

## EVALUATION ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF *Cymbopogon citrates* L. ON FIMA AND PAPC GENES AGAINST *Escherichia coli*

Maha A. Khalaf\*  , A. H. I. AL-Azawi\*\*  

\*Dept. of Field Crops . Coll. Agric. Sci. , Al-Qasim Green University , Iraq .

\*\* Dept. Biotechn. Gen. Eng. And Biotech. Inst. for Post Grad. Studies , University of Baghdad , Iraq .

### ABSTRACT

This study was aimed to evaluate the antibacterial activity on *Cymbopogon citrates* (*C. citrates*) leaves extract and assessment of its effect on *fimA* and *papC* genes in *Escherichia coli* (*E. coli*) isolates. Rotary apparatus were used to prepare aqueous and methanolic extract; total phenolic content and high-performance liquid chromatography (HPLC) were conducted to determine the active compounds in the extract. The results showed that the methanolic and aqueous extracts contain seven flavonoids derivatives (Catechine, Chlorogenic acid, Ferulic acid, Gallic acid, P-Coumaric acid, Quercetin and Kaempferol) in various ratios were identified on the basis of matching retention time with the standards. The total phenolic contents were 19.56 and 47.45 mg/g in 50 mg/ml, in aqueous and methanolic extracts respectively. The antibacterial activity of *C. citrates* leaves extracts showed that the aqueous extract was less effective than methanolic extract in concentration 16 mg/ml. Moreover, the result of the minimum inhibitory concentration (MIC) showed that the methanolic extract on *E. coli* isolates was 16 mg/ml, while the MIC values of aqueous extract were 32 mg/ml. *C. citrates* methanolic leaves extract had antibiofilm activity, which inhibits 90% and 100% of the biofilm formation of *E. coli* in concentrations 16 and 32 mg/ml respectively. The result of the gene expression revealed that there is a decrease in the expression of the *fimA* and *papC* genes.

**Keywords:** Antibacterial activity, Antibiofilm, Gene expression, Total phenol, MIC.

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### INTRODUCTION

The rapid growth of multidrug-resistant (MDR) bacteria and the development of multidrug-resistant types of germs have undeniably created a serious worldwide health issue. Additionally, the emergence of new diseases necessitates the search for novel antimicrobials, with a focus on plant extracts. Lemongrass (*C. citratus*) plant belongs to the Gramineae family (Kumar et al., 2013). *C. citratus*, universally known as Lemongrass is a small herbaceous plant and is found worldwide especially in Asia and Africa. It is used as traditional medicine for treatment of numerous diseases such as fever, sore throats, cough, laryngitis, bronchitis, oral candidiasis, body ache, head ache, digestive problems (Hassan et al., 2021). The pharmacological properties of *C. citratus* include antibacterial

activity, which is helpful in treating bacterial infections that are resistant to multiple drugs. (Vandepitte et al., 2003). The aqueous and methanolic leaves extracts of *C. citratus* contain a variety of bioactive substances, primarily flavonoids, alkaloids, saponin, tannins, and phenolic compounds like quercetin, luteolin, apigenin, isoorientin 2'-O-rhamnoside, and kaempferol, which are known to have numerous advantages, particularly in the fields of pharmacy, food, health, and agriculture (Ncube et al., 2008). Recently other researches were (Sarowska et al., 2019; Salih et al. 2022) studied the aqueous and ethanolic extracts of lemon grass, which are typically known for their bioactive components (*C. citratus*), were tested in-vitro for their antibacterial effects against a number of different clinical isolates. The typical human

intestinal flora contain facultative pathogens called *E. coli*. APEC *E. coli*. Urinary tract infections (UTI), sepsis, and newborn meningitis are just a few of the illnesses that are brought on by extraintestinal pathogenic *E. coli* (ExPEC). The virulence factors (VFs) found in ExPEC strains enable bacterial cells to enter and grow inside the host, facilitating infection. Adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasins are just a few of the virulence factors (VF) associated with the pathogenicity of ExPEC that are numerous and have a variety of functions, from those related to bacterial colonization to those related to virulence. These VF are typically encoded on pathogenicity islands (PAIs), plasmids, and other (N'Guessan, 2007; Schmittgen et al, 2008). This study was aimed to evaluate the antibacterial activity of (*C. citrates*) leaves extract and assessment of its effect on *fimA* and *papC* genes in (*E.coli*) isolates.

#### MATERIALS AND METHODS

**Preparation of Aqueous Extract:** The aqueous extract was prepared according to (Mahdi and Al-Azawi., 2022) . A total 200 gram of *C. citrates* leaves macerated in 1400 ml of distilled water for 72 hours, after extraction, the mixture was filtered through Whatman No.1 paper. The filtrate evaporated to dryness under vacuum at 50°C by a rotary evaporator to eliminate water. The resulting extract stored in amber glass vials at 4°C until analyzed.

**Preparation of Methanolic Extract:** The methanolic extract was prepared according to (Mahdi and Al-Azawi, 2022) by using Soxhlet apparatus. 100 gm of *C. citrates* leaves were put in a thimble and 700 ml of 70% methanol was added within 40-60 C<sup>0</sup> for 6 hours. The solution was filtered through a filter paper Whatman No.1 and evaporated to dryness under vacuum at 40 C<sup>0</sup> by a rotary evaporator to get rid of methanol; the extract was stored in amber glass vials at 4C<sup>0</sup> until analyzed.

#### Determination of Total Phenolic Contents

Total phenolic content of *C. citrates* extracts were determined spectrophotometrically using the Folin-Ciocalteu method described by (Berradre et al., 2014), 2 ml of Folin-Ciocalteu reagent (diluted 10 times) was mixed with 1.6

ml of 7.5% sodium carbonate solution and 0.4 ml of *C. citrates* extracts. The volume was completed to 5 ml by adding distilled water. The tubes were covered with parafilm for 30 min. at room temperature, and then the absorbance was read at 760 nm spectrophotometrically.

**High-Performance Liquid Chromatography (HPLC):** Methanolic extract of *C. citrates* leaves extract were identified by (HPLC) (Shimadzu, Japan) according to (Rebecca and Amanpreet., 2022) (HPLC) is a widely used analytical technique for separating, identifying, and quantifying components in complex mixtures. To analyze the lemongrass aqueous and methanolic extracts, HPLC can be employed to determine the presence and concentration of various compounds present in these extracts.

**Bacterial isolates:** Ten isolates of *Escherichia coli* were obtained 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 using VITEK-2 System.

**Antibiotic susceptibility test:** Kirby-Bauer method was followed as described by World Health Organization (Vandepitte et al., 2003) to conduct an examination of 11 different antibiotics' susceptibility. In order to create a bacterial suspension with a moderate level of turbidity in comparison to the standard turbidity solution, 1-2 isolated colonies of bacteria from the original culture were selected and added to a test tube containing 3 ml of normal saline. This roughly translates to 1.5x10<sup>8</sup> CFU/ml. A portion of the bacterial suspension was transferred using a sterile cotton swab, spread gently and uniformly on Mueller-Hinton agar medium, and then left for 10 minutes. After that, a sterile forceps was used to firmly lay the antimicrobial discs on the agar while ensuring that they made contact with it. The plates were then turned over and incubated for 18 to 24 hours at 37 C<sup>0</sup>. created inhibition zones (CLSI., 2022 ; Humphries et al., 2021)

**Disc diffusion method:** Antibacterial activity was measured using the conventional disc diffusion methodology .(Subramaniam et al.,

2020) determine whether the alcoholic and aqueous extracts of *C. citrates* have antibacterial properties. Using a sterile swab, the bacterial culture, which had been adjusted to the 0.5 McFarland standard, was used to equally inoculate Muller Hinton agar plates. The sensitivity test was conducted after the plates had been dried for 15 minutes. A 500 mg/ml stock solution of plant extract was made by combining 0.5 g of the extracts with 1 ml of each of their corresponding solvents (distilled water for an aqueous extract and dimethyl sulfoxide (DMSO) for an alcoholic extract, respectively). Following that, the stock solution was diluted to contain extract concentrations of 62.5, 125, and 250 mg/ml. Each dilution was impregnated with 20 l into six-millimeter sterile blank discs. Negative controls were DMSO discs and distilled water. Before placing any discs on the Mueller Hinton agar surface, they were all completely dried. For 18 to 24 hours, the plates were incubated at 37°C. By measuring the diameter of the inhibitory zone surrounding the discs after the incubation, the antibacterial activity was assessed. To assure dependability, the test was run three times.

**Minimum Inhibitory Concentration (MIC) determination of *C. citrates* L. Methanolic and aqueous leaves extracts:** Using a 96-well microtiter plate and the broth microdilution technique, the (MIC) of *C. citrate* extracts was determined. The working solution of the plant extracts was prepared at 256 and 512 mg/ml in broth and serial two-fold dilutions of extract were prepared directly on the plate to make the concentrations 128-1 and 256-1 mg/ml for Methanolic and aqueous extracts respectively. 100 µl of the prepared *C. citrates*, Methanolic and aqueous extracts were introduced into the first wells in row A. Rows B-H in columns had 100 µl of the broth alone. Twofold serial dilutions using micropipette were done systematically down the columns (from rows A-H). 100 µl was removed from the starting concentrations in row A and transferred to the next row with the 100µl broth, properly mixed, and the procedure was repeated up to the last row (H) where the last 100µl was discarded. This brings the final volume in all the test wells with the extracts to 100 µl except the

column which had 200 µl of the broth that served as sterility control. 100µl of the 1×10<sup>6</sup> CFU/ ml bacterial inoculum was transferred into all the wells except the negative control. Microtiter plates were incubated at 37°C for 18-20 hrs. To check for any color changes, 20 µl of resazurin dye was added to each well and incubated for 30 minutes. The lowest concentrations of the extracts at which no color changed from blue to pink in the resazurin broth assay were identified as the Minimum Inhibitory Concentrations visually in broth micro dilutions. (Patel and Khara., 2016)

**Biofilm formation Assessment:** Quantification of biofilm formation by *E. coli* was assessed as described by ( Radovanovic et al., 2015). All isolates were grown overnight in Brain Heart Infusion Broth at 37°C. Each isolates was transferred to tryptic soy broth (TSB) containing 1% glucose and mixed well by pipetting. A suspension of the bacterial isolate was adjusted to McFarland No. 0.5 turbidity standard. 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. The adhering cells were stained by adding 200 µl of 0.1% crystal violet to each well for 15 min. Once the staining reaction has completed, the excess stain was removed by repeated washing (2-3 washes) with distilled water. The plate was dried by leaving them at room temperature for approximately 30 min to ensure they were completely dry, finally, 33% acetic acid was added to fix the stain. Optical density (OD) readings were determined using an ELISA auto reader at a wavelength of 630 nm. Average of OD values of sterile medium were calculated and subtracted from all test values. Cut off value (OD<sub>c</sub>) was calculated, which can provide categorization of isolates as biofilm producer or not (Asmaa and Abdulameer., 2023).

**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)

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

The 96-well microtiter plate was used to determine the antibiofilm activity of *C.citrates* methanolic and aqueous extract. The working solution of the plant extracts was prepared at 50 ppm for the methanolic and aqueous extract to make the concentrations (128-1) mg/ml. 200 µl of each sample was introduced into the first wells in row A. Rows B-H in columns had 100 µl of the broth alone. Twofold serial dilutions using micropipette were done systematically down the columns (from rows A-H). 100 µl was removed from the starting concentrations in row A and transferred to the next row with the 100µl broth, properly mixed, and the procedure was repeated up to the last row (H) where the last 100µl was discarded. 100µl of the 1×10<sup>6</sup> CFU/ml bacterial inoculum was transferred into all the wells except the negative control. The same procedure was done as indicated in paragraph (Assessment of biofilm formation).

**Extraction of Genomic DNA:** DNA was extracted from *E. coli* bacteria using a commercial purification system (Genomic DNA Extraction Mini Kit (iNtron®, Korea); this kit was designed to isolate DNA from Gram-positive and Gram-negative bacteria. DNA was extracted by this kit using the bacterial protocol (for Gram-negative bacteria).

**Estimation of the DNA concentration and purity:** The DNA concentration is detected by using the Nanodrop. The Nanodrop uses to measure the optical density (O.D) at wavelength of 260 nm and 280 nm by adding (1 micro liter) of the extracted DNA . The DNA purity ratio estimates according to this formula:

$$\text{DNA purity ratio} = \text{O.D } 260 \text{ nm} / \text{O.D } 280 \text{ nm}$$

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

This step was carried out by adding 12.5 µl from OneTaq (NEB®) mastermix, 5 µl of DNA sample, 1 µl 10 pmol/µl from each primer and 5.5 µl of free-nuclease water, the reaction Odone under the optimal PCR conditions for gene as shown in Table (1). (Naufalin et al., 2019).

**Table 1. PCR conditions of *fimA* and *papC* genes**

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

**Gene expression Analysis Using qRT PCR**

**Technique:** To assess the effect of the *C. citrates* methanolic extract on the gene expression of *fimA* and *papC* related to biofilm formation, the measurement of the gene expression of the two genes in the isolates was done before and after the treatment with the methanolic extract. The sub MIC concentration of the methanolic extract was used to allow bacterial growth. RNA was extracted by using TRIzol™ Reagent according to the the protocol described by the manufacturer. In order to assess the gene

expression of *fimA* and *papC* gene, (Mohammed and Al- Dujaili., 2020) are showed in ( Table 1) ; Moreover, after several trials, the thermo cycler protocol was optimized and the protocol is listed in ( Table 2). The data results of qRT-PCR were calculated as they were a direct comparison of Ct values between target and reference (housekeeping) genes. The genes were analyzed by the relative quantification of gene expression levels (fold change) using the ΔΔCt method .

**Table 2. Primers utilized in this Study**

Primer name		Sequence (5'-3')	Production size ( bp)	Reference
<i>FimA</i>	F	CAGGTTGTCACACTCGGTGA		
	R	GCAACAACAGGATCGCAGTC	110	
<i>PapC</i>	F	GGTTTGTGCGGTGGTTTGAA		(Mohammed and Al- Dujaili., 2020 )
	R	CCCACGGAGTTGAAGAACGA	134	
House Keeping gene	F	GGATCAGAATGCCACGGTGA		
<i>16S rRNA</i>	R	GCAGGTTCCCCTACGGTTAC	170	

**RESULTS AND DISCUSSION**

**Total phenolic content of *C. citrates* leaves extracts:** Numerous phenolic compounds have been studied for their biological properties and benefits to human health . The total phenolic contents of the aqueous and methanolic *C. citrates* leaves extracts were evaluated by using Folin-Ciocalteu reagent. The results showed that the total phenolic content of the *C. citrates* extracts increased gradually with increases of the concentration, with significant differences ( $P \leq 0.05$ ). The highest values were 19.56 and 47.45 mg/g in 50 mg/ml in both aqueous and methanolic extracts respectively as shown in ( Table 3).

**High-performance liquid chromatography (HPLC):** Individual phenolic compositions of *C. citrates* were analyzed by HPLC method

according to (Rebecca and Amanpreet., 2022) In this study, seven flavonoids derivatives (Catechine , Chlorogenic acid , Ferulic acid , Gallic acid , P-Coumaric acid , kaempferol, and quercetin) were detected in methanolic and aqueous extracts (Figure 1), when compared with standard compounds as shown in ( Figures 2) The obtained results showed variations in the concentration ratio of the methanolic and aqueous *C. citrates* leaves extracts. The results of the HPLC explained that Gallic acid and Ferulic acid in both extracts were high concentration than other compounds. The results of this study was agreement with a study by (Al – Azawi . , 2017) which mention that the high flavonoids and high phenolic content present in the methanolic extract of *C. citrates*.

**Table 3. Total phenolic content of *C. citrates* leaves extract**

Concentration (mg/ml)	Mean ± SE		LSD value
	Aqueous extract (mg/g)	Methanolic extract (mg/g)	
12.5	5.68 ±0.07	13.56 ±0.12	0.396 *
25	10.50 ±0.08	25.51 ±0.01	0.231 *
50	19.56 ±0.07	47.45 ±0.02	0.202 *
LSD value	0.260 *	0.250 *	---

\* ( $P \leq 0.05$ ).

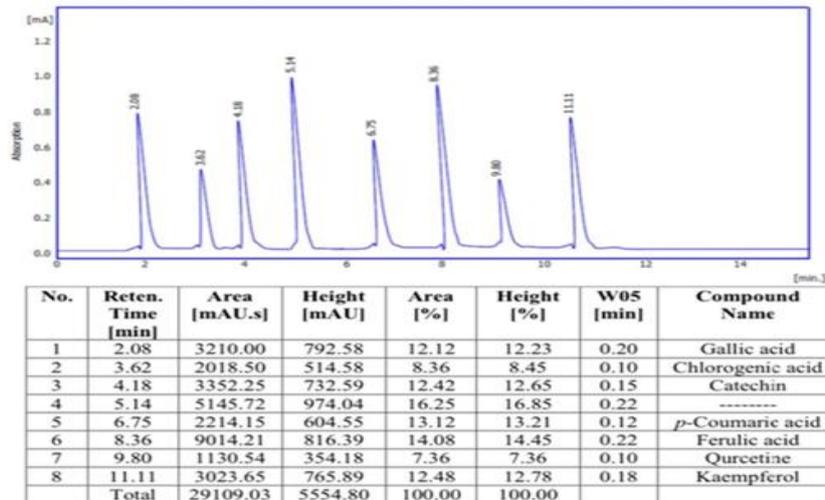


Figure 1. HPLC Chromatography of Phenolic compounds in Aqueous *C.citrates* leaves extracts

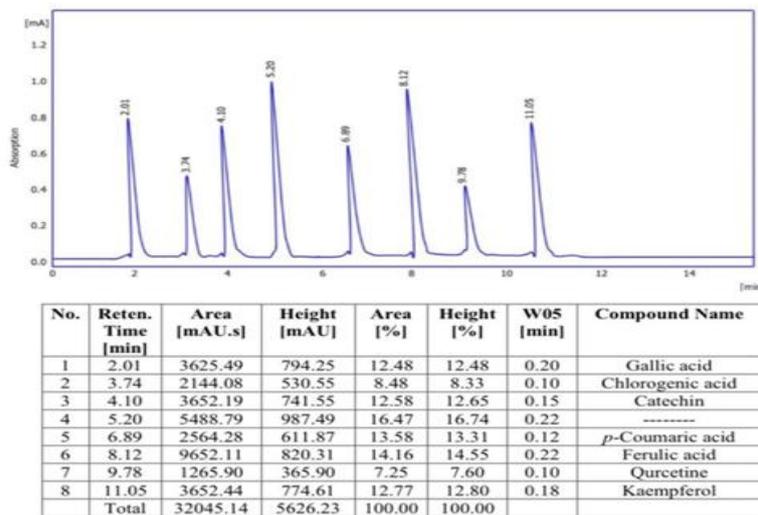


Figure 2. HPLC Chromatography of Phenolic compounds in Methanolic *C.citrates* leaves extracts

**Antibiotics susceptibility test**

The antibiotic susceptibility test revealed that the resistance of *E.coli* isolates was 45.4% - 72.7% for the antibiotics. The highest resistance percentage was found toward (Ticarillin and Cefepime) and the lowest resistance percentage was found toward (colistin ) (as shown in ( Table 4 ). Due to the increase in resistant clinical isolates, there is a paramount need to develop new and

innovative antimicrobial agents. Therefore, researchers are looking for new leads in the discovery of better alternatives against multidrug resistant microbial strains. Among the potential sources of new agents, plants have long been investigated owing to their popular use as remedies for diverse infectious diseases because they contain many bioactive compounds that could be interest in therapeutics ( Djeussi et al . , 2013 ) .

**Table 4. Antibiotic susceptibility test of *E. coli***

Antibiotics	AMC	TIM	FEP	CTR	IPM	CL	TOB	AK	CIP	STX	FOF	% Res.
<b>Isolates</b>												
E <sub>1</sub>	R	R	R	R	S	S	S	S	I	R	R	63.636%
E <sub>2</sub>	I	R	R	R	S	S	S	S	R	R	I	63.636%
E <sub>3</sub>	R	R	R	R	S	S	S	S	R	R	S	54.545%

E <sub>4</sub>	I	R	R	R	S	S	S	I	S	R	R	63.636%
E <sub>5</sub>	R	R	R	I	S	S	S	S	R	R	R	63.636%
E <sub>6</sub>	R	R	R	R	S	S	R	S	R	R	R	72.727%
E <sub>7</sub>	R	R	R	R	R	S	S	S	R	R	R	72.727%
E <sub>8</sub>	R	R	R	R	R	S	S	R	R	I	R	81.818%
E <sub>9</sub>	R	R	R	R	S	S	S	R	S	R	R	63.636%
E <sub>10</sub>	R	R	R	R	R	S	S	S	R	R	S	63.636%
	100%	100%	100%	100%	30%	0%	10%	30%	80%	100%	80%	

(E) : *E.coli* , (Res.) : Resistance, ( I ) intermediate , ( S ) Sensitive , ( % ) : Percentage ( 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 *E. coli* isolates, the bactericidal activity of *C. citrates* L. leaf extracts was assessed using the disk-diffusion method. The results indicated that the aqueous extract was

significantly less efficient than the Methanolic extract at a concentration of 64 mg/ml with a significant difference ( $P \leq 0.05$ ) as seen in ( Table 5) .

**Table 5. Inhibition Zone Diameter ( mm) of Antibacterial Activity methanolic and aqueous extracts of *C. citrates* on *E. coli***

No. of Isolate	Methanolic extract			Aqueous extract			LSD Value
	64	128	256	64	128	256	
	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	
E <sub>1</sub>	12.67±0.33	16.67 ±0.33	21.67 ±0.33	9.67 ±0.33	12.67 ±0.33	17.67 ±0.33	5.19 *
E <sub>2</sub>	11.67 ±0.33	16.00 ±0.57	20.00 ±0.57	9.67 ±0.33	14.00 ±0.57	16.67 ±0.33	4.76 *
E <sub>3</sub>	14.67 ±0.33	18.67 ±0.33	25.00 ±0.57	10.67±0.33	14.67 ±0.33	18.67 ±0.33	5.47 *
E <sub>4</sub>	13.00 ±0.57	16.67 ±0.33	21.67 ±0.33	10.33±0.33	13.33 ±0.33	17.67 ±0.88	5.08 *
E <sub>5</sub>	11.67 ±0.33	15.33 ±0.33	19.66 ±0.88	10.33±0.33	12.67 ±0.33	15.67 ±0.88	5.31 *
E <sub>6</sub>	9.67 ±0.33	12.00±0.57	14.67 ±0.33	7.67 ±0.33	9.67 ±0.33	12.67 ±0.33	5.54 *
E <sub>7</sub>	10.33 ±0.33	12.33 ±0.33	14.67 ±0.33	8.00 ±0.57	9.67 ±0.33	13.00 ±0.57	4.89 *
E <sub>8</sub>	9.00 ±0.57	11.33 ±0.33	13.00 ±0.57	6.67 ±0.33	8.67 ±0.33	10.67 ±0.33	5.02 *
E <sub>9</sub>	11.67 ±0.33	14.67 ±0.33	20.67±0.33	10.33±0.33	12.67 ±0.33	16.00 ±0.57	5.41 *
E <sub>10</sub>	11.67 ±0.33	15.67 ±0.33	21.67 ±0.33	10.33±0.33	11.67 ±0.33	17.00 ±0.57	5.68 *
LSD value	1.16 *	1.16 *	1.45 *	1.07 *	1.07 *	1.64 *	---

\* ( $P \leq 0.05$ ).

Flavonoids subgroups are the most common, and almost ubiquitous, throughout the plant

kingdom . The antibacterial activity of plant extracts comes from bioactive compounds

which they secondary metabolites. Flavonoids subgroups are the most common, and almost ubiquitous, throughout the plant kingdom, flavonoids have been identified as polyphenolic compounds capable of exerting antibacterial activities via various mechanisms of action (Al – Azawi.,2017; Donadio et al., 2021 ) Explain the mechanism of action of flavonoids through suppressing nucleic acid synthesis, cytoplasmic membrane function and energy metabolism, as well as, inhibit peptidoglycan synthesis, damage the microbial membrane structure, modify the bacterial membrane surface hydrophobicity, and modulate quorum sensing ( Cosme et al., 2020)

**Determination of the (MIC) of the *C. citrates* L. leaves extracts:** Broth microdilution method was used to determine the MIC of the plant extracts using the 96-well microtiter plate. A method using the oxidation-reduction colorimetric indicator resazurin has been proposed for the determination of the MIC of the antimicrobial agents against *E.coli*. Resazurin, which is blue in its oxidized state, turns pink when reduced by viable cells and can easily be detected with the naked eyes and the MIC determined even without the aid of a spectrophotometer ( Neuman et al., 2022). The result of the MIC showed that the methanolic extract was more effective than aqueous extract. The MIC values of the aqueous extract for all isolates were from 32-64 mg/ml, and the MIC values of the methanolic extract were from 16-32 mg/ml. The results of this study were in agreement with ( Lorenz and Wright., 1984) who reported that the methanolic extract of *C. citrates* had the highest antibacterial activity. Through the experiments conducted on the *C. citrates* methanolic and aqueous extracts used in this study, it was found that the methanolic extracts were more effective than aqueous extracts. Therefore, the methanolic extracts have been chosen to study the antibiofilm formation and estimation of *FimA* and *PapC* gene expression.

#### **Detection of biofilm formation**

Quantification of biofilm production in plastic Microtiter plates was performed as previously described. The OD 570 of each well was

measured using a microtiter plate reader where the absorbance was determined at 630nm by ELISA reader, and the means of the triplicates were calculated( N'Guessan et al., 2007). The isolates were classified as non-producers, weak, moderate, or strong biofilm producers based on OD of the isolates and the average OD570 of the negative control, according to the methodology used. Briefly, the cut-off OD (OD<sub>c</sub>) was defined as 3 standard deviations above the mean OD of the negative control. Strains were classified as follows: OD ≤ OD<sub>c</sub> = no biofilm producer, OD<sub>c</sub> < OD ≤ 2 OD<sub>c</sub> = weak biofilm producer, 2 OD<sub>c</sub> < OD ≤ 4 OD<sub>c</sub> = moderate biofilm producer, and 4 OD<sub>c</sub> < OD = strong biofilm producer

#### **Anti biofilm Activity of *C. citrates* :**

Biofilm is a densely packed community of microbial cells that attach and grow on living or nonliving surfaces and surround themselves with secreted polymers. Biofilm-associated infections are often difficult to treat because of multi-drug resistance (Lin et al . , 2021) so it is important to identify new and effective molecules against bacterial biofilm formation. The result of this study showed the methanolic leaves extracts inhibited 100 % of the biofilm formation of *E. coli* isolates in 128 mg/ml and reduced the biofilm formation in 64 mg/ml as shown in (table 6), The phytochemical (flavonoids) compounds were shown the antibiofilm activity through the inhibition or reduction in biofilm formation in a concentration – dependent manner, a study by (Patel et al., 2016) showed the correlation analysis referred to the antimicrobial and antibiofilm activities dependent on flavonoids content. Lemongrass leaves extracts have various bioactive compounds including essential oil and phenolic compounds, these compounds possess antibiofilm activity by disrupting biofilm formation and inhibiting bacterial growth through several mechanisms by which *C. citrates* act on biofilm formation in *E. coli* such as inhibition of adhesion, destruction of extracellular polymeric substance, biofilm dispersal, antimicrobial activity and disruption of quorum sensing (Ohikhena et al., 2017).

**Table 6. Biofilm formation of *E.coli* isolates before and after treatment with *C. citrus* methanolic extract**

Isolates	before treatment (control)	After treatment							
		Concentration							
		4	8	16	32	64	128	256	512
E1	Strong	Strong	Strong	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E2	Strong	Strong	Strong	Moderate	Moderate	Moderate	No biofilm	No biofilm	No biofilm
E3	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm
E4	Strong	Strong	Moderate	Weak	Weak	Weak	No biofilm	No biofilm	No biofilm
E5	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm
E6	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm
E7	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm
E8	Strong	Strong	Strong	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm
E9	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm
E10	strong	Strong	Strong	Moderate	Weak	Weak	No biofilm	No biofilm	No biofilm

**Molecular detection of virulence gene**

The virulence genes responsible for biofilm formation (*FimA* and *PapC*) were investigated in *E. coli* isolates using conventional PCR to confirm the presence of these genes by using a primer specific to each gene. The results showed that (90%) of the isolates had *FimA* while *PapC* was present in (60%) of the total isolates as shown in (figures 3 and 4). (Mohammed and Al- Dujaili., 2020) found that the *FimA* gene was present in 70% of *E.coli* isolates but they also found the presence of *PapC* gene was 100%. Another study found the *FimA* present in 82% and *PapC* at 29.5% (Dadi et al., 2020)

**Gene expression of FimA and PapC:** 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 *C. citrates* extract. Total RNA concentrations

were from 7.1 to 99.1 ng / $\mu$ l. Quantitative Real-Time PCR was performed, and the results showed a decrease in gene expression in the *fimA* and *papC* genes as shown in ( Tables 7 and 8). 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 (Somayeh et al., 2014) The CT value rose in isolates that had been treated with methanolic *C. citrates* extract, showing decreased expression of the *fimA* and *papC* genes (folding) necessary for biofilm formation in *E. coli*. Faezah et al. (2020) found that the use of these herbal Compounds decreases the expression of the genes in the presence of the Sub-MIC concentration of

cinnamon essential oil in eight *E. coli* isolates compared to untreated isolates and conclude the plants extract has a good antibacterial effect on *E. coli* and can reduce biofilm production and expression of genes that are effective in causing disease, So medicine plants have an active ingredient that have various medicinal and therapeutic properties, that can reduce the antibiotic resistance of germs, including *E. coli*. Another studies by (Mohammed and Al-Azawi., 2023) they

reported the treatment with the methanolic and aqueous extracts of the *Conocarpus erectus* and *Rosmarinus officinalis* inhibits the biofilm formation and down-regulation the expression of *pelA* and *algD* which responsible for biofilm formation in *E.coli*. So medicine plants have active ingredients that have various medicinal and therapeutic properties, which can reduce the antibiotic resistance of germs, including *E. coli*.

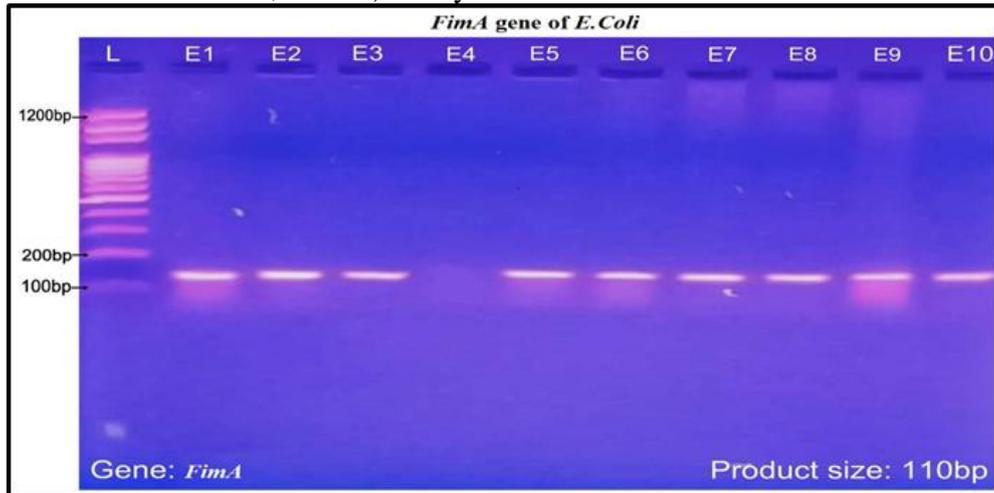


Figure 3. (Gel electrophoresis of amplified *fimA* (110 bp), from *E. coli* using conventional PCR. Agarose 2% stained with Ethidium bromide dye DNA ladder 100-1200 bp

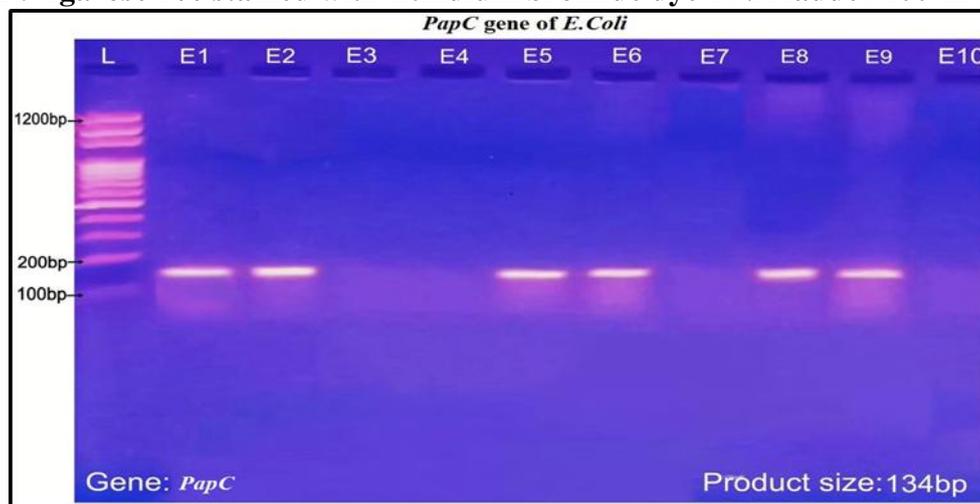


Figure 4. Gel electrophoresis of amplified *papC* (134 bp), from *E. coli* using conventional PCR. Agarose 2% stained with Ethidium bromide dye DNA ladder 100-1200 bp

Table 7. Gene expression for *FimA* genes

Group	Samples	Ct reference gene	Ct target gene	$\Delta$ CT	$\Delta\Delta$ CT	Fold
Before	E1	21.17	19.26	-1.91	0	1
	E2	15.63	22.56	6.93	0	1

	E3	18.99	20.03	1.04	0	1
	E4	-	-	-	-	-
	E5	25.02	18.85	-6.17	0	1
	E6	16.95	20.30	3.35	0	1
	E7	14.78	20.30	5.52	0	1
	E8	15.64	19.12	3.48	0	1
	E9	17.26	17.51	0.25	0	1
	E10	35.77	25.14	-10.63	0	1
After	E1	21.17	19.88	-1.29	0.62	0.650671
	E2	15.63	25.94	10.31	3.38	0.096055
	E3	18.99	24.84	5.85	4.81	0.035649
	E4	-	-	-	-	-
	E5	25.02	20.04	-4.98	1.19	0.438303
	E6	16.95	25.09	8.14	4.79	0.036147
	E7	14.78	23.25	8.47	2.95	0.129408
	E8	15.64	23.63	7.99	4.51	0.043889
	E9	17.26	24.41	7.15	6.9	0.008373
	E10	35.77	25.24	-10.53	0.1	0.933033

**Table 8 Gene expression for *PapC* genes**

Group	Samples	Ct Reference gene	Ct Target gene	$\Delta$ CT	$\Delta\Delta$ CT	Fold
Before	E1	21.17	19.30	-1.87	0	1
	E2	15.63	22.47	6.84	0	1
	E3	-	-	-	-	-
	E4	-	-	-	-	-
	E5	25.02	17.1	-7.92	0	1
	E6	16.95	21.94	4.99	0	1
	E7	-	-	-	-	-
	E8	19.45	13.95	-1.69	0	1
	E9	17.26	18.45	1.19	0	1
	E10	-	-	-	-	-
After	E1	21.17	27.78	6.61	8.48	0.002801
	E2	15.63	28.87	13.24	6.4	0.011842
	E3	-	-	-	-	-
	E4	-	-	-	-	-

E <sub>5</sub>	30.88	23.74	-1.28	1.06	0.478963
E <sub>6</sub>	16.95	28.69	11.74	6.75	0.009291
E <sub>7</sub>	-	-	-	-	-
E <sub>8</sub>	23.54	21.23	5.59	3.19	0.10957
E <sub>9</sub>	17.26	29.57	12.31	11.12	0.000449
E <sub>10</sub>	-	-	-	-	-

### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

### DECLARATION OF FUND

The authors declare that they have not received a fund.

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تقييم النشاط المضاد للبكتيري والفعالية المضادة للاغشية الحيوية لمستخلص اوراق عشبة الليمون *Cymbopogon*

*citrates L.* على جينات *fimA* و *papC* ضد العصيات القولونية

مها عادل خلف الدليمي\* احمد حربي ابراهيم العزاوي\*\*

\*قسم المحاصيل الحقلية ، كلية الزراعة ، جامعة القاسم الخضراء ، بابل ، العراق

\*\*قسم الهندسة الوراثية ، معهد الهندسة الوراثية والتقانات الاحيائية للدراسات العليا ، جامعة بغداد ، العراق

#### المستخلص

الهدف من هذه الدراسة هو تقييم الفعالية المضادة للبكتريا والمضادة لتكوين الغشاء الحيوي لمستخلصات اوراق عشبة الليمون ودراسة تأثيره على التعبير الجيني لبعض الجينات المكونة للغشاء الحيوي (*FimA* and *PapC*). استخدمت طريقة النقع وجهاز السكسكوليت لتحضير المستخلص المائي والكحولي اوراق عشبة الليمون وتم اجراء العديد من الاختبارات على المستخلصات بما في ذلك فحص الكروماتوغرافيا السائلة ذات الاداء العالي (HPLC) والمحتوى الفينولي الكلي لتحديد المركبات الفعالة في المستخلصات. اظهرت النتائج ان المستخلص الميثانولي والمائي يحتوي على سبعة من مشتقات الفينولات هي ( *Catechine* , *Chlorogenic acid* , *Ferulic acid* , *Gallic acid* , *P-Coumaric acid* , *Quercetine* ) (and *Kaempferol*) بنسب مختلفة من خلال مطابقتها مع زمن الاحتجاز للمركبات القياسية. بلغ المحتوى الفينولي الكلي 19.56 و 47.45 ملغم/غرام في المستخلصين المائي والكحولي على التوالي بتركيز 50 ملغم/مليتر، بينت نتائج الفعالية المضادة للبكتريا لمستخلصات اوراق عشبة الليمون ضد عزلات بكتريا القولون ان مستخلص المائي كان اقل فعالية من المستخلص الميثانولي عند تركيز 16 ملغم / مل. علاوة على ذلك بينت نتائج قياس التركيز المثبط الادنى للمستخلص الميثانولي على عزلات بكتريا القولون كانت 16 ملغم /مل بينما نتائج للمستخلص المائي 32ملغم /مل كما ثبت المستخلص الميثانولي لاوراق عشبة الليمون تكوين الاغشية الحيوية بنسبة 90% و 100% للبكتريا المعوية عند تركيز 16 و 32ملغم/



مليلتر على التوالي. بينت نتائج التعبير الجيني عن انخفاض في التعبير الجيني عن جينات *PapC* و *FimA* بعد المعاملة بالمستخلصات.

**الكلمات المفتاحية:** عشبة الليمون، التركيز المثبط الادنى، المحتوى الفيولي، التعبير الجيني.