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Original Research Article

Antibacterial, Antibiofilm Activity of *Cymbopogon citrates* L. Essential oil Leaves Extract and assessment of its effect on *fimA* and *papC* Genes against *Escherichia coli* Isolate

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Abstract: ***Background***: Escherichia coli, sometimes known as E. coli, is a gram-negative bacterium that is typically present in people's intestinal flora. Some forms of E. coli can cause sickness, while the majority of these types are benign and aid in digestion. By creating biofilms, bacteria are able to defend themselves against mechanical, chemical, and biological assaults, giving them exceptional survival benefits. The scented medicinal grass known as lemon grass belongs to the Cymbopogon genus. Lemongrass essential oils include bioactive phytoconstituents that have a wide range of therapeutic benefits, including antibacterial, anticancer, antioxidant, insecticidal, and antimalarial actions. ***Methods*:** The aim of this study is to evaluating the antibacterial activity of **(***C. citrates***)** essential oil leaves extract and assessment of its effect on *fimA* and *papC* genes in *E. coli*. Gas chromatography and mass spectrometry analyses of the *C. citrates* essential oil extract. Forty-eight isolates of *E. coli* from different sources such as (urine, blood and stool. the diagnosis was confirmed using VITEK-2. The experiments use in this study is (Antibiotic susceptibility test, Disc diffusion method, Total phenolic content, Determination of Minimum Inhibitory Concentration (MIC) of *C. citrates* L. m biofilm formation and Genetic analysis). ***Results*:** The objective of this study is to assess the antibacterial and antibiofilm properties of extracts derived from the leaves of C. citrates and the effect on gene expression of *fimA* and *papC* genes after treatment with sub-MIC of *C. citrates* leaves extracts. ***Conclusion***: The finding of this study showed that (90%) of the isolates had *FimA* while *PapC* was present in (60%) of the total isolates. The gene expression revealed that there was a decrease in the expression of *Fim A* and *PapC* genes.

Keywords: *Cymbopogon citrates L.*, *Escherichia coli*, Antibacterial, Antibiofilm, *fimA*, *papC* gene.

INTRODUCTION

The gram-negative bacterium Escherichia coli, sometimes known as E. coli, is a natural component of human gut flora. Some forms of E. coli can cause illness, however the majority are benign and aid with digestion. The rod-shaped (bacilli) E. Coli bacteria usually manifest as solitary cells. Many E. Coli strains can move about in liquid environments because they have flagella. Both oxygen and no oxygen are necessary for its growth. Depending on the amount of oxygen present, it can change its metabolic activities. Some strains of E. coli have gained genes that enable them to cause illnesses, although the majority of strains are harmless [1]. Pathogenic E. coli strains are categorized into various pathotypes, such as enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC). E. coli is widely used in scientific research and biotechnology. Its ease of cultivation, rapid growth, and well-understood genetics make it a valuable model organism for studying various biological processes [2].

Biofilm formation by UPEC poses challenges in the treatment of UTIs as the bacteria within the biofilm can be highly resistant to antibiotics. Disrupting and eradicating the biofilm requires specialized treatment

strategies, such as combination antibiotic therapy or the use of biofilm-disrupting agents [3].

It is possible for bacteria to defend themselves against mechanical, chemical, and biological challenges thanks to the ability to build biofilms, which affords them remarkable survival advantages. Additionally, it encourages bacteria to propagate genes that code for antibiotic resistance. In fact, detergent and antimicrobial treatments are less effective against bacteria found in a biofilm on the surfaces of livestock facilities or inside water pipelines [4].

Lemongrass, also known as Cymbopogon citratus, is a tall, monocotyledonous, aromatic perennial plant with thin, sharp-edged green leaves and a pointed apex that is a member of the Poaceae family [5]. The pharmacological properties of C. citrates include antibacterial action, which is helpful in treating bacterial infections that are resistant to multiple drugs. Because the plant contains chemicals that change resistance, it has an antibacterial effect [6]. Lemongrass essential oils include bioactive phytoconstituents that have a variety of therapeutic effects, including antibacterial, anticancer, antioxidant, insecticidal, and antimalarial actions [7].

The lemon grass-like therapeutic grass belongs to the genus Cymbopogon. It is widespread in tropical and semi-arid regions on the continents of Asia, America, and Africa. This grass' distinctive lemon aroma is one of its identifying traits and results from the high citral content in its oil. The oil is appropriate for usage in perfumes, detergents, and soaps because to its redolence. The pharmaceutical industry also makes use of it [8].

Lemongrass essential oils include bioactive phytoconstituents that have a variety of therapeutic effects, including antibacterial, anticancer, antioxidant, insecticidal, and antimalarial actions [7].

MATERIALS AND METHODS

Plant Collection

Purchasing lemongrass from local Iraqi markets, the expert from the biology department at the University of Baghdad's College of Science identified it as C. citrates. After being washed with water and allowed to air dry, the leaves were ground into a powder in a grinder and stored at 4°C for additional analysis.

Essential Oil Preparation

The Clevenger apparatus was used to steam distill the C. citrates leaves in order to extract the volatile oil. 100 g of the leaves were added, after being cut into small pieces, to the round flask of the distillation apparatus with a capacity of (2) liters, which contained 1000 ml of distilled water in a weight-to-volume ratio of 1:10, at a temperature of 100°C, for 2-3 hours [9].

Gas Chromatography Mass Spectrophotometer Analysis

GC-MS equipment was used to analyze the volatile oil extract from C. citrates. The equipment's experimental circumstances are as follows: Dimensions of the HP-5MS ultra inert capillary non-polar column are 30 mm 0.25 mm, 0.25 mm for the ID, and 0.25 m for the film thickness. The mobile gas has a flow rate of 1 ml/min. For the gas chromatographic component, the oven temperature was 50°C escalated to 300°C at 7°C/min for 10 min. The mass spectrometer was used to determine the composition and structure of substances. A comparison was made between the spectrum of unexplained components and the spectrum of identified components kept in the NIST collection [10, 11].

Bacterial Isolates

Ten Escherichia coli isolates that had previously been collected from patient urine at hospitals in Baghdad and identified by using the VITEK-2 System. The susceptibility to eleven distinct antibiotics was analyzed using the Kirby-Bauer technique developed by the World Health Organization. To create a bacterial suspension with a moderate level of turbidity in comparison to the usual turbidity solution, one or two isolated colonies of bacteria from the original culture were selected and added to a test tube containing three milliliters of conventional saline. This is roughly equivalent to 1.5x108 CFU/ml [13, 14]**.** Using the traditional disc diffusion approach, antibacterial activity was evaluated.to establish whether C. citrates essential oil extracts have antimicrobial qualities. The bacterial culture, corrected to the 0.5 McFarland standard, was used to evenly inoculate Muller Hinton agar plates using a sterile swab. The plates were dried for 15 minutes before to the sensitivity test The unfavorable controls were DMSO discs. Prior to being placed on the Mueller Hinton agar surface, each disc was completely dried. For 18 to 24 hours, the plates were incubated at 37°C. The antibacterial activity was assessed by measuring the inhibitory zone surrounding the discs following incubation. To guarantee reliability, the test was run three times.

Finding the Minimum Inhibitory Concentration (MIC) of Extracts from C. citrates L. essential oil leaves

A 96-well microtiter plate and the broth microdilution method were used to determine the minimum inhibitory concentration (MIC) of C. citrate extracts. The concentrations (32-1 l/ml) for the essential oil extract were created by making consecutive two-fold dilutions of the extract immediately on the plate after the working plant extract solution (64 l/ml) was prepared in broth.

Microtiter plates were incubated at 37C0 for 24 hours. Twenty liters of the resazurin dye were added to each well, and the mixture was incubated for half an hour to check for color changes. The Minimum. In the

resazurin broth assay, inhibitory doses were visually identified in broth microdilutions as the lowest extract concentrations at which no color changed from blue to pink [16].

Biofilm formation assessment

The amount of biofilm that E. coli forms was quantified in accordance with the description in [17]. Every isolate was cultivated overnight at 37°C in Brain Heart Infusion Broth. Using a pipette, each isolate was put to tryptic soy broth (TSB), which contains 1% glucose, and mixed well. The bacterial isolate's suspension's turbidity was fixed to McFarland No. 0.5.

Optical density (OD) measurements were taken using an ELISA auto reader set to operate at 630 nm. After calculating each test result, the average of the sterile medium's OD values was deducted. To categorize isolates as either forming biofilms or not, the cut off value (ODc) was calculated [18].

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

By the calculation of cutoff value (ODc), the result of biofilm detected as below (Table 1):

Table 1: The classification of bacterial adherence

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

Study the antibiofilm activity of *C. citrates* leaves extracts

A 96-well microtiter plate was used to test the C. citrates essential oil extract's antibiofilm properties. The working solution of the plant extracts was prepared at 50 ppm for the essential oil extract in order to attain concentrations of 32-1 (l/ml). The first wells in row A were filled with 200 l of each sample at a time. Only rows B through H of the columns contained 100 l of the broth. Methodically, double serial dilutions using micropipettes were carried out down the columns (beginning with rows A–H). Up until the final row (H), where the final 100 l was thrown away, the procedure was repeated. From the initial concentrations in row A, 100 l were removed and moved to the following row along with the properly mixed 100 l of broth. All of the wells except the negative control received 100 l of the 1 106 CFU/ ml bacterial inoculum. The identical process as described in the previous sentence (Assessment of biofilm formation) was followed.

Polymerase chain reaction (PCR) molecular identification of virulence genes

Genomic DNA extraction

DNA from E. coli bacteria was extracted using a commercial purification technique called the Genomic DNA Extraction Mini Kit (iNtron®, Korea). Both Gram-positive and Gram-negative bacteria can have their DNA isolated using this technique. This kit extracted DNA from Gram-negative bacteria using the bacterial method.

Estimation of the DNA concentration and purity

DNA concentration is measured with the Nanodrop. The Nanodrop measures the optical density (O.D.) at 260 and 280 nm wavelengths after adding one microliter of the isolated DNA. The following formula is used to estimate the DNA purity ratio: O.D. 260 nm / O.D. 280 nm is the DNA purity ratio.

Molecular identification of the genes papC and fimA 12.5 liters of OneTaq (NEB®) mastermix, 5 liters of DNA sample, 1 liter of each primer diluted to 10 pmol/l, and 5.5 liters of free-nuclease water were added at this step. The optimal PCR conditions for the gene, as indicated in Table (2), were used to conduct the reaction.

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

Cycle No.	Step	Temperature	Time
1	Initial Denaturation	94 °C	5 min.
40x	Denaturation	94 °C	30 sec.
	Annealing	60 °C	45 sec.
	Extension	72 °C	45 sec.
1	Final Extension	72 °C	7 min.

Analysis of Gene Expression Applying the qRT-PCR Method To ascertain the effect of the C. citrates essential oil extract on the gene expression of fimA and papC associated with biofilm formation, the expression of the two genes in the isolates was assessed both before and after treatment with the extract. To encourage bacterial growth, the essential oil extract was used at a concentration below the minimum inhibitory

concentration (MIC). RNA was extracted using TRIzolTM Reagent in compliance with the manufacturer's suggested methodology. To assess the expression of the fimA and papC genes, Table (3) lists [19]. As a direct comparison of Ct values between the target and reference (housekeeping) genes, the data findings of qRT-PCR were calculated. The Ct technique was used to evaluate the genes by relative measurement of fold changes in gene expression levels.

Table 3: Primers utilized in this Study

Primer name		Sequence (5´-3´)	Production size	Reference
FimA	F	CAGGTTGTCACACTCGGTGA	110 bp	[19]
	R	GCAACAACAGGATCGCAGTC		
PapC	F	GGTTTGTGCGGTGGTTTGAA	134 bp	
	R	CCCACGGAGTTGAAGAACGA		
House Keeping gene *16S rRNA*	F	GGATCAGAATGCCACGGTGA	170 bp	
	R	GCAGGTTCCCCTACGGTTAC		

RESULTS AND DISCUSSION

Gas Chromatography Mass Spectrometry (GS-MS)

The C. citrates essential oil extract was subjected to gas chromatography and mass spectrometry analyses, which resulted in the identification of ten components, as shown in Figure (1). All identified compounds (Eugenol, Limonene, Linalool, Camphene, Cymene, Citral, Geraniol, a-pinene, Camphor, and Terpinen) were listed along with their respective retention times and percentages of compound in the extract.

The results were compared to a study by [20] who mentions that the Citral content of Cymbopogon citrates oil extract varies from 44.3 and contains other compounds such as Geranyl acetate, Linalool, Geraniol, Eugenol, and Ferulic acid. In this study, Limonene (32.12) and Citral (33.25) was the main element and many other compounds were also present.

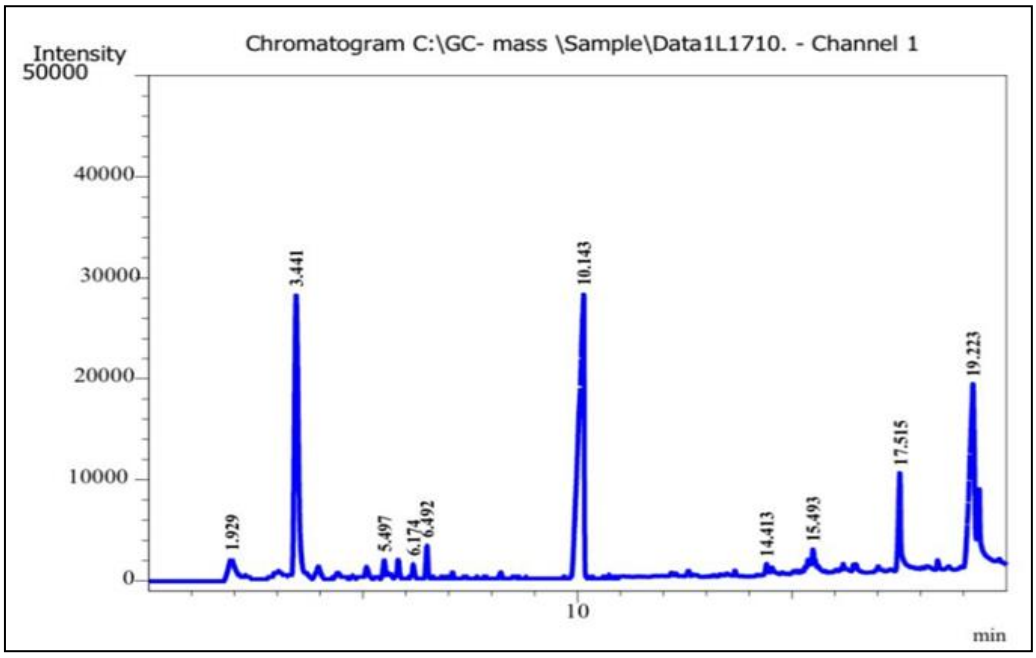


Figure 1: GC-MS for essential oil of *C. citrates*

Total phenolic content of essential oil *C. citrates* leaves extracts

For their beneficial effects on human health and biological characteristics, numerous phenolic substances have been investigated [21]. The essential oil leaves extract had the highest total phenolic content, containing 110.91 mg/g at a concentration of 500 g/ml, as indicated in (Tables 4).

Table 4: Total phenolic content of *Cymbopogon citrates* essential oil leaves extract

Concentration (µg\ml)	Essential oil extract (mg\g)
125	30.12
250	55.18
500	110.91

Test for antibiotic susceptibility

The E. Coli isolates' resistance to the antibiotics ranged from 45.4% to 72.7%, according to the antibiotic susceptibility test. As can be shown in Table (2), the resistance percentages to ticarcillin and cefepime were the highest and to colistin, lower, respectively. The necessity to create fresh and cutting-edge antimicrobial medicines is critical given the rise in resistance clinical isolates. As a result, scientists are searching for fresh information to help them find improved remedies for multidrug resistant bacteria strains. Due to their widespread usage as treatments for a variety of infectious diseases and the abundance of bioactive substances they contain, plants have long been considered one of the possible sources of novel drugs [22].

Table 5: Antibiotic susceptibility test of *E. coli*

Antibiotics / Isolates	AMC	TIM	FEP	CTR	IPM	CL	TOB	AK	CIP	STX	FOF	Resistant (%)
E_1	R	R	R	R	S	S	S	S	I	R	R	54.5%
E_2	I	R	R	R	S	S	S	S	R	R	I	45.4%
E_3	R	R	R	R	S	S	S	S	R	R	S	54.5%
E_4	I	R	R	R	S	S	S	I	S	R	R	45.4%
E_5	R	R	R	I	S	S	S	S	R	R	R	54.5%
E_6	R	R	R	R	S	S	R	S	R	R	R	72.727%
E_7	R	R	R	R	R	S	S	S	R	R	R	72.727%
E_8	R	R	R	R	R	S	S	R	R	I	R	72.727%
E_9	R	R	R	R	S	S	S	R	S	R	R	63.636%
E_{10}	R	R	R	R	R	S	S	S	R	R	S	63.636%
Resistant (%)	80%	100%	100%	90%	30%	0%	10%	20%	70%	90%	70%	

(E): *E. coli*, (R): Resistance, (I): Intermediate, (S): Sensitive, (AMC): Amoxicillin, (TIM): Ticarcillin, (FEP): Cefepime, (CTR): Ceftriaxone, (IPM): Imipenem, (CL): Colistin, (TOB): Tobramycin, (AK): Amikacin, (CIP): Ciprofloxacin, (SXT): Trimethoprim, (FOF): Fosfomycin

Antibacterial activity of *C. citrates* L. leaves extracts

The disk-diffusion method was used to evaluate the bactericidal activity of C. citrates L. leaf extracts on E. coli isolates. As shown in Table (6), the results showed that the essential oil extract was more effective at a concentration of 50 l/ml with a significant difference (P0.05).

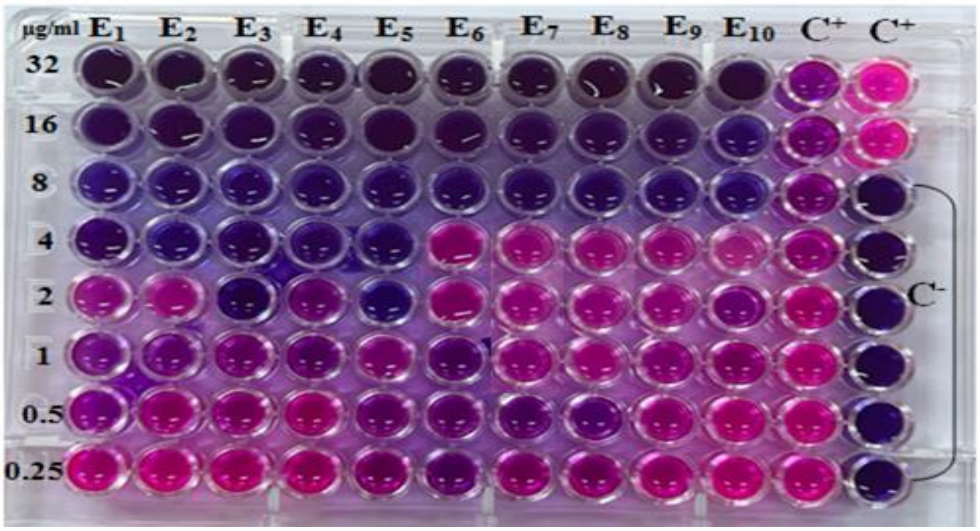
Table 6: Antibacterial Activity of *C. citrates* essential oil on *E. coli*

No. of Isolate	Essential oil		LSD value
	25 µg/ml	50 µg/ml	
E_1	18.67 ±0.33	26.67 ±0.33	5.72 *
E_2	18.33 ±0.33	25.67 ±0.33	4.58 *
E_3	19.67 ±0.3	34.67 ±0.33	5.96 *
E_4	16.67 ±0.33	24.67 ±0.33	5.23 *
E_5	20.67 ±0.33	30.67±0.33	5.04 *
E_6	11.00 ±0.57	20.00 ±0.57	6.14 *
E_7	11.00 ±0.57	21.67 ±0.33	5.63 *
E_8	10.67 ±0.33	19.00 ±0.57	5.03 *
E_9	12.33 ±0.33	21.67 ±0.33	5.83 *
E_{10}	13.67 ±0.33	22.67 ±0.33	5.67 *
LSD value	1.16 *	1.17 *	---
* (P≤0.05).			

Citral, the primary constituent of lemongrass, has been used as a natural preservative and flavoring agent because of its antibacterial activity against gram-negative and gram-positive bacteria like Escherichia coli and Staphylococcus aureus, respectively [23, 24]. This study summarized the superior effectiveness of C. citrates leave essential oil in lowering the number of total microorganisms, coliform bacteria, molds, and yeasts.

Calculating the C. citrates leaf extract's minimum inhibitory concentration (MIC)

The broth microdilution method and 96-well microtiter plates were used to determine the MIC of the plant extracts. The oxidation-reduction colorimetric indicator resazurin has been used to determine the antibiotic's minimum inhibitory concentration (MIC) against Escherichia coli. The MIC can be determined without a spectrophotometer because resazurin dye, which is blue when oxidized but turns pink when reduced by living cells, is easily visible to the unaided eye [25, 26]. The essential oil was found to be more effective based on the MIC of C. citrate extracts. As seen in figure (2), the oil extract's MIC values for each isolate were 2–8 mg/ml.



(E): *E. coli*, **(C^+):** Control positive (Bacteria + Media), **(C^-):** Control negative (Media only)

Figure 2: MIC of *C. citrates* essential oil extract on *E. coli*

Anti biofilm Activity of *C. citrates*:

A biofilm is a densely packed collection of microbial cells that adheres to and grows on either living or nonliving surfaces. It envelops itself in secreted polymers. Biofilm-associated diseases are often difficult to treat because to multi-drug resistance [27], so it is important to identify new and potent chemicals that prevent the formation of bacterial biofilms. On silicone rubber prostheses and other medical devices where C. tropicalis biofilms are present, there is a good chance that the C. citratus oil will be employed as an antifungal and antibiofilm agent, potentially extending the lifespan of the prosthesis [28].

Citrates act on biofilm formation in E. coli through a number of mechanisms, including inhibition of adhesion, destruction of extracellular polymeric substance, biofilm dispersal, antimicrobial activity, and disruption of quorum sensing. Lemongrass leaf extracts contain a variety of bioactive compounds, including essential oil and phenolic compounds (Table 7).

Table 7: Biofilm formation of *E. coli* isolates before and after treatment with *C. citrus* essential oil extract

Isolates	before treatment (control)	After treatment							
		Concentration							
		0.25	0.5	1	2	4	8	16	32
E_1	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_2	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_3	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_4	**Strong**	Strong	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm	No biofilm
E_5	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_6	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_7	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_8	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_9	**Strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E_{10}	**strong**	Strong	Moderate	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm

It is well known that E. coli may create a biofilm, which is an ideal setting for the exchange of resistance determinants [29] draw the conclusion that essential oils can inhibit the growth of E. coli biofilms, reduce adhesion, and produce fewer glycans. This inhibitory effect is related to the non-competitive inhibition of glucose transfer activity brought on by the presence of citral and geraniol; these findings suggest a potential inhibition mechanism of terpenes on the growth of E. coli biofilms.

Molecular detection of *fimA* and *papC* genes

It was determined whether the biofilm-forming virulence genes FimA and PapC were present in E. coli isolates using traditional PCR and primers that were specific to each gene.

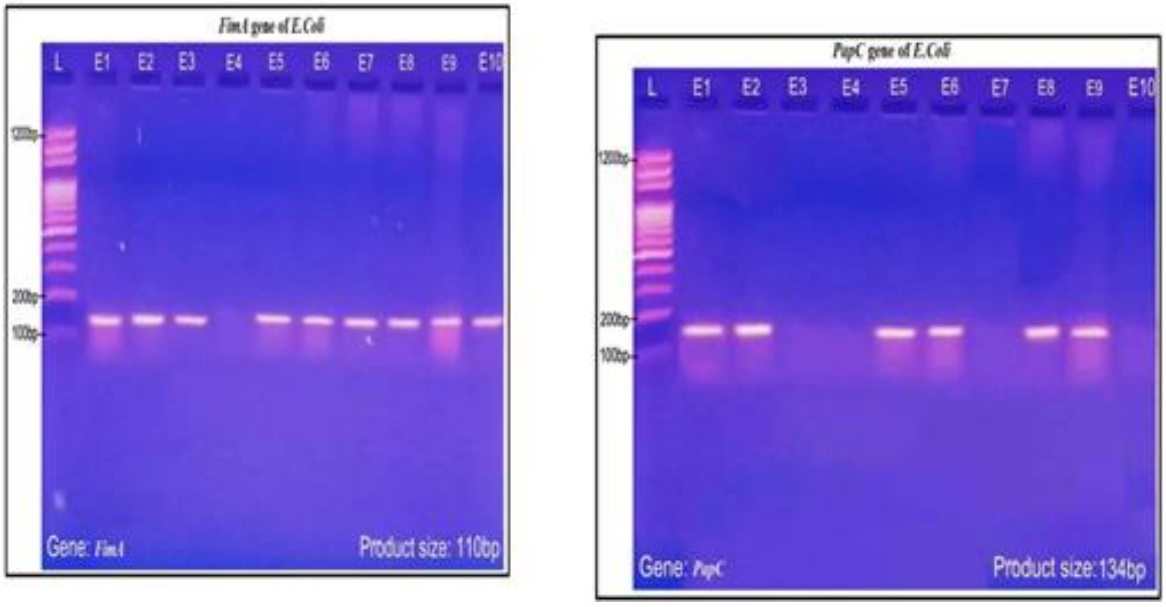


Figure 3: Gel electrophoresis analysis

According to the findings, FimA was present in (90%) of the isolates, while PapC was found in (60%) of all the isolates, as shown in figures (4-17) and (4-18) [19] discovered that while the PapC gene was present in 100% of E. coli isolates, the FimA gene was only present in 70% of E. coli isolates. Another study discovered that 82% of people had FimA and 29.5% had PapC [30].

RNA was isolated from the isolates before and after treatment with the sub-MIC concentration of the essential oil C. citates extract in order to examine the gene expression of the fimA and papC genes. The range of total RNA values was 7.1 to 99.1 ng/l. The fimA and papC genes' gene expression decreased as determined by quantitative real-time PCR, as illustrated in Tables (8 and 9). The amplification was recorded as a Ct value (cycle threshold), indicating that low Ct values imply strong gene expression and high Ct values indicate low gene expression. The housekeeping gene is employed in molecular investigations because it exhibits continuous expression in the cells or tissues under study under various situations [31].

Gene expression of FimA and PapC

The CT value rose in isolates treated with the essential oil C. citrates extract, showing decreased expression of the fimA and papC genes (folding) necessary for biofilm formation in E. coli.

According to a study by [32], the use of these herbal compounds reduces the expression of genes in 8 E. coli isolates when cinnamon essential oil is present in Sub-MIC concentrations compared to untreated isolates. The study's authors conclude that the plant extract has a good antibacterial effect on E. coli and can lower the production of biofilm and the expression of genes that are effective in causing disease.

Table 8: Gene expression results for *FimA* genes before and after treatment with essential oil leaves extracts

Group	Samples	Ct reference gene	Ct target gene	ΔCT	ΔΔCT	Fold
Before	E_1	36.37	12.61	-23.71	0	1
	E_2	33.65	16.52	-17.13	0	1
	E_3	18.99	20.03	1.04	0	1
	E_4	-	-	-	-	-
	E_5	25.02	18.85	-6.17	0	1
	E_6	16.95	20.30	3.35	0	1
	E_7	17.78	20.30	2.52	0	1

Group	Samples	Ct reference gene	Ct target gene	ΔCT	ΔΔCT	Fold
	E_8	34.4	12.3	-22.1	0	1
	E_9	36.36	13.62	-22.74	0	1
	E_{10}	35.19	13.23	-21.96	0	1
After	E_1	35.65	20.73	-14.92	8.84	0.00218
	E_2	15.48	18.24	2.76	1.38	0.384219
	E_3	15.27	19.51	4.24	3.2	0.108819
	E_4	-	-	-	-	-
	E_5	15.01	19.99	4.98	11.15	0.00044
	E_6	16.31	20.13	3.82	0.47	0.721965
	E_7	19.24	24.04	4.8	2.28	0.205898
	E_8	34.65	21.43	-13.82	8.28	0.0032
	E_9	36.28	19.49	-16.79	5.95	0.0161
	E_{10}	33.65	16.52	-17.13	4.83	0.03515

Table 9: Gene expression results for *PapC* genes before and after treatment with essential oil leaves extracts

Group	Samples	Ct Reference gene	Ct Target gene	ΔCT	ΔΔCT	Fold
Before	E_1	21.17	19.3	0.67	0	1
	E_2	15.63	22.47	3.18	0	1
	E_3	-	-	-	-	-
	E_4	-		-	-	-
	E_5	18.99	21.94	6.93	0	1
	E_6	25.02	18.45	2.14	0	1
	E_7	-	-	-	-	-
	E_8	15.64	29.54	8.98	0	1
	E_9	17.26	20.40	-0.16	0	1
	E_{10}	-	-	-	-	-
After	E_1	18.63	29.88	11.25	10.58	0.000653
	E_2	19.29	19.16	-0.13	-3.31	0.917662
	E_3	-	-	-	-	-
	E_4	-	-	-	-	-
	E_5	15.01	24.75	8.44	1.51	0.35111
	E_6	16.31	28.92	9.68	7.54	0.005373
	E_7	-	-	-	-	-
	E_8	15.03	24.62	9.59	0.61	0.655197
	E_9	20.56	20.40	-0.16	0	0.258816
	E_{10}	-	-	-	-	-

Depending on the concentration of the extract, the length of exposure, and the particular genes being researched, the effects of lemongrass extract on gene expression in E. coli may differ. According to a different study, flavonoids can have antibacterial effects via a number of different mechanisms, such as anti-virulence mechanisms, cytoplasmic membrane disruption, energy metabolism, inhibition of folic acid synthesis, inhibition of cell membrane synthesis and function, and inhibition of nucleic acid synthesis [33, 34]. Thus, the antibacterial and down-regulation effects of the phenolic extract in this study may be due to all of these mechanisms.

CONCLUSION

The extracts of C. citrates leaves exhibited antibacterial activity against E. coli isolates, with the essential oil demonstrating greater effectiveness. Molecular identification of FimA and PapC genes and examination of gene expression by comparing the isolates exposed to Sub-MIC of oil C. citrates leaf extracts with the untreated isolates revealed that (90%) of the isolates contained FimA, while PapC was found in (60%) of the entire isolate group. The gene expression showed a reduction in the expression levels of Fim A and PapC genes.

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