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ORIGINAL ARTICLE

Comparison of the antimicrobial and antivirulence activities of Sidr and Tualang honeys with Manuka honey against *Staphylococcus aureus*

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ABSTRACT

Background and Objectives: Honey is one of the oldest traditional remedies that has been widely utilized to cure a variety of human ailments. The objective of this research was to test and compare the antibacterial activity of Sidr honey (SH) and Tualang honey (TH) to that of Manuka honey (MH) against *Staphylococcus aureus*.
Materials and Methods: The antibacterial activity of MH, SH and TH against *S. aureus* was investigated by agar well diffusion, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), time-kill curve, microtiter plate and RT-qPCR analysis.
Results: Agar inhibition assay showed that MH possess highest total antibacterial activity against *S. aureus* with an inhibition zone 25.1 mm compared with that of SH (22.2 mm) and TH (21.3 mm). The findings showed that when compared to SH and TH (MIC: 25% and MBC: 50%), MH honey had the lowest MIC (12.5%) and MBC (25%). After *S. aureus* was exposed to MH, SH, and TH, there was a decrease in colony-forming unit as seen by the time-kill curve. The lowest concentration 20% of MH, SH and TH was significantly found to inhibit *S. aureus* biofilm. The RT-qPCR results revealed that all the selected genes in *S. aureus* were downregulated in gene expression following exposure to each of the tested honeys. Comparing the total antibacterial, antibiofilm, and antivirulence activities of all the tested honeys, MH demonstrated the greatest levels of these properties.
Conclusion: According to this study, various types of each evaluated honey have the capacity to effectively suppress and modify the virulence of *S. aureus* via a variety of molecular targets.

Keywords: *Staphylococcus aureus*; Antibacterial; Anti-virulence; Honey; Virulence factors

INTRODUCTION

Since over 5500 years ago, honey has been used by humans as one of the most appreciated and valued natural products (1, 2). Honey was consumed by the majority of ancient populations, including the Egyptians, Greeks, Romans, Chinese, Mayans, and Babylonians, for both nutritional and therapeutic reasons

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(1, 3). Honey is utilized as a nutritional supplement, in traditional medical treatments, and as an alternative therapy for a variety of clinical diseases (4). The number of identified honey species has increased to about 300 nowadays (1). Due to its phytochemical, antibacterial, anti-inflammatory, and antioxidant properties, honey has a potential therapeutic role in the treatment of disease (5, 6). The many types of nectar that honeybees collect are related to these sorts (7).

High osmolarity, acidity, hydrogen peroxide (H_2O_2) and non-peroxide components, methylglyoxal, and other components of honey may all contribute to its antibacterial properties (8). The sources of nectar, geographical factors like temperature, the origin of the flowers, and humidity all have a significant impact on honey's antibacterial effectiveness (9, 10). Several research has been conducted on various aspects of honey variations, such as their physicochemical qualities, chemical composition, antibacterial activity, and therapeutic use (11). Honey has been shown to have antibacterial action against pathogenic bacteria and *in vivo* activity, making it appropriate for the treatment of ulcers, infected wounds, and burns (12, 13). Sidr and Tualang honeys have been studied in order to assess its scientific value (14, 15). One of the findings from such investigations suggested that Sidr and Tualang honey had a significant therapeutic potential (14, 15). However, the antimicrobial mechanism of action of these honeys is yet unknown, and if these antibiofilm activities, as well as any probable antivirulence activities, might be related to changes in bacterial gene expression. Furthermore, no data on the antibacterial and antivirulence activities of Sidr honey (SH) and Tualang honey (TH) compared to Manuka honey (MH) against *S. aureus* are available in the literature. Therefore, the purpose of this research was to investigate and compare the antibacterial and antivirulence properties of Sidr honey (SH) and Tualang honey (TH) with Manuka honey (MH) against *S. aureus.* The relevance of this work is that it provides *in vitro* support for gene regulation after honey therapy. Differential expression between treated and untreated samples might hypothetically reveal virulence pathways.

MATERIALS AND METHODS

Bacterial train and culture conditions. A reference strain of *Staphylococcus aureus* (ATCC-25923) was purchased from the American Type Culture Collection (ATCC). Petri dishes with sheep blood agar (BA) were purchased directly from supplier in Jordan (Fisher Scientific, UK). This media was used for maintenance of bacteria. *S. aureus* was streaked on both sheep blood agar and mannitol salt agar by using a sterile inoculation loop. The plates were incubated at 37°C for 24 hours (16).

Honey samples. Manuka (MH), Sidr (SH), and Tualang (TH) honeys were obtained from a commercial supplier in Amman, Jordan. Stock samples were kept in dark bottles and stored at room temperature away from light sources. The samples were prepared freshly for each experiment (15, 17).

Agar well diffusion of honeys against *S. aureus*. The agar well diffusion technique was used to assess the susceptibility of honeys. Sterile distilled water was used to dilute MH, SH, and TH to reach honey concentrations of 75%, 50%, 25%, and 12.5% (v/v). The inoculums were prepared by picking parts of similar colonies of *S. aureus* with a sterile wire loop and suspended in sterile normal saline. The inoculum density was adjusted to be equal to 0.5 McFarland standard. After that, a sterile cotton swab was dipped into the bacterial solution and streaked across the whole surface of the plate. A sterile cork borer (6 mm diameter) was used to create agar plate wells. The agar plate wells were labeled and 100 µL of honey concentrations (75%, 50%, 25%, and 12.5% (v/v)) were applied. As a negative control, sterile distilled water was utilized. The agar plates were incubated for 24 hours at 37ºC. The zones of inhibition were measured using a digital venire calliper. The experiment was carried out three times (18-20).

MIC and MBC determination of honeys against *S. aureus*. The minimal inhibitory concentration (MIC) was measured using the broth microdilution technique. The MH, SH, and TH concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125% (v/v) were freshly prepared using NB broth. In brief, the cell density for *S. aureus* was adjusted to 0.5 McFarland standard, as previously described. 100 µl of each concentration of MH, SH, and TH was put into a microtiter plate. The positive control was inoculum without honey, the sterile control was broth medium alone, and the negative control was honey without inoculum. The plates were incubated at 37°C overnight. At

540 nm, absorbance was measured using a microplate reader. The MIC_{50} and MIC_{90} values were calculated using the formulas shown below (21). Following the MIC experiment, the MBC test was done using the streak plate technique. The MIC test determined each honey dilution with no bacterium growth. Two wells of the corresponding honey dilution were randomly selected for each honey concentration with no bacterium growth, and one loopful bacteria suspension was transferred from each well onto two different Mueller Hinton Agar plates (MHA). It was equally distributed and incubated at 37°C for 24 hours. MBC were calculated as the lowest concentration that enabled less than 1% bacterial growth. The experiment was carried out in triplicate (14).

$$\text{Growth inhibition (\%)} = \frac{\text{1-OD of the test well – OD of corresponding negative control} \times 100}{\text{OD of bacterial growth control} - \text{OD of sterility control}}$$

Time-kill curve of *S. aureus* against honeys. Time-kill curve was used to investigate the effects of MH, SH, and TH on cell viability. The *S. aureus* inoculum was adjusted to 1×10^6 CFU/mL. By inoculating 100 µl of *S. aureus* bacteria at 1×10^6 CFU/mL into 10 mL of NB with and without 2 × MIC of MH, SH, and TH. The samples were then incubated for 9 hours at 37°C in a shaking water bath (100 rpm). Following then, samples were obtained every 3 hours for up to 9 hours. The mean Log10 CFU/ml over time was then plotted for each sample. To determine the TVCs, the log reduction (LR) was calculated for each sample by subtracting the Log10 CFU at zero time and the Log10 CFU during 9 hours of incubation (14, 22).

Microcolony formation of *S. aureus* exposed to honeys. In brief, the *S. aureus* inoculum was adjusted to the 0.5 McFarland standard as previously stated. A 100 µl culture was placed into 24-well plates and incubated at 37°C for 24 hours. After incubating for 24 hours at 37°C, 50 µl of planktonic cells were removed and replaced with 50 µl of MIC of MH, SH, and TH. As a positive control, an inoculum without honey was used. After washing with PBS, the plates were dyed with 0.1% (w/v) crystal violet. Light microscopy with oil immersion at 1000× magnification was used to examine the images (14, 22).

The influence of honeys on *S. aureus* biofilm. To investigate the concentrations of MH, SH, and TH needed to inhibit *S. aureus* biofilm. MH, SH, and TH concentrations of 60%, 40%, 20%, 10%, and 5% (v/v) were freshly prepared in NB broth from a stock solution of 100% (v/v) honey. As previously mentioned, the *S. aureus* inoculum was modified to be 0.5 McFarland standard. A 100 µl of each honey concentration was poured into a microtiter plate and inoculated with 100 µl of a diluted overnight culture of *S. aureus* (0.5 McFarland standard). The positive control was inoculum without honey, the negative control was honey without inoculum, and the sterile control was broth only. The plates were incubated at 37°C for 24 hours. By inverting the plate and tapping it, the media was removed. The plate was washed three times with PBS to remove planktonic bacteria before being drained. For four minutes, the plates were dyed with 200 µl of 0.2% crystal violet. The plates were washed under running tap water to remove excess discoloration and dried at room temperature before being solubilized with 95% ethanol. The amount of biofilm produced was quantified using a microplate reader set to 540 nm. The percentage of biofilm inhibition was estimated using the formulas shown below. The experiment was carried out in triplicate (18, 19, 22, 23).

$$\text{Biofilm inhibition (\%)} = \frac{(OD_{540} \text{ of positive control}) - (OD_{540} \text{ of individual or (combined) antimicrobial)} \times 100\%}{(OD_{540} \text{ of positive control})}$$

Effect of honeys on levels of expression of *S. aureus* virulence genes by RT-qPCR. As previously mentioned, the *S. aureus* inoculum was modified to be 0.5 McFarland standard. A 100 µl culture was dispensed into a microtiter plate with 100 µl of MIC of MH, SH, and TH as a positive control, and an inoculum without honey treatment was utilized as a negative control. The plate was incubated at 37°C for 24 hours. Following incubation, the mixture was resuspended in 500 µl of Phosphate-buffered saline (PBS), vortexed, and centrifuged for 30 seconds at 13,000 rpm. The supernatant was discarded, and the pellet was thoroughly washed in PBS twice. The SV Total RNA Isolation System was used to extract total RNA from treated and untreated *S. aureus* (Promega, UK). A NanoDrop was used to measure total RNA. RNA was kept at -80°C. RNA purity was determined using the 260/280 and 260/230 absorbance ratios. Only samples ranging from 1.8 to 2.1 were acceptable for

conversion to cDNA. The samples were then adjusted to 100 ng/µl in order to convert to cDNA. A cDNA conversion kit was used to convert total RNA samples to cDNA (Promega, UK). Following cDNA conversion, samples were stored at -20ºC for further experiment. The primer sequences for *S. aureus* are presented in Table 1. Using sterile ultra-pure water, oligonucleotides that had been lyophilised and desalted were reconstituted. Each reaction's RT-qPCR mastermix was prepared according to the manufacturer's instructions (Promega, UK). One cycle of denaturation at 95ºC for 2 minutes, 40 cycles of amplification at 95ºC for 15 seconds, and 40 cycles of annealing at 60ºC for 1 minute were employed in the PCR process. The manufacturer provided the positive control for the reaction, and nano-pure water was employed to exclude the possibility of contamination. The Applied Biosystems StepOne Software v2.3 was used to perform densitometry. A modified $2^{-\Delta\Delta}$ method was utilized to assess the level of relative gene expression in *S. aureus* samples. The experiment was carried out in triplicate (15, 24, 25).

Statistical analysis. The data were presented as a mean ± standard deviation, or as a number (percentage). Independent student t-test from SPSS version 20 was employed to compare the treatment and control groups. The significance level was set at $P<0.05$.

RESULTS

Antibacterial activity of honeys against *S. aureus.* Using the disc diffusion method, all of the honeys tested exhibited *S. aureus.* When compared to the effects of SH and TH, the antibacterial effects of MH were more strong, as seen by larger inhibition zones. The agar inhibition experiment revealed that the lowest concertation of MH, SH, and TH at 12.5% (v/v) had antibacterial action against *S. aureus* with inhibition zones of 13.1 ± 0.2 mm, 11.2 ± 0.2 mm and 11.1 ± 0.3 mm respectively (Table 2).

MIC and MBC determination of honeys against *S. aureus.* The MICs for MH, SH and TH were 12.5%, 25% and 25% (v/v) respectively, the MBCs for MH, SH and TH were 25%, 50% and 50% (v/v) respectively and the MIC_{50} and MIC_{90} for MH were 12.5% and 25% (v/v) respectively against *S. aureus*. Interestingly, SH and TH demonstrated constant MIC_{50} and MIC_{90} values at 12.5-25% (v/v) against *S. aureus.* MH was shown to be the most effective honey against *S. aureus.* In general, bacterial growth inhibition began at a level lower than the MIC and subsequently in-

Table 1. Primer sequences used in RT-qPCR analysis for *S. aureus*

Gene name	Amplicon size (bp)	Annealing temp (Cº)	Primer sequence (5' → 3')
argF	143	52	Forward: CCAAGCAGAATTCGAAGGA
			Revers: GGATGCGCACCTAAATCAAT
purC	117	62	Forward: GAAGCGCATTTTCTCAACAA
			Revers: CCCTTACCTGCCATTGTGTC
Adh	124	62	Forward: GTTGCCGTTGGTTTACCTGT
			Revers: TTCAGCAGCAAATTCAAACG
scdA	132	56	Forward: CGAAAGCAGCGGATATTTTT
			Revers: GCGAACCTGGTGTATTCGTT
pykA	126	52	Forward: TGCAGCAAGTTTCGTACGTC
			Revers: GGGATTTCAACACCCATGTC
menB	109	56	Forward: CTGGGGAAGGTGATTTAGCA
			Revers: ACCGCCACCTACAGCATAAC
fabG	122	54	Forward: CCGGGACAAGCAAACTATGT
			Revers: CCAAAACGTGCTAACGGAAT
*yqiL**	125	62	Forward: GACGTGCCAGCCTATGATTT
			Revers: ATTCGTGCTGGATTTTGTCC

**yqiL* was used as a reference gene

creased with honey concentration until it reached 50% (Table 3 and Fig. 1).

Time-kill curve of *S. aureus* against honeys. At 3, 6, and 9 hours, the total number of *S. aureus* cells treated with 2 × MIC of MH, SH, and TH was significantly reduced (P<0.05) compared between treated and untreated samples. *S. aureus* showed a 1.4-$\log_{10}$, 0.7-$\log_{10,}$ and 0.6-$\log_{10}$ reduction in cfu/ml after being treated with 2 × MIC of MH, SH, and TH respectively compared to untreated cells at 3-hours incubation. Furthermore, after 6-hours of incubation with 2 × MIC of MH, SH, and TH, *S. aureus* demonstrated a 2.6-$\log_{10}$, 2.1-$\log_{10}$, and 1.9-$\log_{10}$ decrease in cfu/ml respectively compared to untreated cells. *S. aureus* incubated with 2 × MIC MH, SH, and TH for 9 hours had the greatest bactericidal activity, yielding 3.7-$\log_{10}$, 3.3-$\log_{10}$, and 3.0-$\log_{10}$, respectively (Fig. 2).

Table 2. The antibacterial activities of MH, SH, and TH against *S. aureus* using an agar well diffusion assay.

Honey samples	75%	50%	25%	12.5%
Manuka honey (MH)	25.1 ± 0.3	23.4 ± 0.4	19.4 ± 0.2	13.1 ± 0.2
Sidr Honey (SH)	22.2 ± 0.2	20.4 ± 0.3	17.3 ± 0.4	11.2 ± 0.2
Tualang honey (TH)	21.3 ± 0.2	20.1 ± 0.2	17.2 ± 0.4	11.1 ± 0.3

(Inhibition Zone (mm) ± SD)

Table 3. MICs and MBCs values for MH, SH and TH against *S. aureus*

Honey samples	MIC % (v/v)	MIC_{50} % (v/v)	MIC_{90} % (v/v)	MBC % (v/v)
Manuka honey (MH)	12.5%	12.5%	25%	25%
Sidr Honey (SH)	25%	12.5-25%	25-50%	50%
Tualang honey (TH)	25%	12.5-25%	25-50%	50%

Microcolony formation of *S. aureus* exposed to honeys. When compared to untreated cells, MH, SH, and TH were observed to reduce and disrupt microcolony development in *S. aureus* at the MIC of MH, SH, and TH. Light microscopy revealed that *S. aureus* microcolony development was easily interrupted following treatment with all of the tested honeys and indicated that the bacterial cells were loosely associated with each other in a background of planktonic cells (Fig. 3).

The influence of honeys on *S. aureus* biofilm. In the presence of 60%, 40% and 20% (v/v) of MH, SH and TH, the growth of *S. aureus* biofilm was signifi-

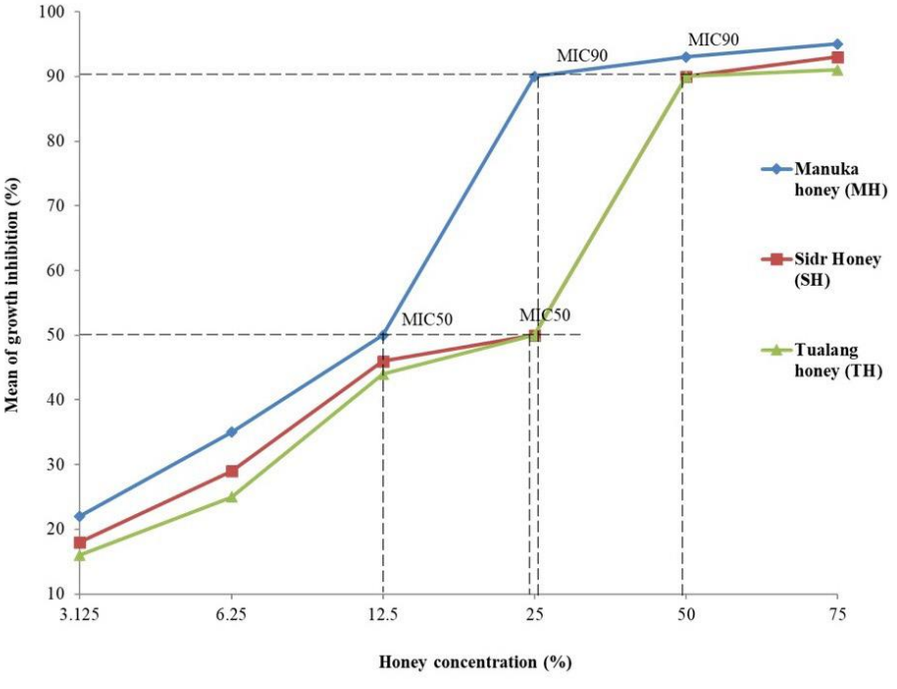
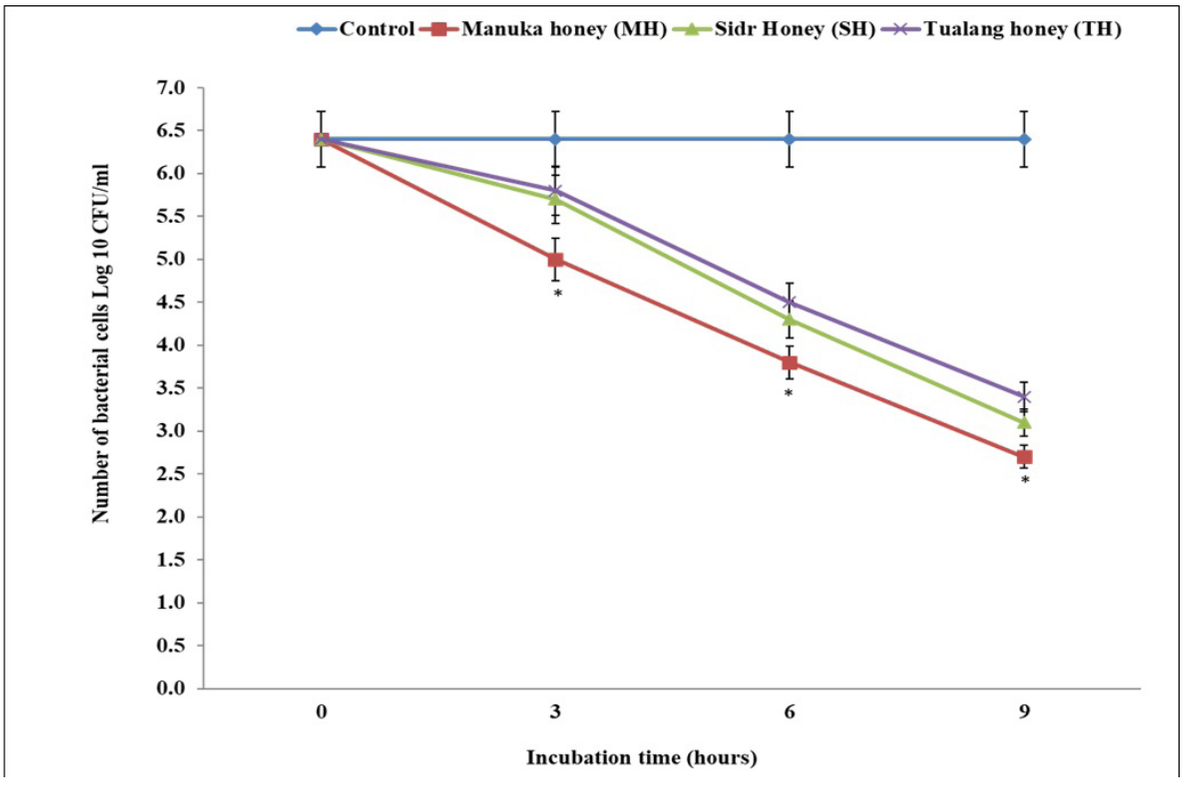


Fig. 1. Growth inhibition of *S. aureus* following exposure to MH, SH and TH.



Asterisks; *P<0.05 indicate statistically significant difference between treated and control samples.

Fig. 2. Time-kill curve of *S. aureus* after exposure to MH, SH and TH.

cantly (P< 0.05) reduced relatively to the untreated cells. However, at 10% and 5% (v/v) concentrations of MH, SH and TH, the growth of *S. aureus* biofilm was not significantly reduced. However, the greatest inhibition of *S. aureus* biofilm was found to be 52%, 46% and 42% after exposure to 60% (v/v) concentration of MH, SH and TH respectively. Furthermore, it was observed that the susceptibility testing of MH, SH and TH concentration below the MIC has decreased the growth of *S. aureus* biofilm (Fig. 4).

Expression levels of *S. aureus* virulence genes exposed to honeys quantified by RT-qPCR. The RT-qPCR method was used in this study to evaluate and compare the level of virulence genes in *S. aureus* after exposure to MIC of MH, SH, and TH. The results are presented as the n-fold change in gene expression levels in *S. aureus* treated with MICs of MH, SH, and TH in comparison to expression levels in untreated and reference gene. In this experiment, RT-qPCR results revealed that all virulence genes in *S. aureus* were downregulated with varying degrees of downregulation found after treatment with MICs of MH, SH, and TH. Seven genes *argF, purC, adh, scdA, pykA, menB* and *fabG* in *S. aureus* showed the significant reduction (P<0.05, $P \leq 0.01$ and $P \leq 0.001$) of gene expression after exposure to MIC of MH, SH and TH when compared to untreated. The expression of *argF, purC, adh, scdA, pykA, menB* and *fabG* genes in *S. aureus* were decreased in a 3.2-fold, 5.4-fold, 5.6-fold, 6.1-fold, 6.4-fold, 7.2-fold and 7.5-fold following treatment with MIC of MH respectively (Figs. 5. and 8). Whereas, the expression of *argF, purC, adh, scdA, pykA, menB* and *fabG* genes in *S. aureus* were decreased in a 2.4-fold, 4.8-fold, 5.1-fold, 5.5-fold, 6.1-fold, 6.5-fold and 7.1-fold after being treated with MIC of SH respectively (Figs 6. and 8). Whilst, the expression of *argF, purC, adh, scdA, pykA, menB* and *fabG* genes in *S. aureus* were decreased in a 2.2-fold, 3.8-fold, 4.5-fold, 5.1-fold, 5.7-fold, 5.9-fold and 6.4-fold after exposure to MIC of TH respectively (Figs. 7 and 8).

DISCUSSION

Honey's antibacterial action is accomplished by a variety of molecules, including hydrogen peroxide,

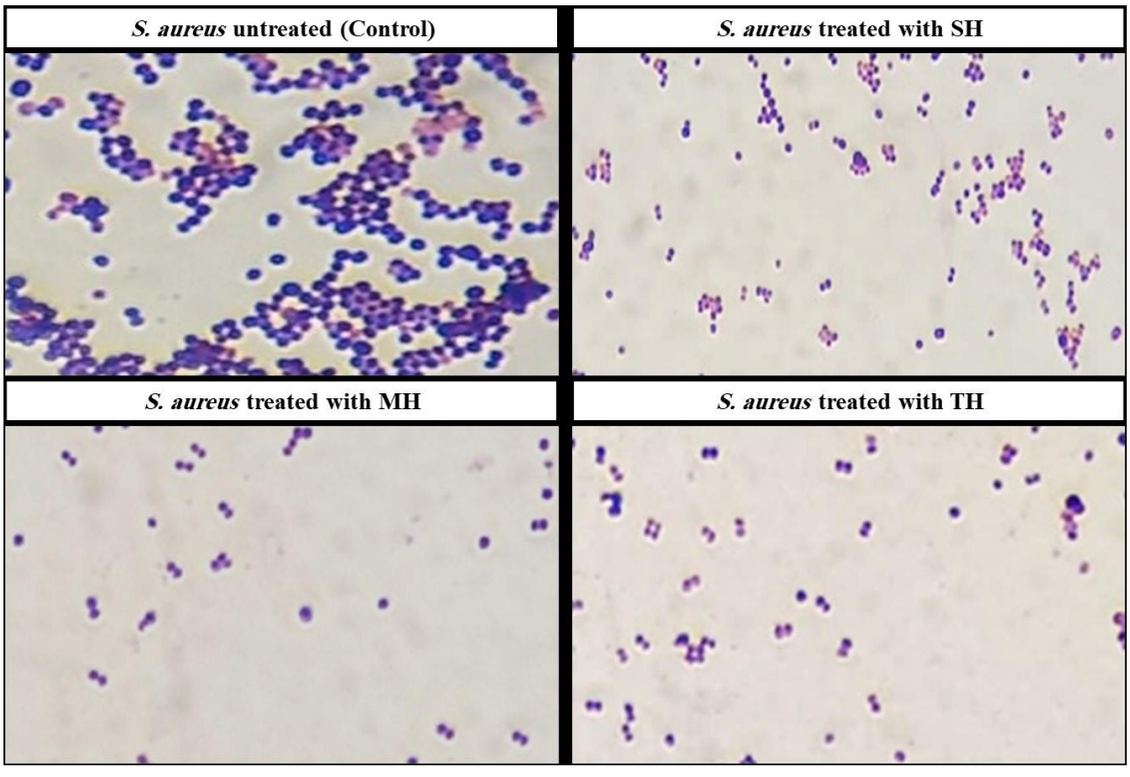
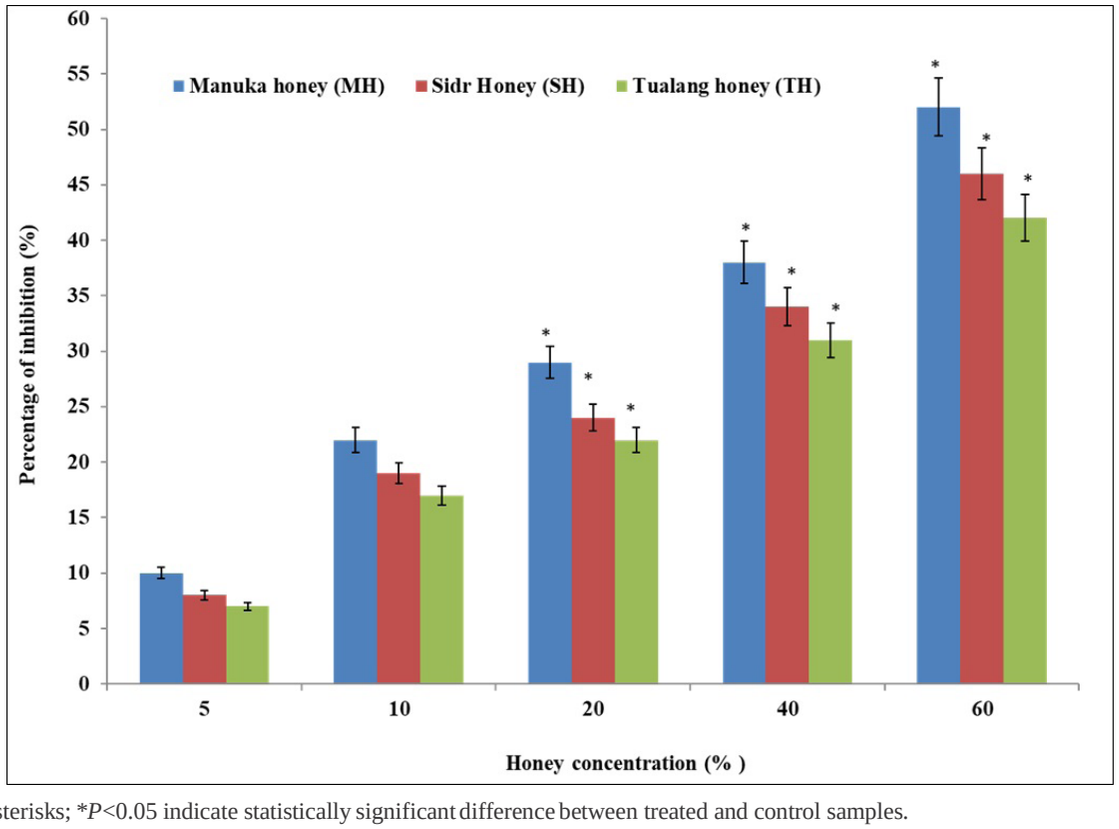


Fig. 3. Microcolony formation of *S. aureus* following exposure to MH, SH, and TH.



Asterisks; *P<0.05 indicate statistically significant difference between treated and control samples.

Fig. 4. The effect of various concentrations of MH, SH, and TH on *S. aureus* biofilm

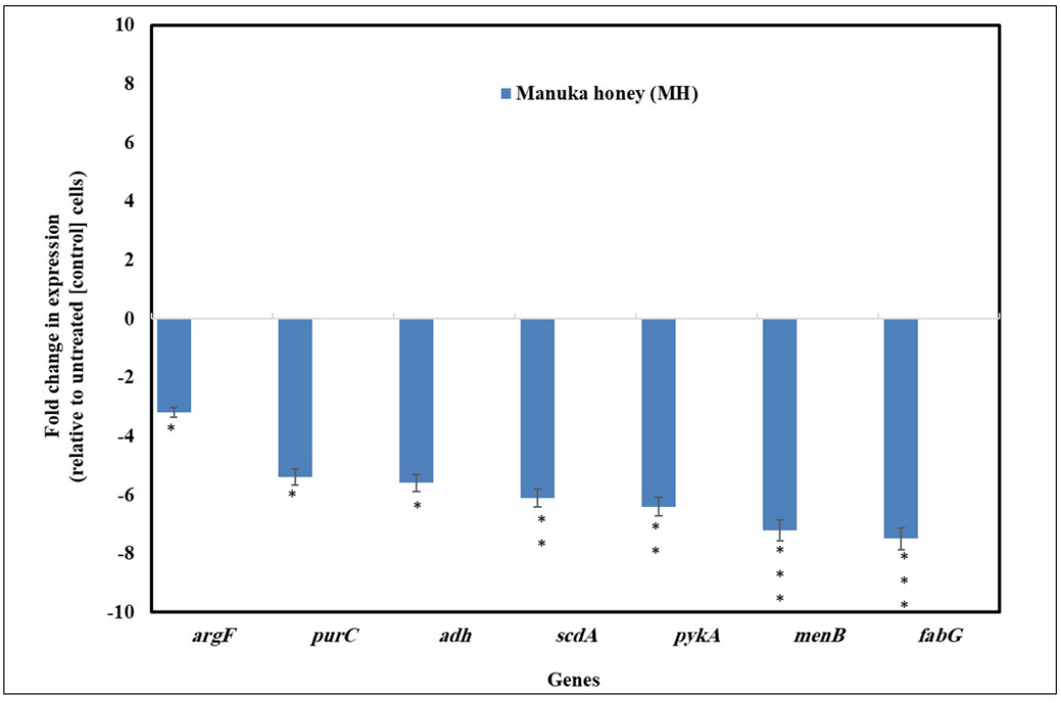


Fig. 5. Changes in gene expression patterns in *S. aureus* after treated with MIC of MH, as determined by RT-qPCR. Mean values of fold changes (± SD) are shown in relation to untreated *S. aureus* cells. Asterisks; * $P<0.05$; ** $P \leq 0.01$; and *** $P \leq 0.001$ indicate statistically significant difference in the expression of each gene between treated and untreated samples. Results are expressed as the mean fold change.

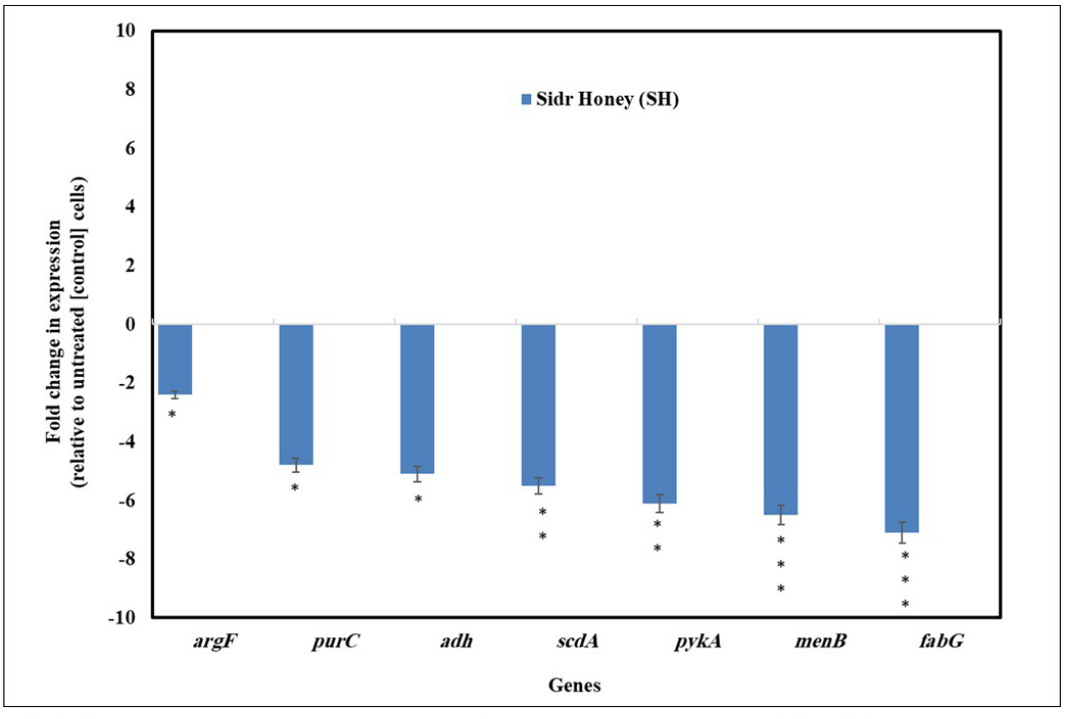


Fig. 6. RT-qPCR analysis of gene expression patterns in *S. aureus* after treatment with MIC of SH. Mean values of fold changes (± SD) are shown in relation to untreated *S. aureus* cells. Asterisks; * $P<0.05$; ** $P \leq 0.01$; and *** $P \leq 0.001$ indicate statistically significant difference in the expression of each gene between treated and untreated samples. Results are expressed as the mean fold change.

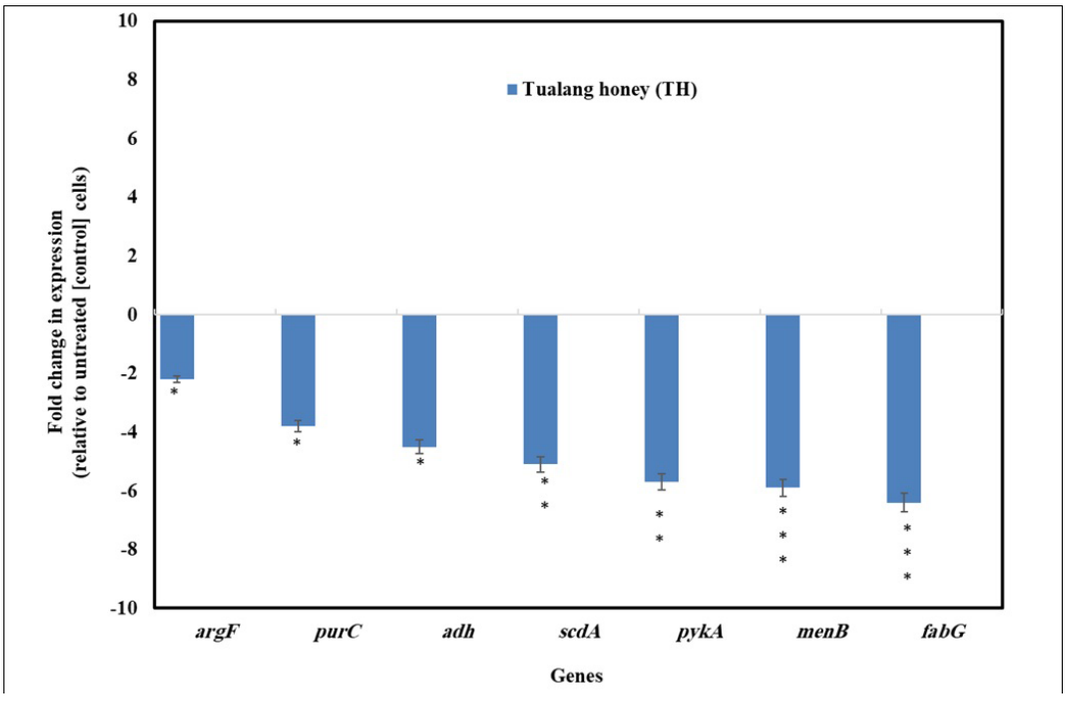


Fig. 7. Alterations in gene expression profiles in *S. aureus* following treatment with MIC of TH as determined by RT-qPCR. Mean values of fold changes (± SD) are shown in relation to untreated *S. aureus* cells. Asterisks; * $P<0.05$; ** $P \leq 0.01$; and *** $P \leq 0.001$ indicate statistically significant difference in the expression of each gene between treated and untreated samples. Results are expressed as the mean fold change.

non-hydrogen peroxide or combination of these two compounds. Two enzymes that regulate hydrogen peroxide are glucose oxidase and catalase. Glucose oxidase produce the hydrogen peroxide to preserve the nutritional compound of honey meanwhile catalase destroy the hydrogen peroxide. The antibacterial action of non-hydrogen peroxide is comprised of flavonoid, phenolic acid, high pH, and sugar content. When given as a supplement, these substances protected free radicals from harming human cells and tissues. We concentrated on the well diffusion assay because honey is a complicated solution composed of various sizes of chemicals and compounds. The use of disks may result in the exclusion of large molecules that are not well absorbed by paper disks, contributing to inaccurate results (21). This study found that the antibacterial effects of MH were more strong, as seen by larger inhibition zones when compared to the effects of SH and TH. Study by Jarrar et al. showed that Manuka and Chilean honeys exhibited higher antibacterial action at 25% concentration of honey (26). Previous study reported that at 25% concentration of honey exhibited lower antibacterial action (27).

The MIC value is utilized to inhibit approximately 99% of bacterial growth, whereas the MBC value is used to eliminate approximately 99% of the bacterial colonies (28). The MICs values were 12.5%, 25% and 25% (v/v) for MH, SH and TH respectively and the MBCs values were 25%, 50% and 50% (v/v) for MH, SH and respectively against *S. aureus*. Previous study demonstrated that the MIC and MBC values for Manuka honey were 12% and 16% against *P. aeruginosa* respectively (29). Study by Tan et al. reported by that Manuka honey (UMF 10+) exhibits higher antibacterial activity than Tualang honey against *E. coli, P. aeruginosa* and *S. aureus* (30). The MIC_{90} and MIC_{50} values are used to inhibit 90% and 50% of *S. aureus* cultures respectively (28). We included the MIC_{90} and MIC_{50} to enhance the bacterial growth inhibition pattern. In the current study, the MIC_{50} and MIC_{90} values for MH against *S. aureus* were 12.5% and 25% (v/v) respectively and less than that for SH and TH. The time-kill curve assay is used to assess the bactericidal or bacteriostatic activity of an antimicrobial agent against a bacterial strain over time (31). In this study, it was noticed that the population of *S. aureus* reduced after exposure

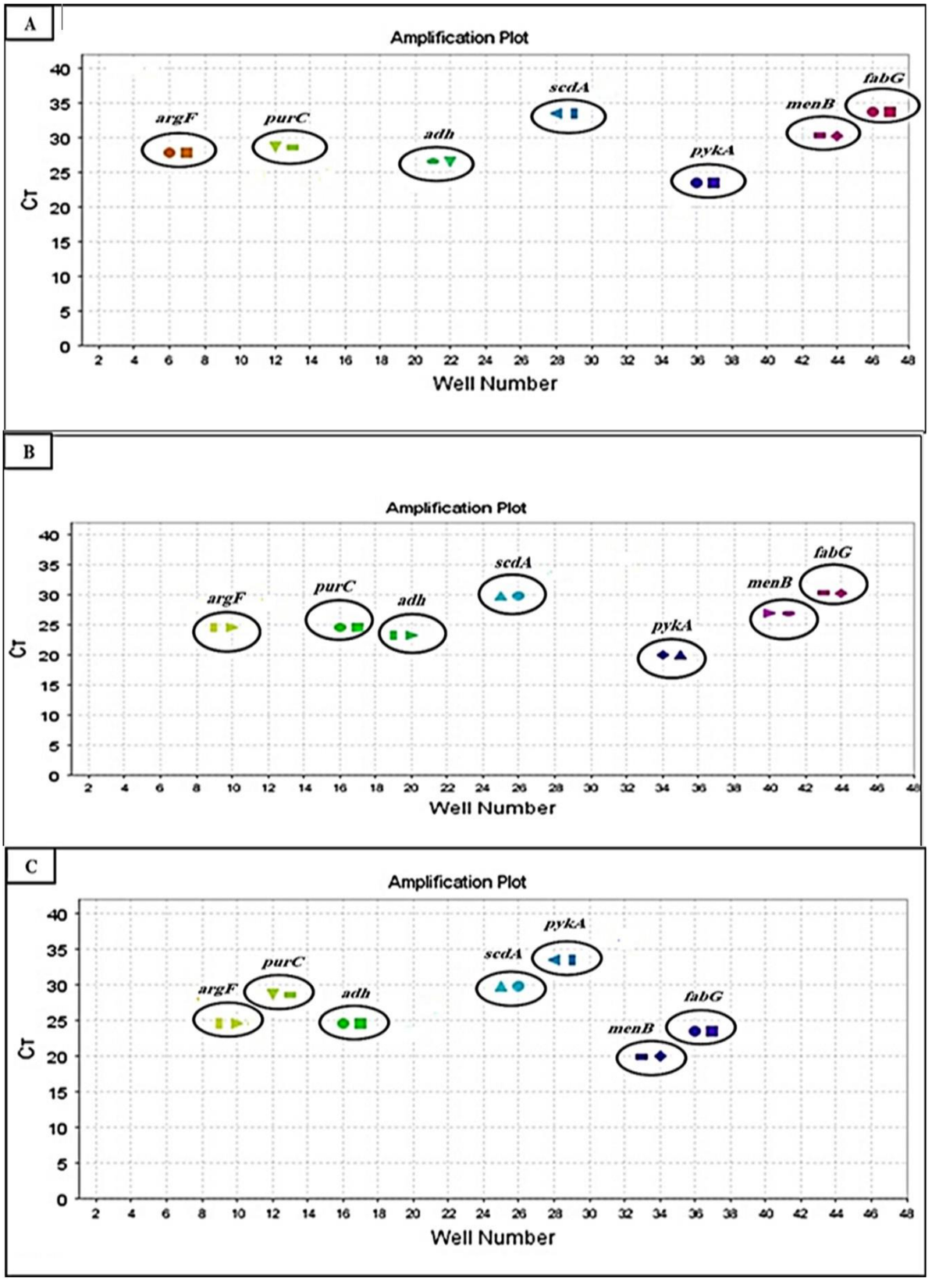


Fig. 8. The amplification of a cDNA target genes was plotted versus the Ct value.
argF, purC, adh, scdA, pykA, menB and *fabG* genes in *S. aureus* (A): MH, (B):SH and (C): TH.

to 2 times MIC of MH, SH and TH at 6 and 9 hours. Previous study showed that Manuka honey inhibited the planktonic growth of *P. aeruginosa* (32). Another study reported that Manuka honey decreased the number of *P. aeruginosa* cells to 6-log reduction (33). Study by Grecka et al. revealed that Polish honey reduced the number of *S. aureus* cells to 4-log (34). Algerian honey had destroyed the number of *E. coli* and *P. aeruginosa* cells after 24 hours (35). In this study, MH, SH and TH was found to inhibit biofilm formation in *S. aureus.* The ability of MH, SH and TH to eradicate *S. aureus* biofilm is due to one or more components present in the honey other than MGO and sugar, such as low pH, hydrogen peroxide, phenolic and other unknown components (36). In comparison to our study, MH had a lower MIC and MBC against *S. aureus* than SH and TH. This might be attributed to the presence of various organic antibacterial components given by MH rather than honey, as well as the floral origin of the nectar.

The RT-qPCR method was used to analyze and compare the levels of virulence genes in *S. aureus* following exposure to MICs of MH, SH and TH. According to RT-qPCR analysis, all genes in *S. aureus* (*argF, purC, adh, scdA, pykA, menB* and *fabG*) were significantly downregulated ranging from 3.2-fold to 7.5-fold after exposed to MH. Meanwhile, the above gene expression was downregulated ranging from 2.4-fold to 7.1-fold after being treated with MIC of SH and from 2.2-fold to 6.4-fold after exposure to MIC of TH. Investigations on the effects of MH, SH, and TH on *S. aureus* gene transcription have revealed a variety of expression patterns that vary depending on the bacterial strain, exposure time between honey and bacteria, and honey doses used in each research. Previous studies showed that the expression of *algD, oprF, fleN, fleQ, fleR, fliA,* and *fliC* in *P. aeruginosa* were downregulated after following treatment with Sidr honey (15) and Manuka honey (37). The study done by Ahmed and Salih (38) demonstrated that the expression of genes *lasI* and *rhl* in *P. aeruginosa* were reduced after treated with local Iraqi honey (38). The *oprH* and *oprB* in *P. aeruginosa* were downregulated after exposure to Manuka honey (37). Certain targeted gene expression in honey was reflected as down-regulation of spaetzle, AMPs abaecin and defensin-1 and up-regulation of lysozyme-2 (39).

CONCLUSION

This is the first study to compare the antibacterial activity of SH and TH against *S. aureus. S. aureus* cell growth was reduced in both the planktonic and biofilm stages after treatment with SH and TH. In comparison to SH and TH, MH had a greater effect on *S. aureus* in both planktonic and biofilm cultures. Differential gene expression in response to MH, SH, and TH exposure revealed downregulation of multiple *S. aureus* virulence genes. In comparison to MH, the results suggest that SH and TH may represent potential antibacterial agents for the treatment and regulation of *S. aureus* infections. According to the findings, each honey may include a critical derivative component capable of efficiently inhibiting the planktonic and biofilm of *S. aureus.*

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