L. nobilis:*

In this study *Laurus nobilis* methanolic leaves extract had antibiofilm activity, it inhibited 90% and 100% of the biofilm formation of *E. coli* in 32 and 64 mg/ml respectively, and reduction in biofilm formation in 16 mg/ml as shown in table (9). The antibiofilm activity of the methanolic extract on *E. coli* isolates inhibited 100% of the biofilm formation in 128 mg/ml.

Table 9: Biofilm formation of *E. coli* isolates before and after treatment by *L. nobilis* methanolic extract

Isolates	(Control)	After treatment concentrations (mg/ml)							
		1	**2**	**4**	**8**	**16**	**32**	**64**	**128**
E_1	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
E_2	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
E_3	Strong	Strong	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
E_4	Strong	Strong	Strong	Strong	Moderate	Moderate	Weak	No Biofilm	No Biofilm
E_5	Strong	Strong	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
E_6	Strong	Strong	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
E_7	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
E_8	Strong	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
E_9	Strong	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
E_{10}	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm

Molecular detection of *fimA* and *papC* genes:

Multiplex PCR was used to detect the genes of *fimA* measuring 110bp and *papC* measuring 134bp. Results showed that 70% of the isolates that had *fimA* and *papC* was present in 100% of the total isolates as shown in figures (3) and 4).

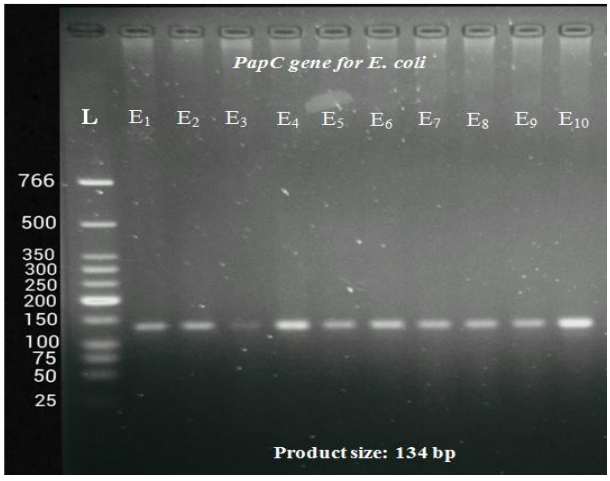

Figure (4): Gel electrophoresis of amplified *papC* (134 bp), from *E. coli* using conventional PCR. Agarose 2% stained with Ethidium bromide dye DNA ladder 25-766 bp

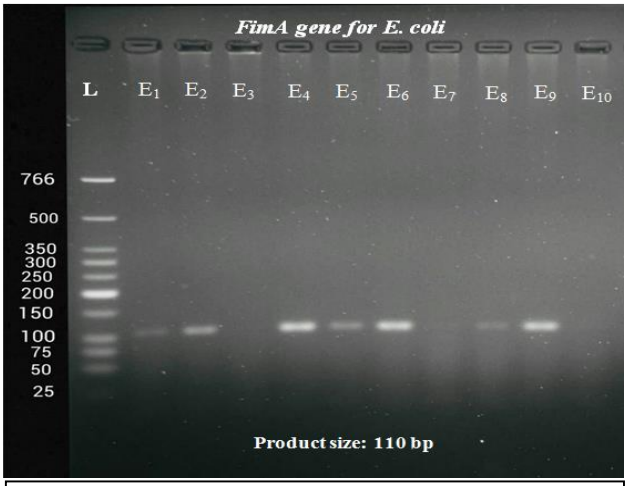

Figure (5): Gel electrophoresis of amplified fimA (110 bp), from *E. coli* using conventional PCR. Agarose 2% stained with Ethidium Bromide dye DNA ladder 25-766 bp

Gene expression Analysis Using qRT PCR Technique:

Total RNA concentrations were from 62.5 to 240 ng /µl. Quantitative Real-Time PCR was performed, and the results showed a decrease in gene expression in the *fimA* and *papC* genes as shown in table (10).

Table (10): Gene expression results for *fimA* before and after treatment with methanolic *L. nobilis* leaves extract (C): control, (E): *E. coli*

group	sample	Ct reference gene	Ct target gene	ΔCT	ΔΔCT	folding
Before treated (Control)	C_1	23.08	12.2	- 10.88	0	1
	C_2	25.3	15.4	- 9.9	0	1
	C_3	21.4	18.3	- 3.1	0	1
	C_4	20.19	17.57	- 2.62	0	1
	C_5	22.09	19.33	- 2.76	0	1
	C_6	22.85	17.51	-5.34	0	1
	C_7	17.56	13.95	-3.61	0	1
	C_8	18.65	14.51	- 4.14	0	1
	C_9	27.09	15.03	-12.06	0	1
	C_{10}	20.98	11.09	- 9.89	0	1
After treated	E_1	22.75	23.74	0.99	11.87	0.000267
	E_2	23.02	20.62	- 2.4	7.5	0.00552
	E_3	18.01	16.03	- 1.98	1.12	0.4600
	E_4	21.2	22.18	0.98	3.6	0.0824
	E_5	22.05	15.09	- 6.96	- 4.2	0.0544
	E_6	22.11	19.3	-2.81	2.53	0.173
	E_7	20.15	18.75	- 1.4	2.21	0.2161
	E_8	23.53	13.09	- 10.44	-6.3	0.0126
	E_9	32.01	22.08	- 9.93	2.13	0.2284
	E_{10}	23.09	19.22	- 3.87	6.02	0.0154

Table (11): Gene expression results for *PapC* before and after treatment with *L. Nobilis* leaves extract

group	sample	Ct reference gene	Ct target gene	ΔCT	ΔΔCT	folding
Before treated (Control)	C_1	25.3	17.1	- 8.2	0	1
	C_2	28.01	23.3	- 4.81	0	1
	C_3	----	----	----	----	----
	C_4	20.32	17.51	- 2.81	0	1
	C_5	26.01	12.09	- 13.92	0	1
	C_6	20.32	17.51	- 2.81	0	1
	C_7	----	----	----	----	----
	C_8	19.45	13.95	- 5.5	0	1
	C_9	22.03	13.99	-8.04	0	1
	C_{10}	----	----	----	----	----
After treated	E_1	30.88	23.74	- 7.14	1.06	0.478
	E_2	22.62	18.09	- 4.54	0.27	0.829
	E_3	----	----	----	----	----
	E_4	21.49	22.03	0.54	3.35	0.0980
	E_5	22.62	20.34	- 2.28	11.46	0.00035
	E_6	25.99	31.24	5.25	8.06	0.00374
	E_7	----	----	----	----	----
	E_8	23.54	21.23	- 2.31	3.19	0.10957
	E_9	19.3	21.01	1.98	10.02	0.000963
	E_{10}	----	----	----	----	----

DISCUSSION

The phytochemicals extracted from medicinal plants are used as drugs [17]. The methanolic and aqueous extracts also contain tannins, phenols, flavonoids and glycosides. These results agree with **Mayara and Viviane [18]** and **Al Chalabi *et al.* [19]**. They reported the presence of alkaloids, tannins, saponins flavonoids, glycosides, phenols and coumarins in aqueous and alcoholic extracts of *Laura nobilis*. The presence of bioactive substances in *L. nobilis* such as polyphenolic compounds, including saponins, flavonoids and tannins endowed with anti-inflammatory activity [20]. The flavonoids are the most generally known that are able to inhibit the oxidants released by leukocytes and phagocytes in the inflammatory area thus maintaining the inflammation [21], The flavonoids also have strong anti-oxidant and water-soluble free radical scavenger properties that are beneficial in avoiding oxidative cell damage [22]. High-performance liquid chromatography (HPLC) analysis findings in methanolic and aqueous extracts were: seven polyphenolic compounds consist of four flavonoids derivatives (Apigenin, Luteolin, Rutin, and kaempferol) and three phenolic acids (Caffeic acid, Gallic acid, and Syringic acid). These findings corroborated the findings of **Nehad** [23] who mentioned that the HPLC analysis on the *L. nobilis* extract found the presence of 11 of phenolic compounds, and several flavonoid derivatives in the extract, which were 81.66 mg/g and 418.37mg/g for Gallaic acid and Rutin derivatives, respectively. Furthermore, **Stefano *et al.*** [24] showed that phytochemicals had many flavonoids. They found 10 flavonoids and the main flavonoid was kaempferol, and their glycosides were also frequently found. A biofilm is an extracellular polymeric (EPS) material that contains a colony of bacteria that have attached to a surface. The main causes of many persistent and chronic infections are the development of these sessile communities and their innate resistance to antimicrobial drugs. In this regard, the previous few decades showed extensive research directed toward examining the anti-biofilm properties of natural compounds, including phytochemicals and extracts from medicinal plants [25]. The phytochemical (flavonoids) compounds showed antibiofilm activity through inhibition or reduction in biofilm formation in a concentration-dependent manner, probably, by partially lysing bacteria, flavonoids likely cause bacterial aggregation. Membrane fusion follows, which lowers active nutrient uptake through a decreased membrane region [26].

The study's results of molecular detection of *fimA* and *papC* genes multiplex PCR agree with **Dadi *et al.*** [27] who found in Ethiopia that *fim* was present in 82% of their isolates but they also found the presence of *papC* gene at 29.5%, which disagree with our result. On the other hand, this result was close to **Sargol *et al.*** [28] result where they found that 90.3% were *fimA* positive and *papC* positive strains (90.4%)**.** In a study of **Fattahi *et al.*** [29] on *E. coli* strains causing UTIs from northwest Iran, they found that there is no connection between presence of *fimA* and the development of biofilm formation. However, they claimed that the capacity to produce biofilms was connected to the existence of *papC*. According to our data, expression of type 1 fimbria is independently linked to the development of biofilms. In order to investigate the gene expression of *fimA* and *papC* genes, RNA was extracted from the isolates before and after treating with the sub MIC concentration of the methanolic *L. nobilis* extract. The amplification was recorded as Ct value (cycle threshold) indicating that high Ct values indicate low gene expression and low Ct value indicates a high gene expression. The housekeeping gene used in molecular studies due to the fact that its expression remains constant in the cells or tissues under investigation and different conditions [30]. Treated isolates with methanolic *L. nobilis* extract increased the CT value, indicating the low expression of the *fimA* and *papC* genes (folding) responsible for biofilm formation in *E. coli*. A study by **Faezah *et al.*** [31] showed that the use of these herbal compounds reduced the expression of the genes in the presence of the Sub-MIC concentration of cinnamon essential oil in 8 *E. coli* isolates compared to untreated isolates and came to the conclusion that the plant extract has good antibacterial effects on E. coli and can lower the production of biofilm and the expression of genes that are effective in causing disease. Another studies by **Mahdi and Al-Azawi** [32] and **Al-Khafaji** and **Al-Azawi** [33] showed that the treatment with the methanolic and aqueous extracts of the *Conocarpus erectus* and *Rosmarinus officinalis* inhibited the biofilm formation and down-regulation of the expression of *pelA* and *algD*, which are responsible for biofilm formation in *P. aeruginosa*. So medicinal plants have active ingredients that possess a variety of medical and therapeutic qualities that can lower bacteria's resistance to antibiotics, including *E. coli*.

CONCLUSION

The antimicrobial activity of *Laurus nobilis* leaves extracts is related to the quantitative of polyphenolic compounds (Apigenin, Caffeic acid, Gallic acid, Luteolin, Rutin, Syringic acid, and kaempferol) present in the methanolic and aqueous extracts. The methanolic extract was more efficient than aqueous extract in antibacterial activity against *E. coli* isolates, which were more resistant to commonly used antibiotics and most of them carried *fimA* and *papC* genes, responsible for biofilm formation. On the other hand, the results of gene expression showed remarkable down-regulation effects of *Laurus nobilis* extract on the *fimA* and *papC* genes for all *E. coli* isolates.

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