CHAPTER 5 : ANTIMICROBIAL ACTIVITY OF AJWA DATES PREPARED FOLLOWING THE PRACTICE OF THE PROPHET (PBUH) TOWARD BACTERIAL CAUSING GASTROENTERITIS

5.1 Introduction

Maintaining health status by applying the prophetic diet in daily life is considered *Sunnah* (Prophetic Tradition), which is known as the narration of Prophet Muhammad Peace Be Upon Him (PBUH). Allah has promised rewards for individuals who make the Prophet Muhammad (PBUH) an example in their lives. Numerous Quranic verse and *Hadith* recommend specific foods with healing properties and are beneficial to human's health, such as honey, barley dates, figs, goat milk, black seeds, and olive oil. Notably, dates are the most favoured food by Prophet Muhammad (PBUH) (Syed, 2003; Farhangi et al., 2014).

People are currently consuming the food recommended by Prophet Muhammad (PBUH), such as the date and honey, and practising the way Prophet Muhammad consumed and prepared food. Among varieties of dates fruits cultivar, Prophet Muhammad (PBUH) preferred eating Ajwa dates. It Nerrated by Sa'd : Allah;s Messenger said, "He who eat seven "Ajwa dates every morning, will not affected by poison or magic on the day he eats them." (Al-Bukhari, 2000). The hadiths guiding the Prophet's consumption of date fruits are also written. Ibn 'Amir and Anas bin Malik narrated, "the Prophet used to eat odd number of dates." (M. Al-Bukhari, 1978), while Sahl bin Sa'd stated that "when Abu Usaid As-Sa`idi got married, he invited the Prophet and his companions. None prepared the food for them and brought it to them but his wife. She soaked some dates in water in a stone pot overnight, and when the Prophet

had finished his food, she provided him with that drink (of soaked dates)." (Al-Bukhari, 2000)

Based on the previous study results, the cold aqueous, hot aqueous, and methanol extracts in Ajwa dates showed significant antibacterial activities. However, no available or published research has performed on the preparation of Ajwa date extraction according to *Sunnah* practice and consumption. Therefore, the antibacterial activity in the preparation of dates was further evaluated, followed by Prophet Muhammad (PBUH) practice by applying three matters stated in the hadith, namely 1) Ajwa dates, 2) consumption of date fruits in an odd number, and 3) soaking of date fruits in water overnight. Therefore, this study aims to investigate the antibacterial activity in Ajwa dates-infused water against six strains of pathogenic bacterial gastroenteritis.

5.2 Methodology

5.2.1 Sample Preparation

Ajwa dates were divided into groups of one date, three dates, five dates, and seven dates. Each group was soaked in a glass of water (240 ml), with each glass being covered using aluminium foil and seated in room temperature (24 ^{o}C). The infused water samples were obtained at different time intervals (zero hour, two hours, four hours, six hours, eight hours, 10 hours, and 24 hours) and subjected to antimicrobial assay using well diffusion assay (methodology **3.5.1**) and phytochemical quantification using TPC, TFC, and TTC (methodologies **3.8.2.1**, **3.8.2.2**, and **3.8.2.3)**.

5.3 Results

5.3.1 Antimicrobial Screening by Well Diffusion Assay

Table 5.1 presents the antibacterial activity of one Ajwa date-infused water. As a result, no antibacterial activity was found in the water against all tested bacteria within the 24 hours.

Table 5.1: Diameter of inhibition zone (mean ± standard deviation) of one Ajwa date-infused water against all the tested bacteria using well diffusion assay.

Times / Bacteria	0h	2h	4h	6h	8h	10h	24h	Ciprofloxacin (0.5mg/ml)
S. aureus	ND	ND	ND	ND	ND	ND	ND	36.67 (±0.58)
S. Typhi	ND	ND	ND	ND	ND	ND	ND	41.33 (±1.15)
S. Typhimurium	ND	ND	ND	ND	ND	ND	ND	39.67 (±0.58)
E. coli	ND	ND	ND	ND	ND	ND	ND	34.67 (±0.58)
V. cholerae	ND	ND	ND	ND	ND	ND	ND	39.67 (±0.58)
S. flexneri	ND	ND	ND	ND	ND	ND	ND	44.67 (±0.58)

ND- Not detected *Inhibition zone included 7mm well hole.

The results for three Ajwa dates-infused water are shown in **Table 5.2**, in which antibacterial activity against *S. aureus* was recorded after eight hours, with diameters of inhibition zone of 27.33 ± 1.15 mm. The diameter of inhibition zone of *S. aureus* was 27.67 ± 0.58 mm at 10 hours, which then remained constant within 24 hours. However, the antibacterial activity against *V. cholerae* and *S. flexneri* was only observed after 24 hours, with inhibition zone diameters of 16.00 ± 1.0 mm and 19.33 ± 0.58mm, respectively, while no antibacterial activity was detected in other bacteria. Among all tested bacteria, there was no significance difference between bacteria inhibition zones at each time interval (p>0.05).

Table 5.2: The diameter of the inhibition zone (mean ± standard deviation) of three Ajwa dates-infused water against the all tested bacteria using well diffusion assay.

Time/ Bacteria	0h	2h	4h	6h	8h	10h	24h	Ciprofloxacin (0.5mg/ml)
S. aureus	ND	ND	ND	ND	27.33 (±1.15)	27.67 (±0.58)	27.67 (±0.58)	36.67 (±0.58)
S. Typhi	ND	ND	ND	ND	ND	ND	ND	41.33 (±1.15)
S. Typhimurium	ND	ND	ND	ND	ND	ND	ND	39.67 (±0.58)
E. coli	ND	ND	ND	ND	ND	ND	ND	34.67 (±0.58)
V. cholerae	ND	ND	ND	ND	ND	ND	16.00 (±1.0)	39.67 (±0.58)
S. flexneri	ND	ND	ND	ND	ND	ND	19.33 (±0.58)	44.67 (±0.58)

ND- Not detected *Inhibition zone included 7mm well hole.

The results for five Ajwa dates-infused water are presented in **Table 5.3**, in which antibacterial activity against *S. aureus* and *S. flexneri* was recorded in the water after six hours, with the inhibition zone diameter at 28.33 ± 0.58 mm and 16.33 ± 0.58 mm, respectively. At the eighth hour, the Ajwa dates-infused water was recorded with antibacterial activity against *S. aureus* and *S. flexneri*, with the inhibition zone diameters of 29.00 ± 1.00 mm and 18.67 ± 0.58 mm, respectively. At the 10th hour, antibacterial activity against *S. aureus* was found in the water, with the inhibition zone diameter of 31.33 mm, which also remained constant within 24 hours. Meanwhile, the inhibition zone diameter demonstrated by *S. flexneri* increased from 21.33 ± 0.58 mm to 22.67 ± 0.58 mm within 10 to 24 hours. The antibacterial activity of Ajwa dates against *V. cholerae* was only recorded after 24 hours with the inhibition zone diameter of 20.33 ± 0.58 mm, while no antibacterial activity was detected for other bacteria. Among all tested bacteria, there was no significance difference between bacteria inhibition zones at each time interval (p>0.05).

Table 5.3: The diameter of the inhibition zone (mean ± standard deviation) of five Ajwa dates-infused water against all the tested bacteria using well diffusion assay.

Time/ Bacteria	0h	2h	4h	6h	8h	10h	24h	Ciprofloxacin (0.5mg/ml)
S. aureus	ND	ND	ND	28.33 (±0.58)	29.00 (±1.0)	31.33 (±1.15)	31.33 (±0.58)	36.67 (±0.58)
S. Typhi	ND	ND	ND	ND	ND	ND	ND	41.33 (±1.15)
S. Typhimurium	ND	ND	ND	ND	ND	ND	ND	39.67 (±0.58)
E. coli	ND	ND	ND	ND	ND	ND	ND	34.67 (±0.58)
V. cholerae	ND	ND	ND	ND	ND	ND	20.33 (±0.58)	39.67 (±0.58)
S. flexneri	ND	ND	ND	16.33 (±0.58)	18.67 (±0.58)	21.33 (±0.58)	22.67 (±0.58)	44.67 (±0.58)

ND- Not detected. Asterisk* indicate significantly different at p <0.05 between bacteria

The results for seven Ajwa dates-infused water are presented **Table 5.4**, in which antibacterial activity against *V. cholerae* and *S. flexneri* after four hours and against *S. aureus, S. Typhi, S. Typhimurium*, and *E. coli* after six hours. The diameters of inhibition zone for *S. aureus*, *S. Typhi, S. Typhimurium,* and *E. coli* within six to 24 hours of water samples increased from 35.00 mm to 36.00 mm, 16.33 mm to 18.13 mm, 16.00 mm to 18.67 mm, and 15.67 mm to 17.33 mm, respectively. Meanwhile, the diameter of the inhibition zone within four to 24 hours of the water samples against *V. cholerae* and *S. flexneri* increased from 15.33 mm to 31.67 mm, and 20.00 mm to 27.00 mm, respectively. Among all tested bacteria, *S. aureus* is the most sensitive organism and showed the significantly higher diameter of inhibition zones compared to *S. Typhi* at 6h, 8h, and 10h; and *E. coli* at 24 hours ($p<0.05$).

Table 5.4: Diameter of inhibition zone (mean ± SD) of seven Ajwa dates-infused water against the all tested bacteria using well diffusion assay.

Times / Bacteria	0h	2h	4h	6h	8h	10h	24h	Ciprofloxacin (0.5mg/ml)
S. aureus	ND	ND	ND	35.00*[b] (±0)	35.00*[b] (±1.0)	35.33*[b] (±0.58)	36.00*[d] (±1.0)	36.67 (±0.58)
S. Typhi	ND	ND	ND	14.33 (±0.58)	15.67 (±0.58)	17.33 (±0.58)	18.13 (±1.15)	41.33 (±1.15)
S. Typhimurium	ND	ND	ND	16.00 (±0.58)	16.33 (±0.58)	16.33 (±1.0)	18.67 (±0.58)	39.67 (±0.58)
E. coli	ND	ND	ND	15.33 (±1.15)	16.67 (±0.58)	16.67 (±1.15)	16.67 (±1.15)	34.67 (±0.58)
V. cholerae	ND	ND	15.33 (±0.58)	21.33 (±1.15)	26.33 (±0.58)	30.67 (±1.15)	31.67 (±0.58)	39.67 (±0.58)
S. flexneri	ND	ND	20.33 (±0.58)	20.00 (±0.0)	21.33 (±0.58)	24.33 (±0.58)	27.00 (±1.0)	44.67 (±0.58)

ND- Not detected. Asterisk (Al-Farsi* & Lee) indicate significantly different at $p<0.05$ between bacteria.
a: *S. aureus* vs other tested organisms
b: *S. aureus* vs *S. Typhi*
c: *S. aureus* vs *S. Typhimurium*
d: *S. aureus* vs *E. coli*

5.3.2 Phytochemical Quantification of Ajwa dates by Colorimetric Assay

5.3.2.1 Total Phenolic Content

The data of total phenolic content of Ajwa dates-infused water are presented in **Table 5.5**. In general, the TPC values of Ajwa date infused in water were dose-dependent and time-dependent. Furthermore, the TPC values from within zero to 24 hours for one Ajwa date infused in water increased from 3.96 to 27.94 mg GAE/sample, three Ajwa dates increased from 24.71 to 59.06 mg GAE/sample. Meanwhile, five Ajwa dates increased from 35.99 to 80.79 mg GAE/samples, while seven Ajwa dates increased from 43.50 to 102.61 mg GAE/samples. A comparison was performed between total phenolic content (TPC) and the number of Ajwa dates used in each time interval (see **Figure 5.1**). Although the TPC values increased with the rising number of used Ajwa dates, there was no significant difference between each group ($p > 0.05$).

Table 5.5: Total phenolic content of Ajwa date dates-infused water. Data are presented as mean ± standard deviation, while values are represented in mg gallic acid equivalence/ 240ml of samples (mg GAE/samples).

	Total Phenolic Content (mg GAE/ 240ml samples)						
	0h	2hs	4hs	6hs	8hs	10hs	24hs
One Ajwa dates	ND	3.96 (±0.06)	8.79 (±0.26)	14.58 (±0.50)	17.84 (±0.23)	20.31 (±0.68)	27.94 (±0.49)
Three Ajwa dates	ND	24.71 (±0.44)	35.32 (±1.04)	46.16 (±0.53)	52.83 (±1.02)	54.46 (±0.72)	59.06 (±0.20)
Five Ajwa dates	ND	35.99 (±0.79)	44.90 (±0.50)	58.22 (±1.23)	62.32 (±0.80)	71.37 (±1.73)	80.79 (±0.68)
Seven Ajwa dates	ND	43.50 (±1.22)	66.68 (±0.23)	83.12 (±1.43)	89.62 (±4.16)	98.32 (±2.23)	102.61 (±0.54)

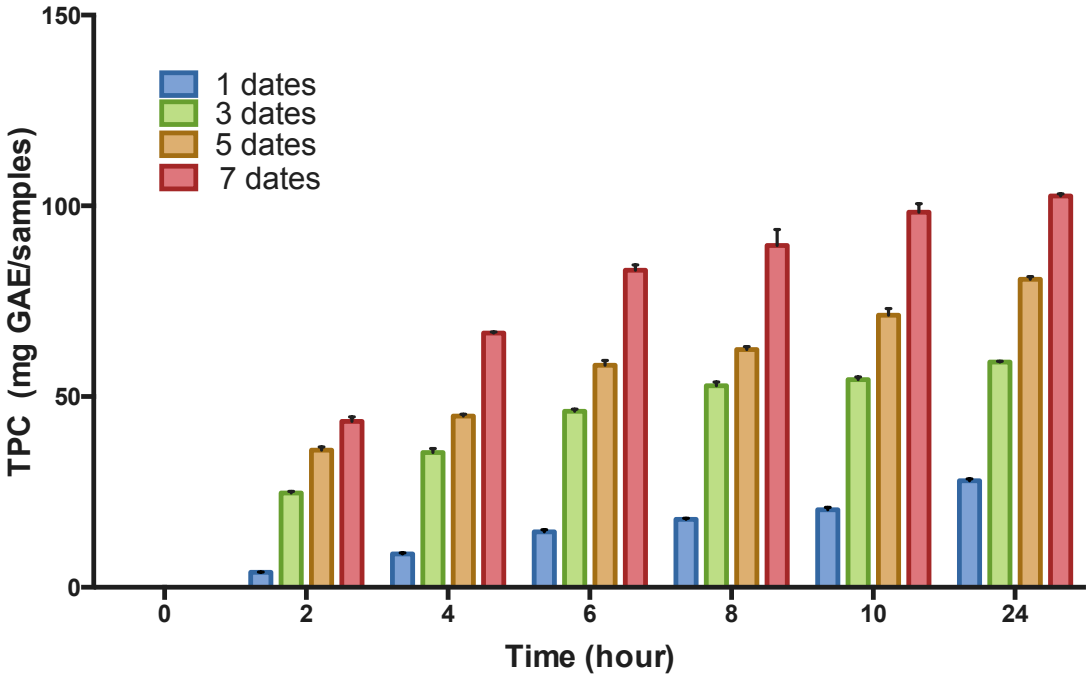

Figure 5.1: Total phenolic content of Ajwa dates-infused water at each interval time.

5.3.2.2 Total Tannin Content (TTC)

The data of total tannin content of Ajwa dates-infused water are presented in **Table 5.6**. In general, the TTC values of Ajwa dates infused in water were dose-dependent and time-dependent. The TTC values from zero to 24 hours for one Ajwa date infused in water increased from 0.64 to 11.13 mg GAE/samples as three Ajwa dates TTC values increased from 7.48 to 33.51 mg GAE/samples, while five Ajwa dates TTC values increased from 11.75 to 30.83 mg GAE/samples. On the other hand, seven Ajwa dates TTC values increased from 12.29 to 32.44 mg GAE/samples.

Furthermore, the total tannin content of Ajwa dates-infused water at each interval hour is illustrated in **Figure 5.2**. Although the TTC values increased with the number of used Ajwa dates, only the group of three Ajwa dates was recorded with significantly higher TTC values compared to the group of one Ajwa date within 24 hours ($p = 0.0395$), while other groups were recorded with no significant difference ($p > 0.05$).

Table 5.6: Total tannin content values (mean ± SD) of Ajwa dates-infused water, while the values are presented in mg Gallic acid equivalence/240 ml of samples (mg GAE/samples).

	Total Tannin Content (mg GAE/ 240ml samples)						
	0h	2h	4h	6h	8h	10h	24h
One Ajwa dates	ND	0.64 (±0.06)	2.59 (±0.30)	5.44 (±0.38)	5.33 (±0.19)	7.14 (±0.64)	11.13 (±0.64)
Three Ajwa dates	ND	7.48 (±0.39)	10.17 (±1.09)	17.47 (±0.54)	17.68 (±1.08)	17.52 (±1.05)	33.51 (±0.30)
Five Ajwa dates	ND	11.75 (±0.86)	10.36 (±0.67)	18.87 (±1.51)	22.09 (±0.72)	24.16 (±1.78)	30.83 (±0.54)
Seven Ajwa dates	ND	12.29 (±1.21)	16.38 (±0.10)	26.67 (±2.69)	29.07 (±4.17)	32.41 (±3.38)	32.44 (±1.31)

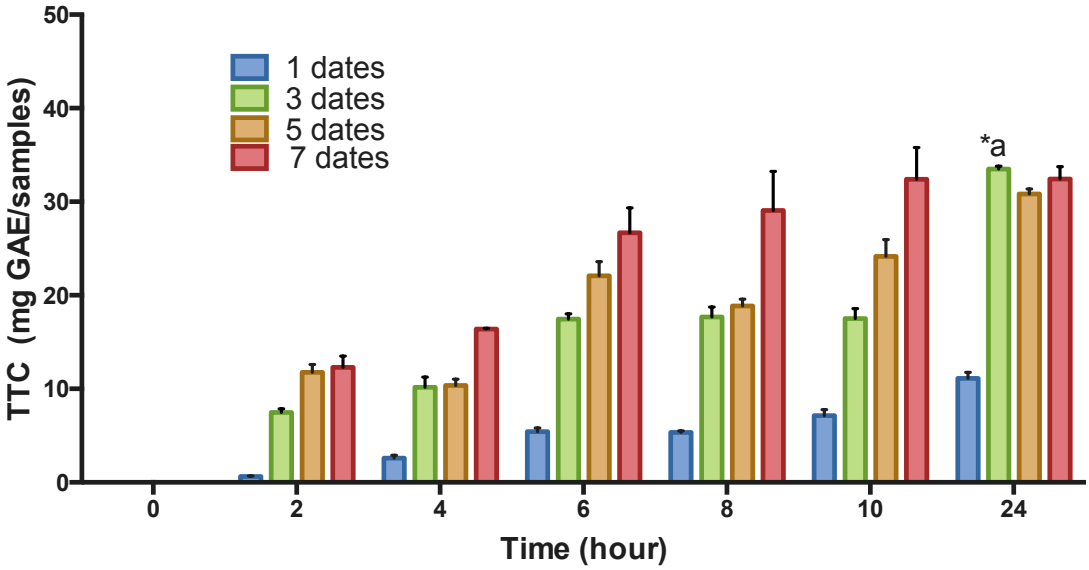

Figure 5.2: Total tannin content of Ajwa dates-infused water at each interval time. The asterisks above bar indicate significance difference between groups (p<0.05)
***a**: One date vs. three dates

5.3.2.3 Total Flavonoids Content (TFC)

In general, the TFC values are dose- and time-dependent. The TFC values from zero to 24 hours for one Ajwa date infused in water increased from 2.98 to 6.29 mg RE/ samples as three Ajwa dates TFC values increased from 6.64 to 13.05 mg RE/samples, while five Ajwa dates TFC values increased from 9.39 to 24.32 mg RE/ samples. On the other hand, seven Ajwa dates TFC values increased from 16.99 to 25.22 mg RE/ samples.

Furthermore, a comparison was conducted between the total flavonoid content of Ajwa dates-infused water at each interval hour (**Figure 5.3**). Although the TFC values increased with the addition of the number of used Ajwa date, there was no significance difference between each group (p > 0.005). The data of total flavonoids content of Ajwa dates-infused water are presented in **Table 5.7**.

Table 5.7: Total flavonoid content of Ajwa date infused in the water. The data are presented as mean ± standard deviation, while the values are presented in mg Rutin equivalence/240 ml of samples (mg RE/samples)

	Total flavonoid Content (mg RE/samples)						
	0h	2h	4h	6h	8h	10h	24h
One Ajwa dates	ND	2.98 (±0.22)	3.86 (±0.17)	4.22 (±0.19)	4.85 (±0.31)	5.16 (±0.39)	6.29 (±0.38)
Three Ajwa dates	ND	6.64 (±0.16)	8.43 (±0.28)	9.68 (±0.22)	12.60 (±0.21)	14.91 (±0.53)	13.05 (±0.08)
Five Ajwa dates	ND	9.39 (±0.27)	11.07 (±0.08)	12.49 (±0.08)	17.35 (±0.87)	19.29 (±0.38)	24.32 (±0.44)
Seven Ajwa dates	ND	16.99 (±0.03)	19.85 (±3.36)	20.26 (±0.06)	25.44 (±0.31)	26.68 (±0.08)	25.22 (±0.81)

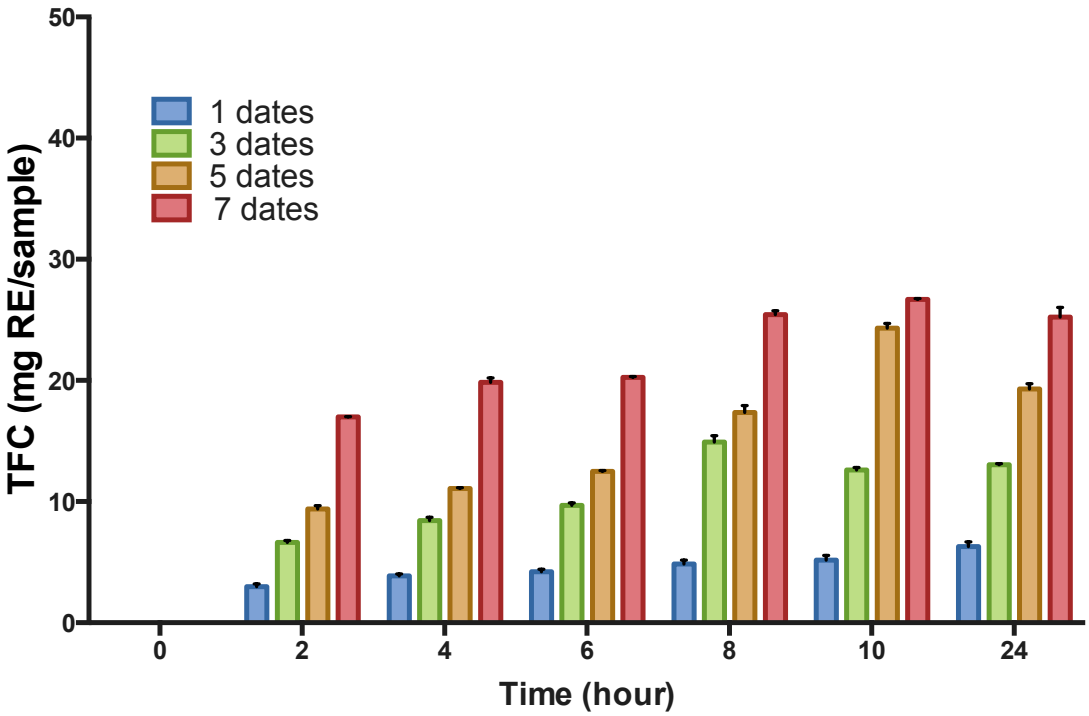

Figure 5.3: Total flavonoid content of Ajwa dates-infused water at each interval time.

5.3.3 Correlations Between Bacterial Inhibition Zone with Phytochemical Analysis

5.3.3.1 Correlation Between Bacterial Inhibition Zone And TPC

The Spearman correlation coefficient was computed to assess the relationship between bacteria inhibition zones and total phenolic content (TPC) of Ajwa dates. As a result, a significant positive correlation between the two variables (TPC and bacteria inhibition zone) was found on all tested bacteria, with *S. aureus* ($r = 0.9016$, $p < 0.0001$), *S. Typhi* ($r = 0.7746$, $p = 0.0002$), *S. Typhimurium* ($r = 0.7609$, $p = 0.0007$), *E. coli* ($r = 0.7379$, $p = 0.0018$), *V. cholerae* ($r = 0.8580$, $p < 0.0001$), and *S. flexneri* ($r = 0.9447$, $p < 0.001$).

A scatterplot of bacteria inhibition zone with TPC values of Ajwa dates is demonstrated in **Figure 5.4**. The increase in TPC values has correlated the increase in bacterial inhibition zone. The correlation coefficient was classified into 'excellent' (0.76 - 1.00), 'good' (0.51 - 0.75), 'fair' (0.26 -0.50), and poor (< 0.25) (Salleh & Rani, 2013). Overall, an excellent, positive correlation between bacterial inhibition zones and TPC values of Ajwa dates was found on *S, aureus, S. Typhi, S, Typhimurium, V. cholerae*, and *S. flexneri*, while a good and positive correlation was present between bacterial inhibition zones and TPC values on *E. coli*.

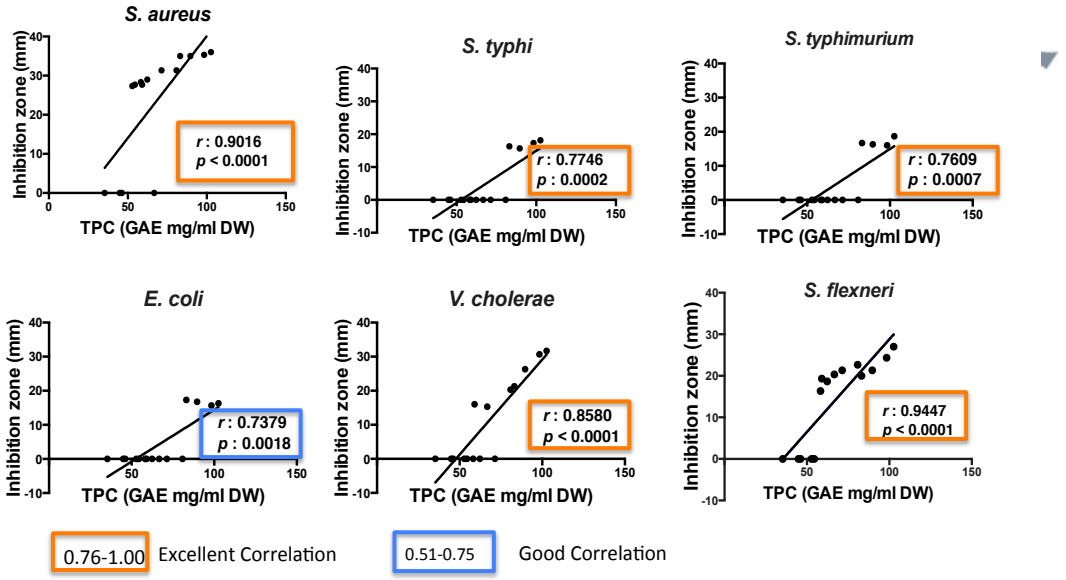

Figure 5.4: Scatterplot of each bacterial inhibition zone against TPC values. The Spearman rank correlation graphs show the positive correlation between bacterial inhibition zone and TPC values.

5.3.3.2 Correlation Between Bacterial Inhibition Zone And TFC

A Spearman correlation coefficient was computed to assess the relationship between bacterial inhibition zones and the total flavonoid content (TFC) of Ajwa dates. A significant positive correlation was present between the two variables on all tested bacteria, which included *S. aureus* ($r = 0.8257$, $p = 0.0003$), *S. Typhi* ($r = 0.7242$, $p = 0.00023$), *S. Typhimurium* ($r = 0.7013$, $p = 0.00037$), *E. coli* ($r = 0.6967$, $p = 0.0040$), *V. cholerae* ($r = 0.7631$, $p = 0.0016$) *and S. flexneri* ($r = 0.7009$, $p < 0.0001$).

Meanwhile, the scatterplot of bacteria inhibition zone with TFC values of Ajwa dates is presented in **Figure 5.5**. The increase in TFC values was correlated with the increase in bacterial inhibition zone. Overall, an excellent, a positive correlation for *S. aureus* and *V. cholerae* was found between bacterial inhibition zone and TFC values of

Ajwa dates. This correlation was also found for *S. Typhi, S. Typhimurium*, *E. coli*, and *S. flexneri* between bacterial inhibition zone and TFC values of Ajwa dates.

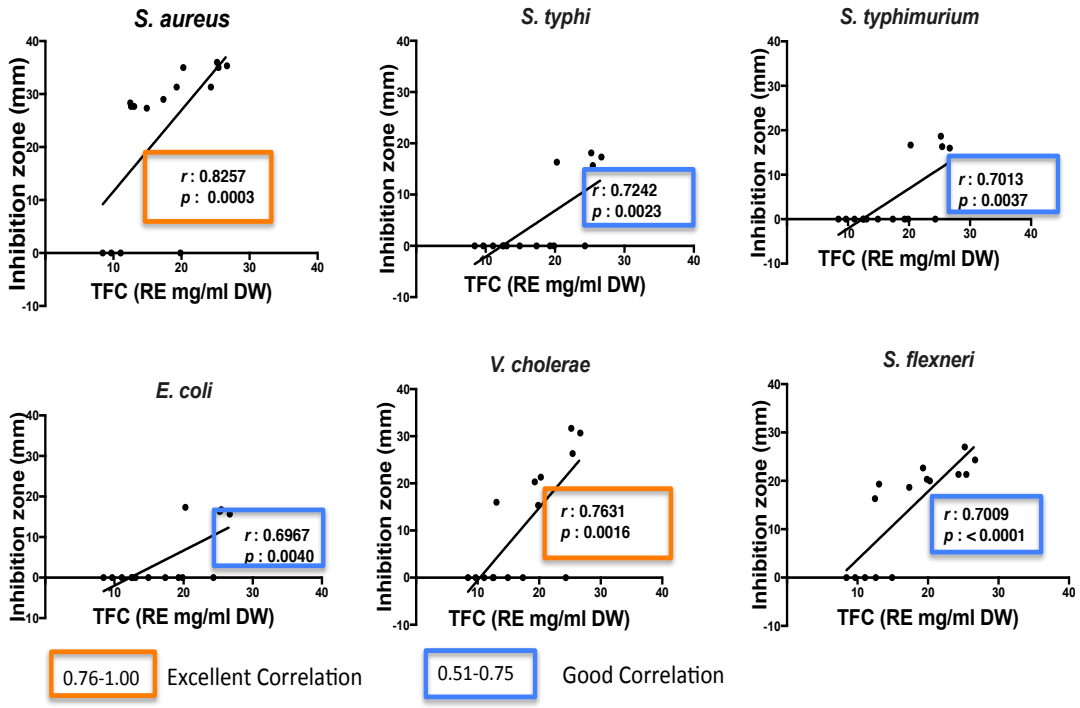

Figure 5.5: Scatterplot of each bacterial inhibition zone against TFC values. Spearman rank correlation graphs illustrate the positive correlation between bacterial inhibition zone and TPC values.

5.3.3.3 Correlation Between Bacterial Inhibition Zone And TTC

A Spearman correlation coefficient was computed to assess the relationship between bacterial inhibition zone and the total tannin content (TTC) of Ajwa dates. A significant positive correlation was recorded between the two variables on all tested bacteria; with *S. aureus* ($r = 0.8239$, $p = 0.0003$), *S. Typhi* ($r = 0.5775$, $p = 0.0254$), *S. Typhimurium* ($r = 0.5546$, $p = 0.0342$), *E. coli* ($r = 0.5225$, $p = 0.047$), *V. cholerae* ($r = 0.7476$, $p = 0.0022$), and *S. flexneri* ($r = 0.7645$, $p < 0.0015$).

A scatterplot of bacteria inhibition zone with TTC values of Ajwa dates is demonstrated in **Figure 5.6.** The increase in TTC values was correlated with the increase in bacterial inhibition zone. Overall, an excellent, positive correlation for *S. aureus* and *S. flexneri* was found between bacterial inhibition zone and TTC values of Ajwa dates. Similarly, this correlation was also found for *S. Typhi, S. Typhimurium, V. cholerae,* and *E. coli* between bacterial inhibition zone and the TTC of Ajwa dates.

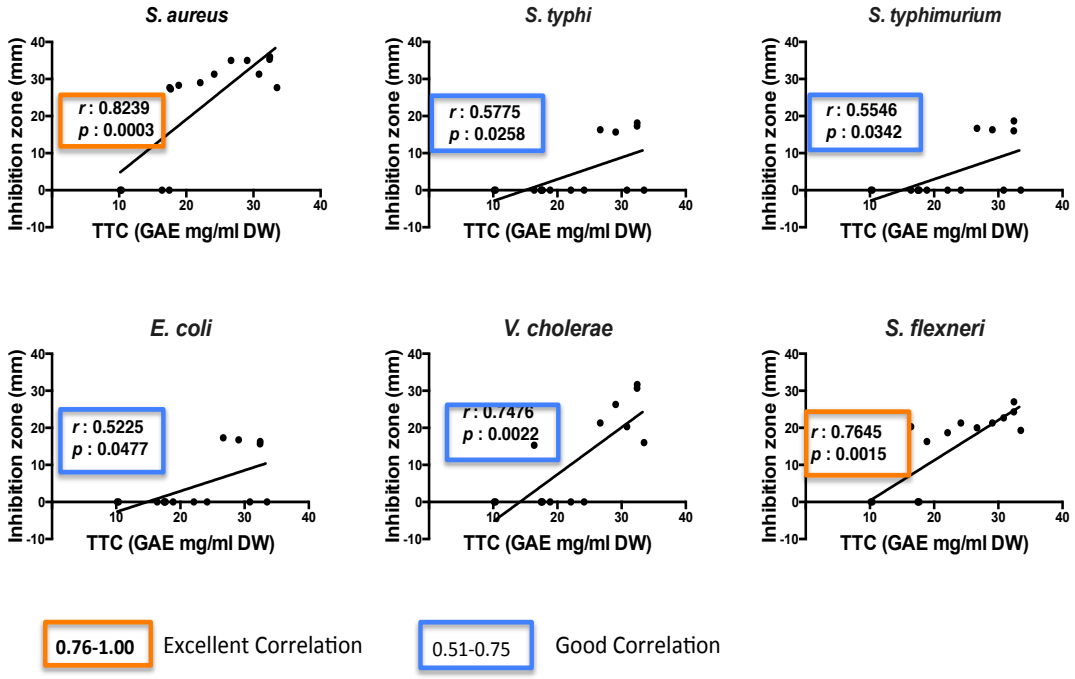

Figure 5.6: Scatterplot of each bacterial inhibition zone against TTC values. The spearman rank correlation graphs indicate the positive correlation between bacterial inhibition zone and TPC values.

5.4 Conclusion

In conclusion, preparation of Ajwa dates-infused water according to the Prophet Muhammad's (PBUH) practice, showed that 7 Ajwa dates exhibited antibacterial activity against all tested bacteria. In addition, there was also a positive correlation between phytochemical content in the Ajwa dates-infused water. And its antibacterial activity. Therefore, the antibacterial properties of Ajwa dates were possibly due to the presence of phenolic, tannin, and flavonoid.