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Expression Variation of Serine Protease Autotransporters Genes of Uropathogenic *E. coli* in Response to Tea Tree Essential Oil (*Melaleuca alternifolia*) and its Polymer

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Abstract

In this study, the antibacterial activity of tea tree essential oil and its polymer on Uropathogenic *E. coli* was determined. In addition to their effect on the gene expression of two serine protease autotransporter genes, *sat* and *vat*. Five hundred clinical urine samples have been collected from individuals aged between 15 and ≥65 years old with urinary tract infections in Babylon. One hundred *E. coli* isolates were collected and isolated using selective and differential culture media, then verified by polymerase chain reaction (PCR). The minimum inhibitory concentration (MIC) was determined using the agar dilution method to assess the antibacterial activity of tea tree essential oil and its polymer. The MIC values of tea tree essential oil was 10000 ppm for 2 strains (3.3%), 5000 ppm for 51 strains (85%), and 2500 ppm for 7 strains (11.7%), while MIC of the polymer was 20000 ppm for 58 strains (96.7%), and 10000 ppm for 2 strains (3.3%). Gene expression was measured using the RT-qPCR method. Tea tree essential oil and its polymer were highly effective antibacterial agents against E. coli bacteria, and their effect on decreasing gene expression of the *sat* gene was more pronounced than that of the *vat* gene. This effect indicates the potential of tea tree essential oil and its polymer as alternative antibacterial agents that target bacterial virulence, providing a strategy for combating antibiotic resistance and pathogenicity.

Keywords: Uropathogenic *E. coli*, Gene expression, Serine protease autotransporters, Tea tree essential oil, polymerization.

التغاير في التعبير الجيني لجينات *serine protease autotransporters* في بكتيريا *E coli* المسببة لأمراض المسالك البولية استجابةً لزيت شجرة الشاي وبوليمره

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الخلاصة

تضمنت هذه الدراسة تحديد التأثير المضاد للبكتيريا لزيت شجرة الشاي وبوليمره على بكتيريا *E coli* المُمرضة للمسالك البولية. كما تم قياس تأثير كل منهما على التعبير الجيني لأثنين من الجينات، وهما *sat*

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و *vat*. تم جمع 500 عينة ادرار من أفراد مُصابين بالتهابات المسالك البولية. تم عزل 100 عينة من بكتريا *E coli*باستخدام أوساط زراعة انتقائية وتفريقية ، ثم تم التحقق منها باستخدام الطرق الجزيئية باستخدام تفاعل البلمرة المتسلسل(PCR) .تم قياس قيم الحد الأدنى للتركيز المثبط (MIC) لزيت شجرة الشاي وبوليمره لتحديد تأثيرهما المضاد للبكتيريا. بلغ الحد الأدنى للتركيز المثبط للزيت العطري ppm10000 لسلالتين، و ppm5000 لـ 51 سلالة، و ppm2500 لـ 7 سلالات. بينما بلغ الحد الأدنى للتركيز المثبط للبوليمر ppm20000 لـ 58 سلالة، و ppm10000 لسلالتين. تم قياس التعبير الجيني بأستخدام RT–qPCR اظهر زيت شجرة الشاي وبوليمره تأثير فعال كمضاد بكتيري ضد بكتريا *E coli*, وكان تأثيرهما على التعبير الجيني لجين *sat* اكثر منه على التعبير الجيني لجين *vat* . يشير هذا التأثير إلى امكانية زيت شجرة الشاي العطري وبوليمره كعوامل مضادة للبكتيريا بديلة تستهدف ضراوة البكتيريا، مما يوفر استراتيجية لمكافحة مقاومة المضادات الحيوية والامراضية.

1. Introduction

Uropathogenic *Escherichia coli* (UPEC), a member of the harmful extraintestinal Enterobacteriaceae strains, represents a considerable health risk. UPEC is recognized as the main contributory factor of urinary tract infections (UTIs), which rank as the second most frequently diagnosed infectious disease in humans globally. Pathogenic *Escherichia coli* is recognized as a highly significant group of bacteria that necessitates the rapid advancement of new antibiotics and innovative treatment strategies. The treatment of UTIs imposes a significant financial burden on healthcare systems [1].

Serine protease autotransporters represent a family of secreted virulence proteins synthesised by pathogenic *E. coli*. These are exported through the type V secretion system. Sat (Secreted autotransporter toxin) and Vat (Vacuolating autotransporter toxin) are two of these proteins identified in UPEC. Both proteins exert cytopathic effects on host cells, making them suitable targets for anti-virulence strategies. Inhibiting these proteins could lead to attenuation of UPEC. [2,3]

Individuals experiencing a symptomatic UTI are usually given antibiotics for their condition; however, such treatments may lead to extended changes in the normal flora of both the gastrointestinal and vaginal environments, as well as contribute to the emergence of multidrug-resistant organisms [4]. Such changes to the normal flora in the gastrointestinal and vaginal tract due to the overuse of antibiotics can result in kidney and liver damage, an imbalance in flora, and various other complications. It is essential that alternative therapies be developed to cope with the growing problem of antibiotic resistance and enhance the efficacy of antibiotics [5].

A variety of promising strategies are currently under development, utilizing knowledge gained from an understanding of the biology of UTI pathogenesis and targeting particular virulence pathways. The ability of UTI pathogens to induce disease can be efficiently countered by these anti-virulence therapeutics, while maintaining the integrity of the gut commensal microbiota [6,7]. Medicinal plants have received growing attention among researchers and specialists in the field [8]. Indeed, the study of such plants has expanded considerably, focusing on the therapeutic compounds they contain, their efficacy in treating and preventing UTIs, and the mechanisms through which they operate [9].

Essential oils consist of complex natural combinations of volatile secondary metabolites derived from plants. Essential oils present several potential benefits, including their availability, reduced side effects, lower toxicity, and enhanced biodegradability when compared to synthetic antibiotics and preservatives. [10,11]. The essential oil of *Melaleuca alternifolia*, commonly

known as tea tree, shows a wide range of antimicrobial properties. Reports indicate that exposure of Gram-negative bacteria, Gram-positive bacteria, and yeast to tea tree essential oil resulted in increased permeability of bacterial and yeast plasma membranes as well as inhibited respiration. Tea tree essential oil induced potassium ion leakage in the case of *E. coli.* [12]

Antimicrobial polymers are substances that possess the ability to eliminate or limit the proliferation of microorganisms on surfaces or in the environment. There has been significant interest in these substances within both academic and industrial contexts, revealing that these polymers could have important characteristics such as reduced toxicity, minimized resistance, enhanced activity, and prolonged lifespan. Consequently, efforts have concentrated on creating antimicrobial polymers that possess all the necessary attributes for maximum effectiveness. [13,14]

The aim of this study is to determine the antibacterial activity of tea tree essential oil and its polymer on UPEC and their effect on the gene expression of two serine protease autotransporter genes, *sat* and *vat*.

2. Materials and methods

2.1 Isolation and identification of bacteria

In this study, 500 clinical urine samples were collected from UTI patients aged between 15 and ≥65 years old with UTIs in Babylon, conducted from October 2023 to December 2023. The initial diagnosis of bacterial isolates has been done by using traditional selective and differential culture media, followed by confirmation via molecular methods using polymerase chain reaction (PCR). A loopful of each urine sample was cultured on MacConkey agar. After incubation at 37°C for 24 h, suspected UPEC colonies were subculture on Eosin Methylene Blue (EMB) agar and chromogenic agar. The presence of *E. coli* was confirmed by PCR using primer pairs targeting the *uidA* gene.

2.2 Antibiotic susceptibility test

The antibiotic susceptibility of *E. coli* was determined using the Kirby-Bauer method, which was performed on Mueller-Hinton agar. Eighteen antibiotic discs were used to assess the susceptibility of the isolated UPEC (Table 4). These antibiotics are frequently prescribed by physicians for patients with UTIs. The results were documented based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints of 2024, categorising them as resistant, sensitive, or intermediate.

2.3 Genomic DNA extraction

Sixty isolates of UPEC were chosen for molecular identification based on the resistance rates of these bacteria to 18 antibiotics utilized in the study. Genomic DNA was extracted using a commercial extraction kit (Favorgen Genomic DNA Extraction Kit) provided by Favorgen Biotech (Ping Tung, Taiwan). After extraction, the concentration and purity of the DNA were measured by using a nanodrop, and then detected by gel electrophoresis.

2.4 PCR amplification and gel electrophoresis

The *uidA* primers were specifically employed to amplify the target *uidA* gene, acting as a species-specific primer for *E. coli*. The amplification reaction was carried out in a total volume of 20µl, which contained 10µl of the master mix, 1.5µl of each primer (forward and reverse), 3µl of the DNA genome and 4 µl of nuclease-free water. The amplification condition is displayed in Table 1. Following amplification, the PCR products were run on gel electrophoresis using a 1.5% agarose gel for 45min and compared with a 100 bp ladder

(Promega). Following the run, the DNA bands on the agarose gel were visualized using ultraviolet light.

Table 1: PCR conditions and gene-specific primers

Target gene	Primer sequence (5′-3′)	Amplicon size (bp)	PCR program run	Reference
uidA	F: ATGGAATTTCGCCGATTTTGC R: ATTGTTTGCCTCCCTGCTGC	166	Initial denaturation:95C° for 4 mins Denaturation: 95C° for 40 sec Annealing: 59.5C° for 40 sec Extension: 72C° for 40 sec Final extension: 72C° for 5 mins 30 cycles	[15]

2.5 Tea tree essential oil sample

Melaleuca alternifolia (tea tree) oil was purchased from the local market (First botany cosmeceuticals/ Australia)

2.6 Polymerization of tea tree essential oil

Tea tree essential oil (22.14 ml) and 30.14g of phthalic anhydride were mixed in a 250 ml flask equipped with an electric agitator, a reflux condenser, a thermometer, and a dropping funnel. Gently heat the mixture until the phthalic anhydride has completely melted, and then continue heating it up to 145°C. After that, a small amount of p-toluene sulfonic acid catalyst was added to the flask. The reaction was completed at 145 °C for 1.5–2 hours. After reaction, the pure terpene maleic adduct was recovered by extraction using distilled water two to three times, then distillation under vacuum was followed to eliminate the unreacted terpene. [16,17]. 3g of the mixture from the first step was then combined with 2gm of gelatine that had been dissolved in a 50:50 mixture of ethanol: water and refluxed for six hours at 70 °C after obtaining a reddish-brown precipitate, washed with 10 ml of diethyl ether, and dried at 50°C in a vacuum oven [18], as shown in Figure 1.

Figure 1: Tea tree essential oil after polymerization with gelatin

2.7 Determination of minimum inhibitory concentration (MIC) of tea tree essential oil and polymer

After antibiotic sensitivity assessment, 60 MDR isolates were selected and subjected to tea tree essential oil and its polymer to identify the MIC. The dilution range for tea tree essential oil was set between 40000 and 625 ppm. The stock solution of tea tree essential oil at a concentration of 500000 ppm was prepared by dissolving 5ml of oil in 5 ml of DMSO. From prepared stock solution, 7 diluted concentration was prepared. The dilution range for the

polymer was set between 40000 and 5000 ppm, and the stock solution was prepared by dissolving 5gm of polymer in 5 ml of DMSO [19].

The agar dilution method has been used to determine MIC. Melted Muller-Hinton agar was mixed with the desired concentration of oil and its polymer stock solution in order to prepare the treatment solution in a total volume of 20 ml. The mixture was then poured into empty and sterile Petri dished and left to solidify. A bacterial suspension was prepared by mixing a few fresh cultured *E coli* bacterial colonies with normal saline, and the concentration was adjusted and standardized to 0.5 McFarland turbidity. After that, using a micropipette, 3 µl of this suspension was added to the surface of agar. The Petri dishes were inverted and incubated at 37ºC for 18-24 h. After incubation, and based on the absence of visible growth, the minimum inhibitory concentration was determined.

2.8 Analyse the polymerized compound using FT-IR

An FT-IR device was used to examine samples (powdered polymers). The sample spectroscopy was performed within the range 400-4000 cm^{-1}, and the results were compared with the reference spectra [20].

2.9 Total RNA extraction and estimation of gene expression of sat and vat genes using qRT-PCR

Ten isolates of UPEC were chosen for total RNA extraction and estimation of gene expression of both *sat* and *vat* genes. These ten isolates were selected based on their MIC results after treatment with tea tree essential oil and its polymer. The MIC values for all ten isolates were 5000 ppm for tea tree essential oil and 20000 ppm for the polymer. Total RNA was extracted after exposure to a concentration below MIC (Sub-MIC) for each sample.

RNA was extracted using GENEzol™ TriRNA Pure Kit (Geneaid, Biotech. Taiwan). The concentration of total RNA was measured using QuantiFluor® (Promega, USA) in order to unify the concentration of RNA before proceeding with qRT-PCR using the formula:C1V1= C2V2.

The qRT-PCR and Livak method (2-ΔΔCT Livak method) was applied to measure the expression of target genes *sat* and vat. The gene expression was compared with *GADPH* gene expression as the housekeeping gene (Reference gene) for normalization of gene expression. The ΔΔCT was used for determining gene expression. The ΔΔCT value was calculated by determining the difference between the ΔCT value of the treated group and the ΔCT value of the untreated control group, and the fold change was measured using the 2-ΔΔCT formula [21,22]. Primer sequences are listed in Table 2.

Table 2: RT-qPCR primers with their sequence

Target gene	Primer sequence (5′-3′)	PCR program run	Reference
sat	F: ACAATGTTGTCTCTGGCTGTTGCC R: GTGATTGTTACGTCTGTTGCTCCG	Reverse Transcription:55°for 10 mins Initial Denaturation: 95°for 1 min 1 cycle Denaturation: 95°for 10 sec Extension: 60° for 30 sec 40-45 cycle Melt Curve: 95-60° various	[23]
vat	F: TACCGTAACCAGCTCATCAACAG R: CATACCCACCTGTTACCCAATGT		[24]
GAPDH (HKG)	F: ACTTACGAGCAGATCAAAGC R: AGTTTCACGAAGTTGTCGTT		[25]

The investigation was conducted using the HiScript II one-step qRT-PCR SYBR Green kit (Vazyme, Biotech, China) according to the manufacturer's instructions. Each of the reaction tubes contains a final volume of 20 µL(Table 3). The qRT-PCR was carried out using qTOWER³ (Analytik Jena, Germany)

Table 3: qPCR master mix preparation

qPCR master mix	The volume
Luna Universal One-Step Reaction Mix	10 µL
Luna WarmStart® RT Enzyme	1 µL
Forward primer	0.5 µL
Reverse primer	0.5 µL
RNA	According to sample
Nuclease-free water	According to sample
Total volume	20 µL

2.10 Statistical Analysis

Data was analysed using SPSS (version 26, SPSS Inc. Chicago, Illinois, USA). The results were expressed as a percentage (%). Statistical analysis was performed using the chi-square test.

3. Results and Discussion

3.1 Isolation and identification

A total of 100 *E. coli* isolates were examined in this study. These isolates of *E. coli* were obtained by culturing in various media. MacConkey agar identified lactose-fermenting pink colonies of *E. coli*, whereas EMB agar was used to assess the ability of each isolate to produce green metallic sheen colonies. Chromogenic agar confirmed the presence of *E. coli*, yielding pink colonies.

From a total of 100 UPEC isolates, 60 were selected for PCR amplification for genetic identification of *E. coli*, and 96.6% (58/60) of *E. coli* were successfully amplified and produced a single band of the target *uidA* gene as a species-specific gene with a molecular weight of 166 bp, as shown in Figure 2. The result in this study was similar to the study performed by other researchers [26].

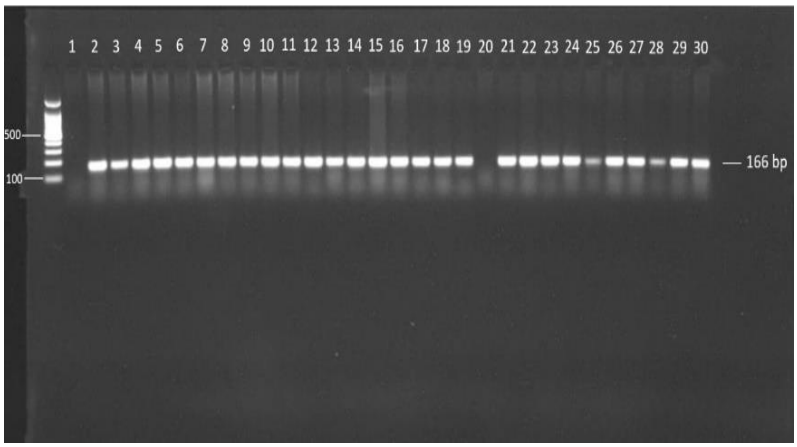


Figure 2: The PCR amplification of *uidA* (166 bp) in UPEC. Gel electrophoresis of amplified gene in agarose 1.5%, 80 V/cm for 45 min., stained with safe red dye and visualized on a UV transilluminator. In the presence of 100 bp DNA ladder. Lane (1-30): Amplicons of *uidA* gene.

3.2 Antibiotic sensitivity test

The development of multiple antibiotic-resistant bacteria associated with UTIs has become a significant public health problem worldwide [27]. In this study, isolated bacteria showed different susceptibility toward 18 antibiotics (Table 4).

In this regard, 64% of isolates in this study were resistant to multiple antibiotic classes, including MDR (63%) and XDR (1%). Other studies have also investigated the multiple antibiotic resistance [28,29]. This increase in MDR strains might have resulted from various factors, such as excessive use of antibiotics or self-medication without prescription. Additionally, careless use of antibiotics contributes to the emergence of MDR, leading to the spread of antibiotic-resistant pathogens in clinical settings [30].

Table 4: Antibiotic sensitivity results in UPEC isolates

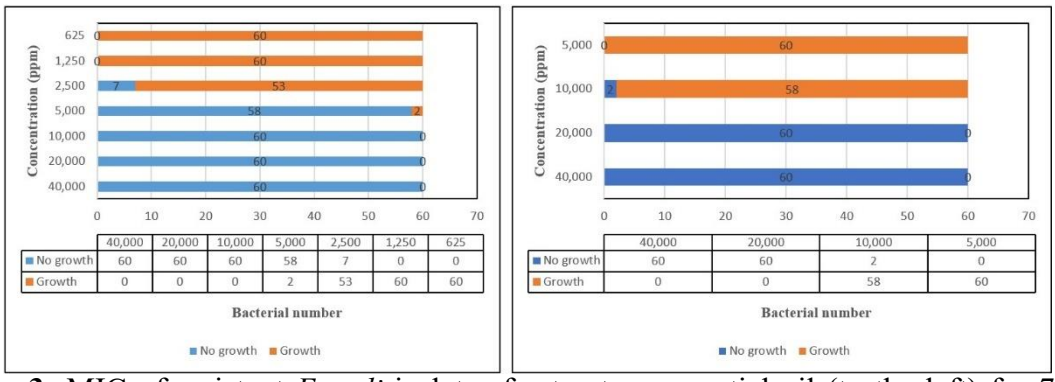
Antibiotics	Sensitive	Intermediate	Resistant	p-value
	No. (%)			
Ampicillin	8	0	92	0.0001**
Pipracillin	20	0	80	0.0001**
Cefixime	34	7	59	0.0001**
Ceftazidime	35	8	57	0.0001**
Ceftriaxone	42	1	57	0.0001**
Azithromycin	44	10	46	0.0001**
Cefepime	46	13	41	0.0001**
Trimethoprim	47	0	53	0.0001**
Sulfa-Trimethoprim	50	2	48	0.0001**
Aztreonam	58	7	35	0.0001**
Ciprofloxacin	69	7	24	0.0001**
Levofloxacin	73	3	24	0.0001**
Norfloxacin	77	0	23	0.0001**
Gentamycin	85	1	14	0.0001**
Nitrofurantoin	94	4	2	0.0001**
Fosfomycin	95	0	5	0.0001**
Amikacin	97	1	2	0.0001**
Meropenem	99	0	1	0.0001**
p-value	0.0001**	0.0001**	0.0001**	

**. Refer to Significant difference at $p \leq 0.01$.

3.3 Minimum inhibitory concentration (MIC) of essential oil against E. coli resistant isolates

E. coli isolates with multiple antibiotic resistance were tested to determine the minimum inhibitory concentration of essential oil and its polymer, by utilizing the agar dilution method with serial concentrations of essential oil (40000 to 625 ppm), and the polymer with serial concentrations (40000 to 5000 ppm). The results showed that the minimum inhibitory concentration of essential oil was 10000 ppm for 2 strains (3.3%), 5000 ppm for 51 strains (85%) and 2500 ppm for 7 strains (11.7%), while the minimum inhibitory concentration of the polymer was 20000 ppm for 58 strains (96.7%) and 10000 ppm for 2 strains (3.3%) figure (3).

In vitro, the possible use of tea tree essential oil in treating diseases caused by different pathogens was supported by its several antibacterial actions. The effect of tea tree essential oil is explained by different mechanisms, which depend on the dose used and the investigated pathogen properties. Structural modification of cell membrane which cause reducing in the membrane potential is suggested is the most significant mechanism. It has been documented that tea tree essential oil can alter potassium ion channel homeostasis and interfere with glucose-dependent respiration, therefore impacting bacterial membrane integrity for *E coli*. [31,32].



	40,000	20,000	10,000	5,000	2,500	1,250	625
No growth	60	60	60	58	7	0	0
Growth	0	0	0	2	53	60	60

	40,000	20,000	10,000	5,000
No growth	60	60	2	0
Growth	0	0	58	60

Figure 3: MIC of resistant *E. coli* isolates for tea tree essential oil (to the left) for 7 serial concentrations 40,000 ppm, 20,000 ppm, 10,000 ppm, 5000 ppm, 2500 ppm, 1250 ppm, and 625 ppm. and polymer (to the right) for 4 serial concentrations 40,000 ppm, 20,000 ppm, 10,000 ppm, and 5000 ppm.

3.4 FT-IR analysis of polymer

FT-IR analysis of this polymer shows significant absorption bands that indicate structural changes occurring throughout the polymerisation process. This analysis shows that, at (3398) cm^{-1}, there are strong intensity bands which refer to the hydroxyl groups, and a weak intensity band at (2956) and (2800) cm^{-1} due to the (C-H) that can verify the existence of long aliphatic hydrocarbon chains characteristic of fatty acid residues. The peak at (1715) cm^{-1} assigned to (C=O) stretching of carboxylic groups confirming the retention of ester linkage from triglyceride structure and proposing the potential generation of oxidized carbonyl compounds throughout the polymerisation process, (1647) cm^{-1} assigned to characteristic absorption of (N -C=O) amid, the presence of this combination of C=O and C-N could suggest that an amidation reaction may have taken place during the polymerization process. (1451cm-1) peak of (C=C) suggesting the presence of conjugated unsaturated systems or residual double bonds, which are features of partially polymerized oils. While at (1375) cm^{-1} band refers to (C-N), indicating the existence of aliphatic or aromatic amine groups, which implies potential for chemical modification or crosslinking, and showed a strong band at (1264) cm^{-1} due to the (C-O) that indicate the existence of ester linkage and that the polymerization process may have produced additional ether bonds (Figure 4).

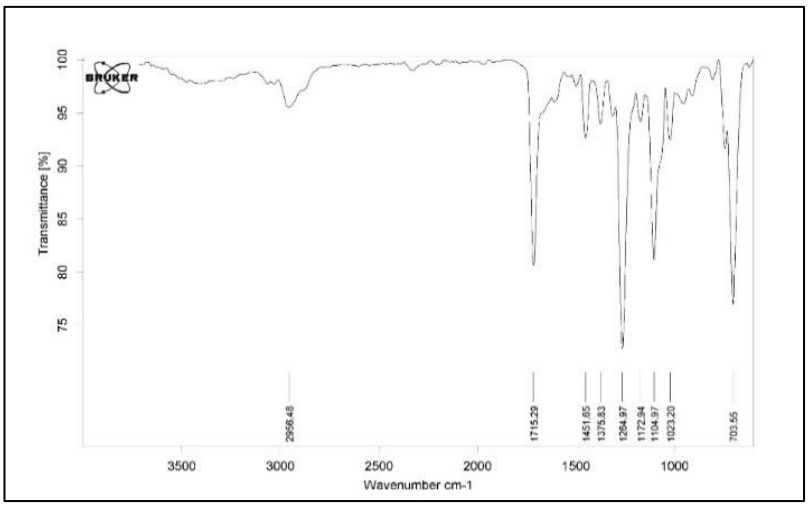


Figure 4: FT-IR analysis results of polymerzed tea tree oil

3.5 Gene expression of target genes by Quantitative Real Time PCR (qRT-PCR)

The expression of *sat* and *vat* genes in the absence of tea tree essential oil and its polymer was measured and compared with their expression in the presence of them to determine the effect of this treatment on gene expression by using the concentration below MIC (Sub-MIC) for each sample. Gene expression was measured using the reverse transcription quantitative polymerase chain reaction (RT-qPCR) method, which is characterized by its accuracy, sensitivity, and rapid results.

Upregulation and downregulation in the expression of both *sat* and *vat* genes were evaluated to determine how the essential oil influences the gene expression of *E. coli*. Following gene normalisation, changes in gene expression of these two genes under several treatment conditions were investigated. The results revealed that several treatments produced varied patterns of gene expression.

Essential oils have been known for their ability to interfere with bacterial gene expression because their primary active compounds can inhibit RNA synthesis and alter gene expression. This reduction in RNA synthesis could lead to a significant alteration in the adaptation mechanisms of bacteria, ultimately resulting in cell death [33]. In *E. coli*, the effect of essential oils on gene expression was assessed. It has been found that essential oils can inhibit the gene expression of some virulence factors, such as adhesion, biofilm formation, and the induction of stress response mechanisms; as a result, the pathogenesis will be reduced [34,35].

According to the current study, it can be concluded that there are changes in *sat* gene expression after treatment with tea tree essential oil; all samples experienced a decrease in gene expression, except that there was an increase in gene expression for sample 44 and no change in sample 40. On the other hand, after treatment with the polymer, all samples experienced a decrease in gene expression, except for sample 35, which showed an increase in gene expression, and sample 58, which showed no change. In general, the effect of tea tree essential oil on *sat* gene expression was more effective than that of its polymer, as shown in Table 5 and Figure 5.

Table 5: Gene expression profile of *sat* gene represented by fold change

Isolates	Tea tree oil	Polymer
10	0.11907975	0.46009383
16	0.00872881	0.48971015
17	0.05076577	0.20732989
35	0.30566007	2.9896985
37	0.04181024	0.41465977
39	0.13584186	0.27547628
40	1.07922824	0.952638
44	1.34723358	0.73713461
50	0.02076072	0.73713461
58	0.0960547	1.02101213

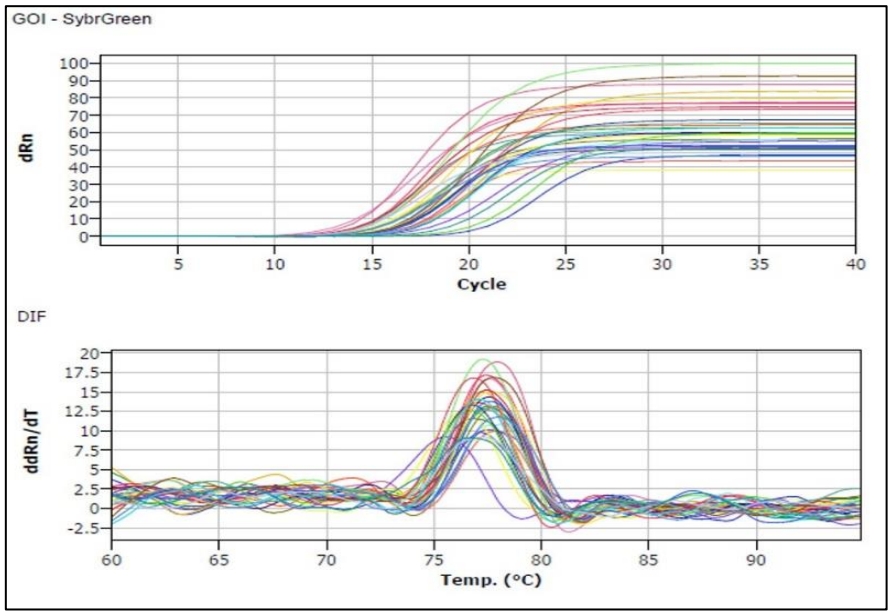


Figure 5: Amplification curve plot and melt curve for *sat* gene

The *vat* gene showed completely different and unstable gene expression in response to treatment. The gene expression decreased for most of the samples after treatment with tea tree essential oil but increased for samples 16, 37, and 50. On the contrary, for treatment with polymer, the gene expression of most samples increased after treatment, while a few of them 17, 37, and 39 showed decreased gene expression. Samples 40 and 44 showed significantly higher *vat* gene expression, which may indicate intrinsic biological variability or an unequal response to therapy. Generally, the effect of tea tree essential oil on *vat* gene expression was also more effective than its polymer, as shown in Table 6 and Figure 6.

Table 6: Gene expression profile of *vat* gene represented by fold change

Isolate no.	Tea tree oil	Polymer
10	0.193445624	4.377174805
16	3.138336392	183.5462717
17	0.000161073	0.297301779
35	0.10153155	2.4794154
37	43.11147446	0.190782401
39	0.024180703	0.079109787
40	0.368567304	843.3572017
44	0.817902059	770.6863347
50	6.96267E-05	1.558329159
58	0.444421341	1.765405993

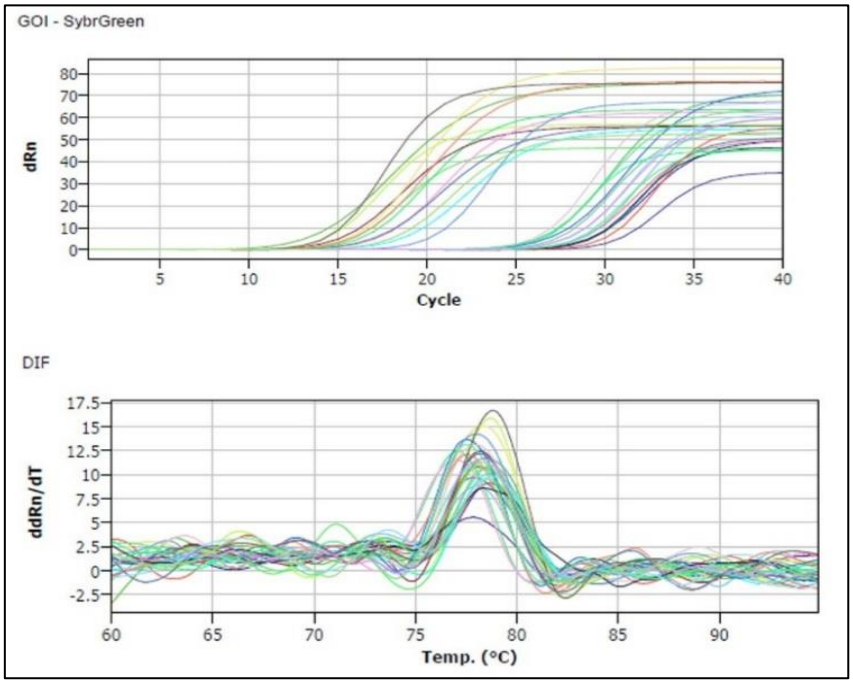


Figure 6: Amplification curve plot and melt curve for *vat* gene

According to the results, the *sat* gene expression was more affected by treatment than the *vat* gene. This may result from different regulatory processes, potentially controlled by distinct promoters, transcription factors, or regulatory pathways. Our treatment could interfere with the pathway regulating the expression of the sat gene but not with that of the *vat* gene. As a result, treatment will downregulate *sat* activity, which includes cytotoxicity, which is their ability to induce morphological changes in kidney, bladder, and other cell lines. Also, it will minimize their ability to increase paracellular permeability. Finally, this treatment can reduce the pathogenesis of bacteria and, as a result, lead to cell death.

4. Conclusion

Numerous plant-derived products, such as essential oils, demonstrate antibacterial characteristics, making them suitable options for countering antibiotic resistance. The domain of antimicrobial polymers is expanding rapidly, involving many applications in healthcare and

medical devices. Tea tree essential oil and its polymer exhibited effective antibacterial activity against *E. coli* bacteria, and their effect on the gene expression of the *sat* gene was more pronounced than that of the *vat* gene. Further studies are required in order to use plant-based antimicrobials as safe and effective alternatives in modern medicine.

5. Acknowledgements

I would like to express my gratitude to the College of Science at the University of Baghdad for providing support to this research project.

6. Ethical approval

The ethics committee of the College of Science, University of Baghdad, approved this work according to document number (CSEC/1023/0089 on October 28, 2023). All participants agreed to provide the researcher with the specimens. All participants gave informed consent in accordance with the Declaration of Helsinki.

7. Disclosure and conflict of interest

There is no conflict of interest

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