CHAPTER 6 :COMPARISON OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL CONTENTS OF 3 TYPES OF DATE FRUITS VARIETIES

6.1 Introduction

The previous finding indicated that Ajwa date exhibited good antibacterial activity against all tested bacteria. Besides the Ajwa dates, over 5000 different date varieties are present, with one variety being well-known in the world market, such as Medjool and Deglet Noor (Amer, 1994). Each date fruit variety comprises a wide range of nutritional and phytochemical content.

Date fruits comprise good antibacterial activity. Therefore, phytochemical screening assay is a simple, fast, and inexpensive procedure, which swiftly provides an answer regarding various types of phytochemicals or secondary metabolites found in plants. According to Jeong et al. (2012), numerous analytical methods were developed, which might facilitate the structural determination of the bioactive compound, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), HPLC/electrospray ionisation tandem mass spectrometry (MS/MS), capillary electrophoresis, ion spray mass spectrometry (MS), gas chromatography/MS (GC/MS), and nuclear magnetic resonance (NMR). Mass spectroscopy provides highly specific chemical information, which is directly related to the chemical structures, such as accurate mass, isotope distribution patterns for elemental formula determination, and

characteristic fragment ions for structural elucidation or identification through spectral matching with authentic compound data. Moreover, the high sensitivity of MS allows the detection and measurement of picomole to femtomole levels of many primary and secondary metabolites (Sumner et al., 2003; Lei et al., 2011).

Accordingly, this chapter aims to compare the antimicrobial activities and phytochemical composition between three date varieties, namely Ajwa, Medjool, and Mariami, against bacterial gastroenteritis. The antibacterial activity was assessed using a well diffusion assay, MIC, MBC, and anti-adhesion assay. While the phytochemicals composition in date fruits extracts were identify using liquid chromatography, mass spectroscopy and the quantification of active phytochemicals are using a colorimetric assay.

6.2 Result

6.2.1 Antibacterial Screening by Well Diffusion Assay

Table 6.1 presents the data of inhibition zone diameter of cold aqueous extracts of Ajwa, Medjool, and Mariami dates against all tested bacteria. Antibacterial activity against all tested bacteria was found from three varieties of dates. While the diameters of inhibition zone for Ajwa date extracts ranged from 15.67 mm to 39.33 mm, Medjool dates ranged from 17.67 mm to 34.67 mm. Furthermore, Mariami dates ranged from 21.00 mm to 38.00 mm.

As for the cold aqueous extracts, four out of the six tested organisms, Mariami dates, showed the highest inhibition zones compared to Medjool and Mariami dates against *S. Typhimurium, S. flexneri, V. cholerae*, and *E. coli*. Ajwa dates showed the highest inhibition zone compared to Medjool and Mariami dates against *S. aureus* and *S. Typhi.* This was followed by the second highest inhibition zones, including the Ajwa dates against *S. Typhimurium, S. flexneri, and V. cholerae*, and Mariami dates against *S. aureus* and *S. Typhi.* Meanwhile, the lowest inhibition zones were Medjool dates against *S. aureus, S. Typhi*, *S. Typhimurium, S. flexneri,* and *V. cholerae*, also Ajwa dates against *E. coli.*

Significant differences were present between each date variety against all tested bacteria. The Dunn's test (post-hoc) analysis (**Figure 6.1**) found that Ajwa dates had significantly higher inhibition zone diameters compared to Medjool dates against *S. aureus* ($p = 0.0177$) and *S. Typhi* ($p = 0.0158$). Mariami dates also showed significantly higher inhibition zone compared to Medjool dates against *S. Typhimurium* ($p = 0.0370$), *S. flexneri* ($p = 0.0289$). Mariami also showed significantly higher inhibition zone

diameter compared to Ajwa against *E. coli* ($p = 0.0177$), while this significant difference was not present between the inhibition zone diameters of Ajwa and Mariami against *S. aureus, S. Typhi, S. Typhimurium,* and *E. coli* ($p > 0.05$).

Out of all the tested organisms, *S. aureus* was the most sensitive organism after the treatment with Ajwa, Medjool, and Mariami dates, which comprised the largest inhibition zone ranging from 34.67 to 39.33 mm. This was followed by *S. flexneri*, *V. cholerae, and S. Typhi*, and *E. coli*, which was the least sensitive organism to Ajwa dates and Mariami dates. *S. Typhimurium* were the least sensitive organisms to Medjool dates.

Table 6.1: Diameter of inhibition zone (mean ± standard deviation) of the cold aqueous extract against the tested bacteria using well diffusion assay method.

Bacteria	Inhibition zone (mm)			
	Ajwa (500mg/ml)	Medjool (500mg/ml)	Mariami (500mg/ml)	Gentamicin (1mg/ml)
S. aureus	39.33 (±0.58)	34.67 (±0.58)	38.00 (±0.00)	36.33 (±0.58)
S. Typhi	24.00 (±0.00)	19.50 (±0.58)	21.00 (±0.00)	28.00 (±1.15)
S. Typhimurium	19.67 (±0.58)	17.33 (±0.58)	20.33 (±0.58)	27.33 (±0.58)
S. flexneri	29.00 (±1.00)	27.67 (±0.58	31.67 (±0.58)	26.33 (±0.58)
V. cholerae	26.67 (±0.58)	24.00 (±0.00)	27.00 (±0.00)	27.33 (±0.58)
E. coli	15.67 (±0.58)	17.67 (±0.58)	21.00 (±0.00)	27.33 (±0.58)

*Inhibition zone included 7mm well hole

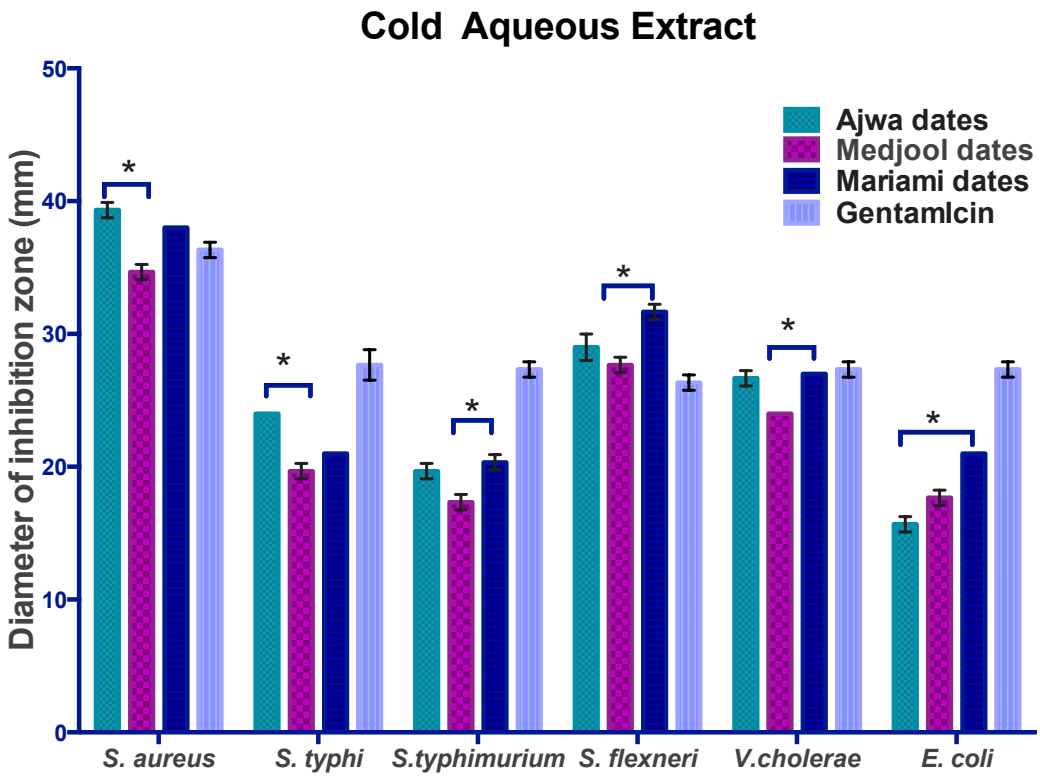

Figure 6.1: Diameter of inhibition zone of Ajwa, Medjool, and Mariami cold aqueous extract against all bacteria using well diffusion assay method. The asterisk* represents the significant value (*$p < 0.05$) between the date varieties.

Table 6.2 presents the data of inhibition zone diameter of hot aqueous extracts of Ajwa, Medjool, and Mariami dates against all tested bacteria. The extracts of all three dates variety were recorded with the antibacterial activity against all tested bacteria. The diameters of inhibition zone for Ajwa date extracts ranged from 18.33 mm to 38.33 mm, while the Medjool date extracts ranged from 17.33 mm to 41.33 mm. Mariami date extracts ranged from 19.33 to 40.33 mm.

In the case of the hot aqueous extracts, three out of six tested organisms, Ajwa dates, were showed with the largest inhibition zones compared to Medjool and Mariami dates against *S. Typhi, V. cholerae,* and *E. coli*. Mariami was showed with the highest inhibition zones against *S. Typhimurium* and *S. flexneri*, while Medjool presented the highest inhibition zones against *S. aureus.* This was followed by the second largest

inhibition zones, namely Mariami dates against *S. aureus, S. Typhi, V. cholerae,* and *E. coli*, including the Ajwa dates against *S, Typhimurium* and *S. flexneri.* Meanwhile, the smallest inhibition zones were the Medjool dates against *S. Typhi*, *S. Typhimurium, S. flexneri,* and *V. cholerae*, including Ajwa dates against *S. aureus* and *S. flexneri.*

Significant differences were recorded between the date varieties against all tested bacteria. The Dunn's test (post-hoc) analysis (see **Figure 6.2**) found that the Ajwa dates comprised significantly larger inhibition zone diameters compared to the Medjool dates against *S. Typhi* ($p = 0.0252$), *E. coli* ($p = 0.0197$), and *V. cholerae* ($p = 0.0289$). Similarly, Mariami dates showed a significantly larger inhibition zone diameter compared to Medjool dates against *S. Typhimurium* ($p = 0.0394$). However, this difference was not present between the inhibition zone diameters of Ajwa and Mariami dates against all tested bacteria ($p > 0.005$), including the inhibition zone diameter between Ajwa and Medjool dates against *S. Typhimurium,* and *S. flexneri* ($p > 0.005$).

Out of all the tested organisms, *S. aureus* was the most sensitive organism after the treatment with Ajwa , Medjool, and Mariami dates with the highest inhibition zone ranging from 38.33 to 41.33 mm. This was followed by *S. flexneri*, *V. cholerae, E. coli,* and *S. Typhi*, with the *S. Typhimurium* being the least sensitive organism to Ajwa, Medjool, and Mariami dates.

Table 6.2: Diameter of inhibition zone (mean ± standard deviation) of the hot aqueous extract against the tested bacteria using well diffusion assay method.

Bacteria	Inhibition zone (mm)			
	Ajwa (500mg/ml)	Medjool (500mg/ml)	Mariami (500mg/ml)	Gentamicin (1mg/ml)
S. aureus	38.33 (±0.58)	41.33 (±0.58)	40.33 (±0.58)	36.33 (±0.58)
S. Typhi	22.50 (±0.58)	19.00 (±0.00)	21.00 (±0.58)	28.00 (±1.15)
S. Typhimurium	18.33 (±0.58)	17.33 (±0.58)	19.33 (±0.58)	27.33 (±0.58)
S. flexneri	29.67 (±0.58)	30.33 (±0.58)	31.00 (±0.00)	26.33 (±0.58)
V. cholerae	28.67 (±1.53)	24.67 (±0.58)	26.67 (±0.58)	27.33 (±0.58)
E. coli	24.33 (±0.58)	20.33 (±0.58)	22.67 (±0.58)	27.33 (±0.58)

*Inhibition zone included 7mm well hole

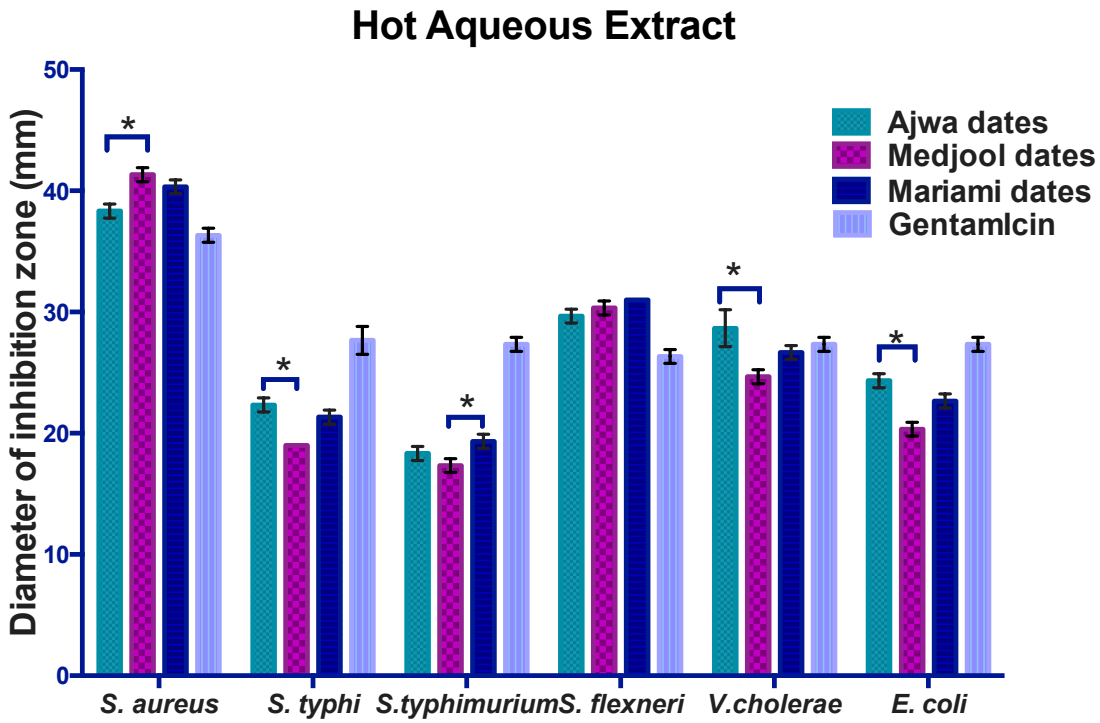

Figure 6.2: Diameter of inhibition zone of Ajwa, Medjool, and Mariami hot aqueous extract against all bacteria using well diffusion assay method. Asterisk above the bar represents significant value (*p <0.05) between dates varieties.

Table 6.3 presents the data of the inhibition zone diameter of the methanol extracts of Ajwa, Medjool, and Mariami dates against all tested bacteria. The variety extracts of all three dates were recorded with antibacterial activity against all the tested bacteria. It was found that the diameters of inhibition zone for Ajwa date extract ranged from 19.67 to 41.33 mm, while the Medjool date extract ranged from 19.33 to 41.33 mm. Besides, Mariami date extract ranged from 20.00 to 41.0 mm.

As for the methanol extracts, Ajwa and Medjool dates showed similar inhibition zone of 41.33 mm against *S. aureus*. Similarly, Ajwa and Mariami dates exhibited similar inhibition zones of 29.33 mm against *S. flexneri*. Four out of the six tested organisms, Ajwa dates, showed the largest inhibition zones compared to Medjool and Mariami dates against *S. aureus, S. Typhi, V. cholerae,* and *E. coli*, while Mariami dates exhibited the highest inhibition zones against *S. Typhimurium*. Furthermore, Medjool dates appeared with the largest inhibition zones against *S. aureus* and *S. flexneri*, followed by the second largest inhibition zones, namely Mariami dates against *S. aureus, S. Typhi,* and *V. cholerae*, including Ajwa dates against *S. Typhimurium and S. flexneri.* The smallest inhibition zones were observed from Mariami dates against *S. aureus* and *E. coli*, including Medjool dates against S. *typhi. V. cholerae*, and *S. Typhimurium.*

Significant differences were present between each date variety against all tested bacteria. It was found from the Dunn's test (post-hoc) analysis (see **Figure 6.3**) that Ajwa dates comprised significantly larger inhibition zone diameter compared to Medjool dates against *S. Typhi* ($p < 0.0001$). The same result was recorded from Ajwa

dates compared to Mariami dates against *E. coli* ($p = 0.0003$). However, there was no significant difference between the inhibition zone diameters of Ajwa, Medjool, and Mariami dates against *S. aureus, S. Typhimurium, S. flexneri,* and *E. coli* ($p > 0.005$).

Overall, *S. aureus* was the most sensitive organism after the treatment with Ajwa, Medjool, and Mariami dates as its largest inhibition zone ranged from 41.00 to 41.33 mm. This was followed by *S. flexneri*, *V. cholerae, E. coli,* and *S. Typhi*, while *S. Typhimurium* was the least sensitive organism to Ajwa, Medjool, and Mariami methanol extracts.

Table 6.3: Diameter of inhibition zone (mean ± standard deviation) of methanol extract against the tested bacteria using well diffusion assay method.

Bacteria	Inhibition zone (mm)			
	Ajwa (500mg/ml)	Medjool (500mg/ml)	Mariami (500mg/ml)	Gentamicin (1mg/ml)
S. aureus	41.33 (±0.58)	41.33 (±0.58)	41.00 (±0.00)	36.33 (±0.58)
S. Typhi	25.00 (±0.00)	20.50 (±0.58)	24.50 (±0.58)	28.00 (±1.15)
S. Typhimurium	19.67 (±0.58)	19.33 (±0.58)	20.00 (±2.00)	27.33 (±0.58)
S. flexneri	29.33 (±1.15)	30.33 (±0.58)	29.33 (±0.58)	26.33 (±0.58)
V. cholerae	27.33 (±0.58)	25.67 (±0.58)	26.67 (±0.58)	27.33 (±0.58)
E. coli	25.33 (±0.58)	25.00 (±0.00)	21.67 (±0.58)	27.33 (±0.58)

*Inhibition zone included 7mm well hole

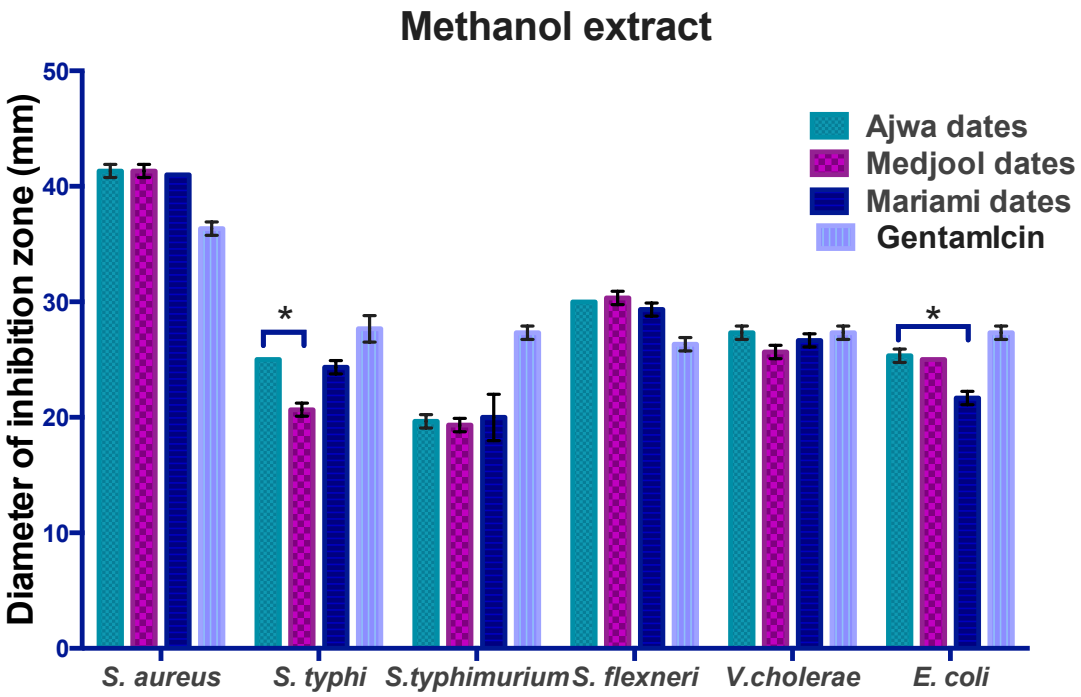

Figure 6.3: Diameter of inhibition zone of Ajwa, Medjool, and Mariami methanol extract against all bacteria using well diffusion assay method. Asterisk* above bar represents significant value (*$p < 0.05$) between date varieties

Kruskal-Wallis test was performed to compare bacteria inhibition zones with control (gentamicin), as shown in **Figure 6.4**. Based on the result, Ajwa methanol extract, Mariami hot aqueous, and Mariami methanol extracts showed significantly larger inhibition zone compared to control (gentamicin) against S. *aureus* ($p < 0.05$). Meanwhile, Ajwa methanol extract, Medjool hot aqueous, Medjool methanol extracts, and Mariami cold aqueous extracts were recorded with the same result compared to the control against *S. flexneri* ($p < 0.05$). However, there was no significant difference between the diameters of inhibition zone, which were significantly smaller compared to the control against *S. Typhi, S. Typhimurium, V. cholerea,* and *E. coli.*

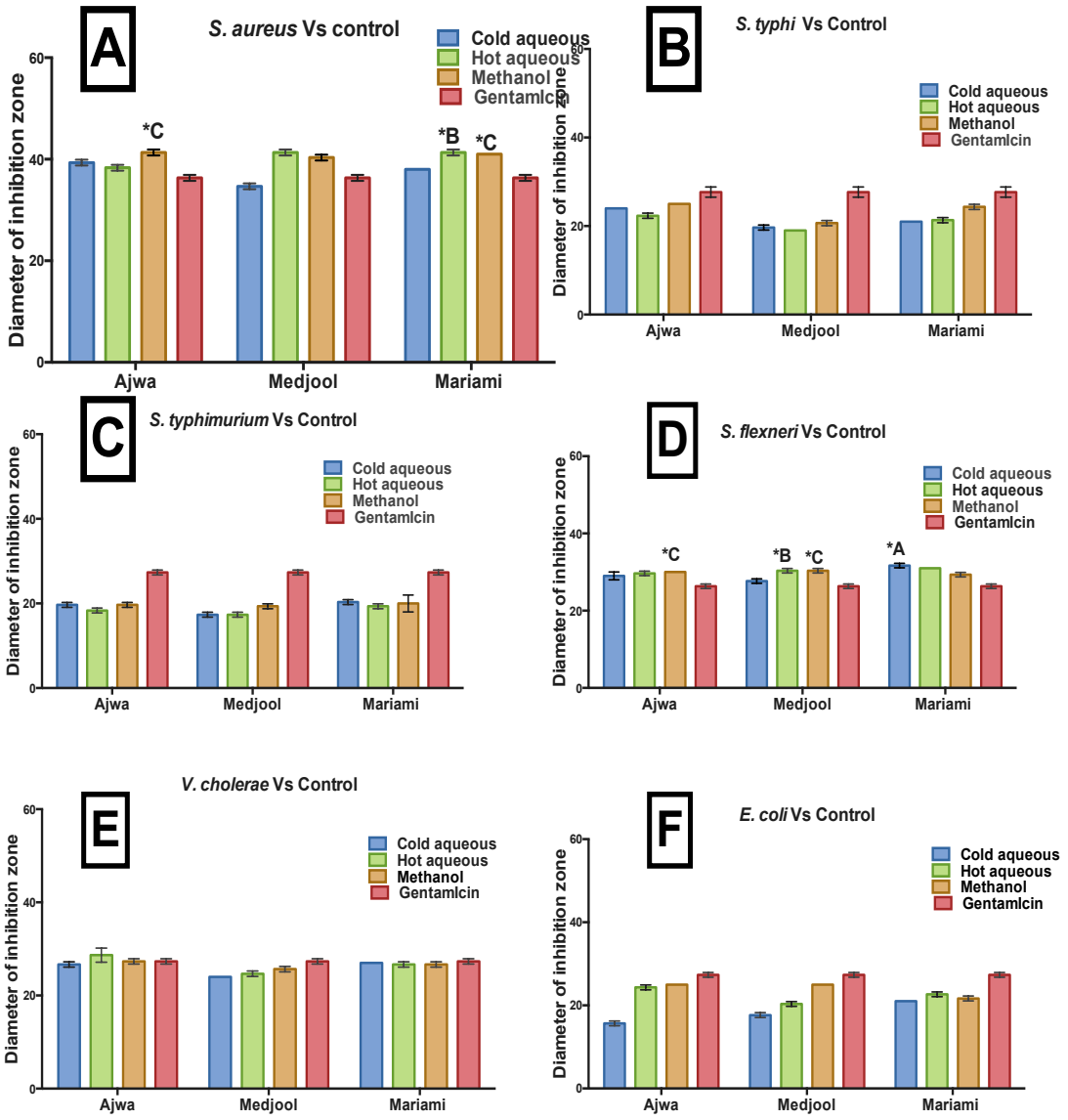

Figure 6.4: Diameter of Inhibition Zone of Date Fruits Extract Against All Bacteria Using Well Diffusion Assay Method. Asterisk* Above Bar Represents Significant Value (*p<0.05) Between Treatment and Control(Gentamicin). **A**- Cold aqueous extract vs control, **B**- Hot aqueous extract vs control **C**- Methanol extract vs control

6.2.2 Minimum Inhibitory Concentration (MIC)

The MIC values for Ajwa, Medjool, and Mariami date are presented in **Table 6.4**, in which the MIC for Ajwa, Medjool, and Mariami cold aqueous extract ranged from 500 mg/ml to 1000 mg/ml. The hot aqueous extract ranged from 250 mg/ml to 500 mg/ml, while the methanol extract ranged from 250 mg/ml to 500 mg/ml.

As for the cold aqueous extract, the lowest MIC values for all date varieties was at 500 mg/ml, while the lowest MIC values for Ajwa dates at 500 mg/ml against *V. cholerae* and *E. coli.* Medjool dates had the lowest MIC values of 500 mg/ml against *V. cholerae*, while the lowest MIC values for Mariami dates was 500 mg/ml against *E. coli*. Besides, the MIC value for Medjool and Mariami date were equal with 1000 mg/ml against *S. Typhi* and *S. flexneri*. The MIC value for Ajwa and Medjool date was 500 mg/ml against *V. cholerae.* Notably, Ajwa date MIC value could not be determined against *S. Typhi* and *S. flexneri*, while the MIC value of Medjool date could not be determined against *S. Typhimurium* and *E. coli.*

As for hot aqueous extract, the lowest MIC for all date varieties was at 250 mg/ml, while the lowest MIC values for Ajwa dates was 250 mg/ml against *S. aureus, V. cholerae,* and *E. coli.* The lowest MIC values for Medjool dates was at 250 mg/ml against *S. aureus* and *V. cholerae*, while the lowest MIC values for Mariami extract was at 250 mg/ml against *E. coli.* Equal MIC values were recorded for Ajwa, Medjool, and Mariami date at 500 mg/ml against *S. Typhi*, *S. Typhimurium*, and *S. flexneri.*

In the case of the methanol extract, the lowest MIC for all date varieties was at 250 mg/ml, while the MIC values for Ajwa, Medjool, and Mariami were equal with a concentration of 250 mg/ml against *S. aureus* and *V. cholerae.* Ajwa dates lowest MIC values were at 250mg/ml against *S. aureus, V. cholerae* and *E. coli.* As for the Medjool dates, the lowest MIC values was at 250 mg/ml against *S. aureus*, *S. Typhi, S. flexneri*,

and *V. cholerae*. The lowest MIC values for Mariami dates was at 250 mg/ml against *S. aureus, S. flexneri*, and *V. cholerae.*

Table 6.4: Minimum Inhibitory Concentrations (MIC) for Ajwa, Medjool, and Mariami dates extracts. The MIC values are presented as mg/ml of three replicates.

Test organism	Minimum inhibitory concentration (MIC)								
	Cold aqueous extract			Hot aqueous extract			Methanol extract		
	AJ	MJ	MR	AJ	MJ	MR	AJ	MJ	MR
S. aureus	1000	1000	1000	250	250	500	250	250	250
S. Typhi	ND	1000	1000	500	500	500	500	250	500
S. Typhimurium	1000	ND	1000	500	500	500	500	500	500
S. flexneri	ND	1000	1000	500	500	500	500	250	250
V. cholerae	500	500	1000	250	250	500	250	250	250
E. coli	500	ND	500	250	ND	250	250	500	500

N.D.=Not determined AJ-Ajwa dates, MJ-Medjool dates, MR-Mariami dates.

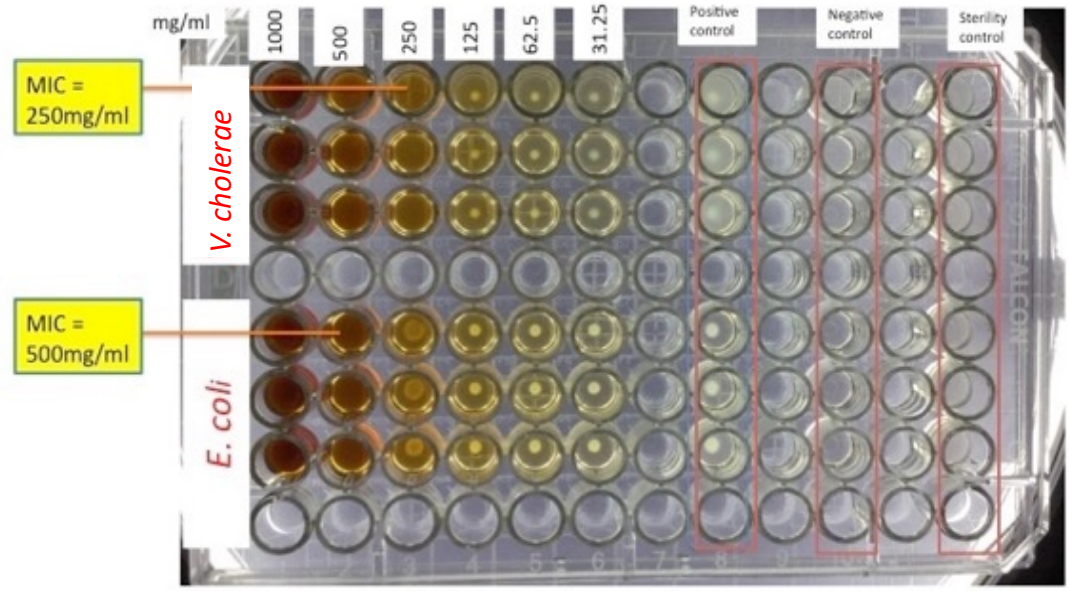

Figure 6.5: MIC determination using 96 wells plate wells, visible no growth considered as MIC values.

6.2.3 Minimum Bactericidal Concentration (MBC)

Table 6.5 presents the MBC values for Ajwa, Medjool, and Mariami dates against all tested bacteria. The MBC was found to have the lowest concentration required to kill a particular organism (**Figure 6.6**). The MBC values of Ajwa, Medjool, and Mariami dates ranged from 500 mg/ml to 1000 mg/ml for cold aqueous, 250 mg/ml to 500 mg/ml for hot aqueous, and 250 mg/ml to 500 mg/ml for methanol extract against all tested bacteria.

In the case of cold aqueous extract, the lowest MBC of Ajwa extract was at 500mg/ml against *V. cholerae and E. coli.* The lowest MBC of Medjool amounted to 500 mg/ml against *V. cholerae,* while the lowest MBC of Mariami extract was recorded at 500 mg/ml against *E. coli*. Furthermore, the MBC value for Ajwa, Medjool and Mariami date was equal at 1000 mg/ml against *S. aureus*, while the MBC value for Medjool and Mariami date was equal at 1000 mg/ml against *S. Typhi* and *S. flexneri*. Besides, the MBC value for Ajwa and Medjool date was equal at 500 mg/ml against *V. cholerae*. However, this value for Ajwa date could not be determined against *S. Typhi* and *S. flexneri*, including the MBC value of Medjool date against *S. Typhimurium* and *E. coli.*

As for the hot aqueous extracts, the lowest MBC value of Ajwa extract was at 250 mg/ml against *S. aureus* and *E. coli*, while the lowest MBC of Medjool extract was at 250 mg/ml against *S. aureus* and *V. cholerae*. The lowest MBC of Mariami extract was 250 mg/ml against *E. coli*, while this value for Ajwa, Medjool, and Mariami dates was equal at 500 mg/ml against *S. Typhi, S. Typhimurium*, and *S. flexneri*. While this value of Ajwa and Medjool dates was equal at 250 mg/ml against *S. aureus*, it was equal at 250 mg/ml against *V. cholerae* for Ajwa and Mariami dates. However, the MBC of Medjool date could not be determined against *E. coli.*

As for the methanol extracts, the lowest MBC value of Ajwa extract was at 250 g/ml against *S. aureus*, *V. cholerae*, and *E. coli.* The lowest MBC value of Medjool extract was 250mg/ml against *S. aureus*, *S. Typhi, S. flexneri*, and *V. cholerae*, while the lowest MBC value of Mariami extract was 250 mg/ml against *S. aureus, S. flexneri*, and *V. cholerae.* Furthermore, the MBC value for Ajwa, Medjool, and Mariami dates was equal at 250 mg/ml against *S. aureus* and *V. cholerae*. Similar MBC value at 500 mg/ml was acquired for Ajwa, Medjool, and Mariami dates against *S. Typhimurium*. The MBC value for Ajwa and Mariami was equal at 500 mg/ml against *S. Typhi*, followed by the value for Medjool and Mariami against *S. flexneri* and *E. coli* at 250 mg/ml and 500 mg/ml, respectively.

Table 6.5: Minimum Bactericidal Concentrations (MBCs) for Ajwa, Medjool, and Mariami date extracts. The MBC values presented in mg/ml of three replicates.

Test organism	Minimum Bactericidal Concentration (MBC)								
	Cold aqueous extract			Hot aqueous extract			Methanol extract		
	AJ	MJ	MR	AJ	MJ	MR	AJ	MJ	MR
S. aureus	1000	1000	1000	250	250	500	250	250	250
S. Typhi	ND	1000	1000	500	500	500	500	250	500
S. Typhimurium	1000	ND	1000	500	500	500	500	500	500
S. flexneri	ND	1000	1000	500	500	500	500	250	250
V. cholerae	500	500	1000	500	250	500	250	250	250
E. coli	500	ND	500	250	ND	250	250	500	500
N.D.=Not determined AJ-Ajwa dates, MJ-Medjool dates, MR-Mariami dates.									

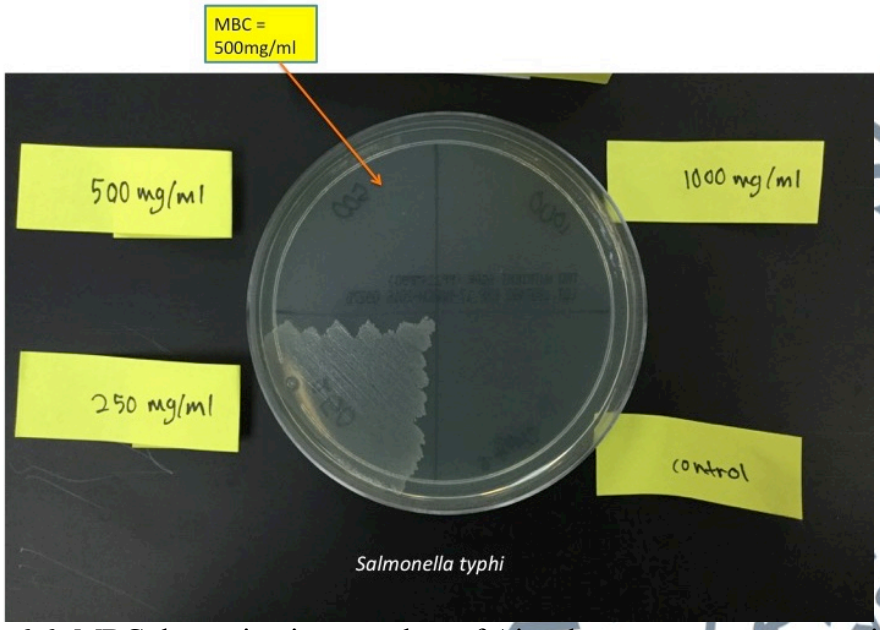

Figure 6.6: MBC determination on plate of Ajwa hot aqueous extract against *S. Typhi*, MBC showed at 500mg/ml.

6.2.4 Bacterial Adhesion Assay

Ajwa, Medjool, and Mariami date extracts were recorded with anti-adhesion activity against all tested bacteria. The bacterial adhesion to Caco-2 cells incubated with date fruits extracts was compared with the untreated control. Furthermore, the data of anti-adhesion activity for cold and cold aqueous extracts and methanol extracts for Ajwa, Medjool, and Mariami dates are presented in **Table 6.6**, **Table 6.7**, and **Table 6.8**. All bacteria showed the relative percentage of bacteria adhesion to Caco-2 cells ranging from 5.15% to 92.35% after the treatment with cold aqueous extract, 4.84% to 72.22% after the treatment with hot aqueous extract and 14.48% to 82.22% after the treatment with methanol extracts of Ajwa, Medjool, and Mariami dates. The best anti-adhesion activity of dates extracts was presented by the lowest relative percentage of bacteria adhesion to caco-2 cells.

As for the cold aqueous extract, Ajwa dates were recorded with the highest anti-adhesion activity on three out of six tested organisms with *S. aureus, V. cholerae* and *S. flexneri.* Medjool dates also recorded with the highest anti-adhesion activity on three out of six tested organisms against *S. Typhimurium, S. Typhi,* and *E. coli.* The second highest anti-adhesion activity was Mariami dates against *S. aureus*, *V. cholerae,* and *S. flexneri,* including Ajwa dates against *S. Typhi, S. Typhimurium,* and *E. coli*. Notably, the lowest anti-adhesion activity was found in Medjool dates against *S. aureus* and *S. Typhi*, including Mariami dates against S. *Typhimurium, S. flexneri, V. cholerae,* and *E. coli*.

Significant differences were present between each date variety against all the tested bacteria. As seen in Dunn test **Figure 6.7**, the post-hoc analysis found that the relative percentage of bacterial adhesion to caco-2 cells was significantly lower after the treatment with Ajwa dates extract compared to Medjool date extracts on *S. flexneri* (p = 0.0219). The percentage of bacterial adhesion to Caco-2 cells was significantly lower after the treatment with Medjool dates extract compared to Mariami date extracts on *S. Typhimurium* (p = 0.0219). Meanwhile, the percentage of bacterial adhesion to Caco-2 cells was lower after the treatment with Ajwa dates compared to Mariami dates extracts against *S. aureus, S. Typhi, S. Typhimurium, V. cholerae,* and *E. coli*, although no significant difference was present between Ajwa and Mariami.

Table 6.6: Relative percentage of bacterial adhesion to Caco-2 cell monolayer after treatment with 100 mg/ml of cold aqueous extract of Ajwa, Medjool and Mariami date. Data are given as mean percentage ± SD of three samples.

Bacteria	The relative percentage of bacterial adhesion (%)		
	Ajwa	Medjool	Mariami
S. aureus	37.65 (±4.79)	92.35 (±32.97)	53.74 (±17.08)
S. Typhi	37.74 (±8.70)	28.93 (±8.50)	42.82 (±3.92)
S. Typhimurium	34.24 (±3.52)	28.93 (±8.50)	48.18 (±10.78)
S. flexneri	18.17 (±6.01)	73.89 (±23.35)	77.22 (±5.93)
V. cholerae	5.15 (±0.59)	8.41 (±2.15)	10.62 (±3.85)
E. coli	56.65 (±0.28)	55.98 (±2.42)	74.39 (±5.93)

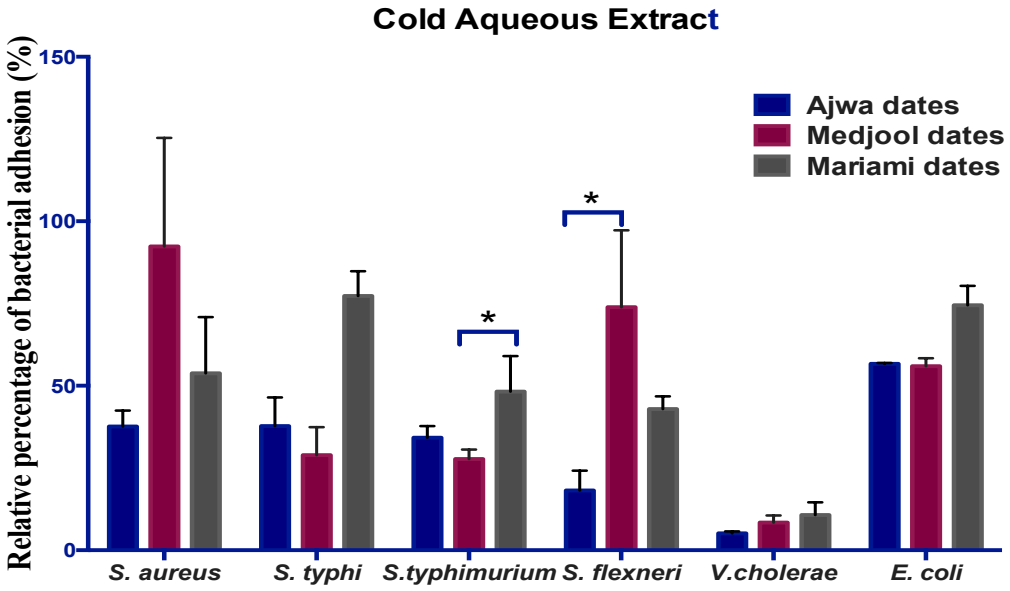

Figure 6.7: Relative percentage of bacterial adhesion to Caco-2 cell monolayer after treatment with 100 mg/ml of Ajwa, Medjool, and Mariami cold aqueous extracts. Asterisk* above the bar indicates a significant difference (*$p < 0.05$) between each date type.

For the hot aqueous extract, Ajwa dates were recorded with the highest anti-adhesion activity on five out of six tested organisms with *S. aureus, S. Typhimurium, S. flexneri, E. coli,* and *V. cholerae* with lowest relative percentage of bacterial adhesion to caco-2 cells after the treatment with Ajwa dates extracts. Mariami dates were also recorded with the highest anti-adhesion activity against *S. Typhi.* The second highest anti-adhesion activity was Ajwa dates against *S. Typhi* and Medjool dates against *S. aureus, S. typhimurium, S. flexneri, V. cholerae,* and *E. coli.* The lowest anti-adhesion was the Mariami dates against *S. aureus, S. typhimurium, S. flexneri, V. cholerae,* and *E. coli.*

Significant differences were present between each date variety against all tested bacteria. The Dunn's test (post-hoc) analysis (see **Figure 6.8**) found that the relative percentage of bacterial adhesion to Caco-2 cells was significantly lower after the treatment with Ajwa dates compared to Medjool and Mariami dates against *S. Typhimurium* ($p < 0.05$), *S. flexneri* ($p < 0.05$), and *V. cholerae* ($p < 0.05$). Meanwhile, Medjool extracts showed a significantly lower percentage of bacterial adhesion to Caco-2 cells compared to Mariami extract against *S. Typhimurium* ($p = 0.0196$) and *S. flexneri* ($p = 0.0192$). There was no significant difference between Ajwa, Medjool, and Mariami extracts against *S. aureus, S. Typhi,* and *E. coli.*

Table 6.7: Relative percentage of bacterial adhesion to Caco-2 cell monolayer after the treatment with 100 mg/ml of hot aqueous extract of Ajwa, Medjool, and Mariami date. The data are presented as the mean percentage ± SD of three samples.

Bacteria	The relative percentage of bacterial adhesion (%) - hot		
	Ajwa	Medjool	Mariami
S. aureus	19.11 (±7.57)	40.68 (±10.31)	58.97 (±29.99)
S. Typhi	40.28 (±5.04)	48.23 (±10.07)	34.43 (±9.04)
S. Typhimurium	18.21 (±8.61)	40.56 (±2.99)	60.27 (±5.87)
S. flexneri	21.06 (±3.87)	42.22 (±6.94)	72.22 (±14.37)
V. cholerae	4.84 (±0.58)	7.63 (±0.87)	8.74 (±0.11)
E. coli	29.75 (±13.33)	37.57 (±13.18)	40.39 (±15.20)

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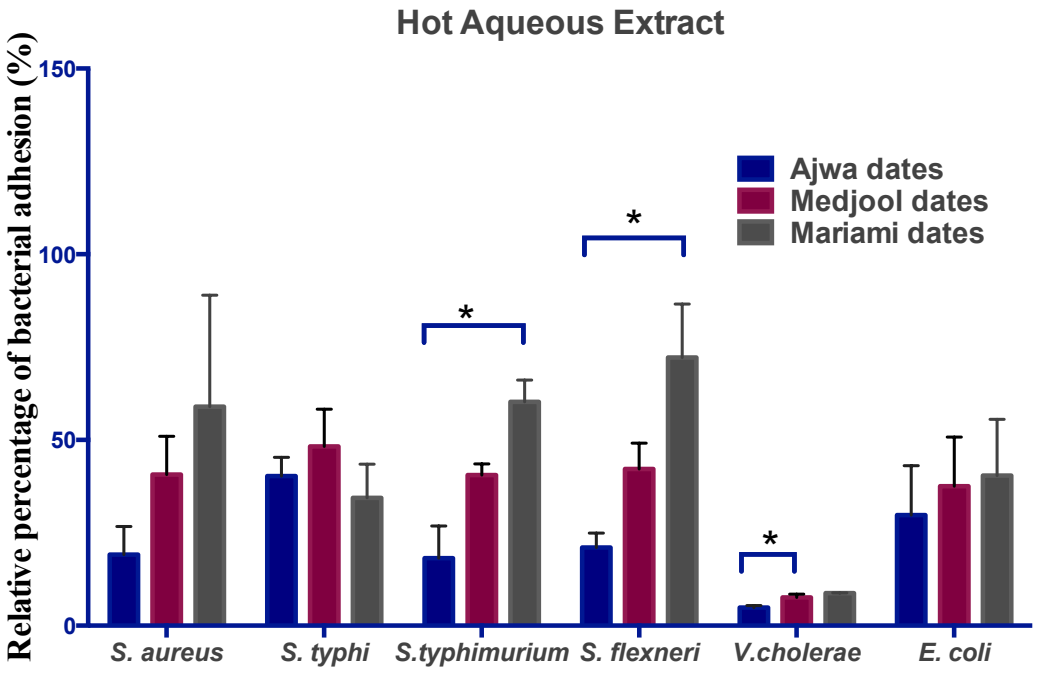

Figure 6.8: The relative percentage of bacterial adhesion to Caco-2 cell monolayer after treatment with 100 mg/ml of Ajwa, Medjool, and Mariami hot aqueous extracts. Asterisk* above bar indicates a significant difference (*$p < 0.05$) between each dates type.

In the case of the methanol extract, Mariami dates were recorded with the highest anti-adhesion activity on three out of six tested organisms with *S. Typhi* and *V. cholerae*, while *E. coli* was recorded with the relative percentage of bacterial adhesion to caco-2 cells after treated with Ajwa dates extracts. Medjool dates also showed the highest anti-adhesion activity against *S. aureus, S. Typhimurium and S. flexneri*, while the second-highest anti-adhesion activity took place in Ajwa dates against *S. aureus*, *S. Typhi, S. Typhimurium, S. flexneri*, and *E. coli*, including Medjool dates against *V. cholerae.* The lowest anti-adhesion activity took place in Mariami dates against *S. aureus, S. Typhimurium,* and *S. flexneri*, including Medjool dates against *V. cholerae* and Ajwa dates against *E. coli.*

Significant differences were present between each date variety against all tested bacteria. Dunn's test (post-hoc) analysis (see **Figure 6.9**) found that the relative percentage of bacterial adhesion to Caco-2 cells was significantly lower after the treatment with Ajwa dates and Medjool dates compared to Mariami dates against *S. aureus* ($p < 0.05$), *S. Typhimurium* ($p < 0.05$), and *S. flexneri* ($p < 0.05$). However, this difference was not present between Ajwa, Medjool, and Mariami extracts against *S. Typhi, V. cholerae*, and *E. coli*.

Table 6.8: Relative percentage of bacterial adhesion to Caco-2 cell monolayer after treatment with 100mg/ml of methanol extract of Ajwa, Medjool and Mariami date. Data are given as mean percentage ± SD of three samples.

Bacteria	The relative percentage of bacterial adhesion (%) - methanol		
	Ajwa	Medjool	Mariami
S. aureus	58.47 (±1.85)	41.47 (±7.74)	82.22 (±10.57)
S. Typhi	31.71 (±9.28)	42.04 (±14.83)	42.82 (±3.92)
S. Typhimurium	33.19 (±5.98)	25.93 (±3.30)	51.69 (±4.68)
S. flexneri	45.66 (±5.09)	32.11 (±6.11)	81.67 (±10.41)
V. cholerae	18.20 (±4.65)	17.04 (±9.40)	14.48 (±2.09)
E. coli	61.01 (±12.24)	64.80 (±4.54)	55.59 (±5.75)

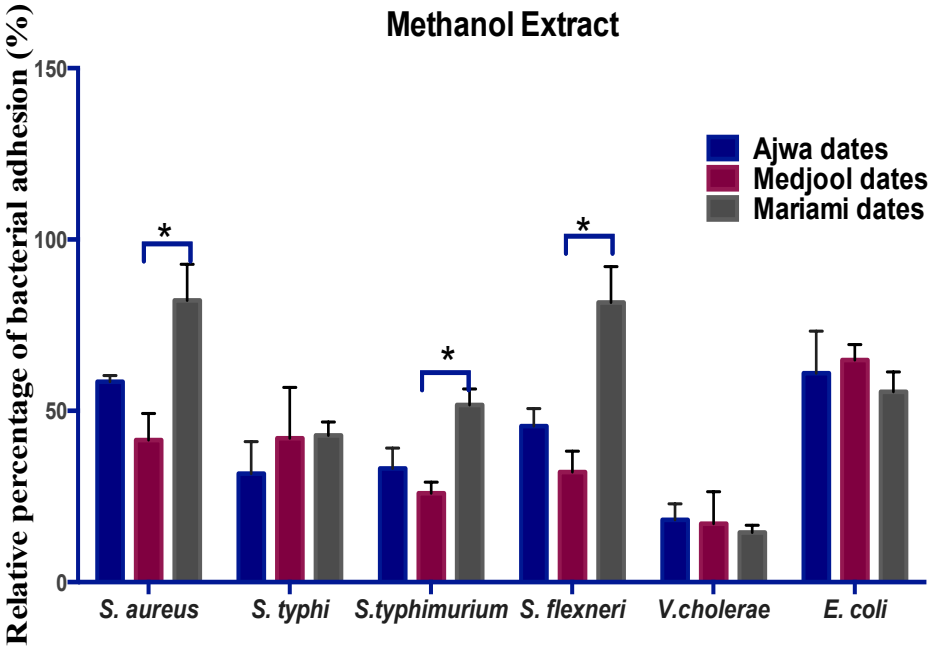

Figure 6.9: Relative percentage of bacterial adhesion to Caco-2 cell monolayer after treatment with 100 mg/ml of Ajwa, Medjool, and Mariami methanol extracts. Asterisk* above bar indicates a significant difference (*p< 0.05) between each dates type.

6.2.5 Screening of Active Compound in Date Fruits Extracts Using UHPLC-ESI-QTOF-MS/MS

From the combination of hot aqueous, cold aqueous, and methanol extracts, Ajwa dates detected a total of 94 compound peaks (see **Appendix 1 - 3**), while Mariami dates detected a total of 78 compound peaks (see **Appendix 4 - 6**), and Medjool detected a total of 83 compound peaks (see **Appendix 7 - 9**). After the removal of the same compound mass peaks on all type of extracts, Ajwa dates showed the highest compound peaks, with 78 compound peaks being detected compared to the Mariami dates, which detected 45 compounds peaks. Additionally, only 46 compounds peaks were detected in Medjool dates.

Sciex internal natural product database tentatively characterised 16 compounds in Ajwa date (see **Appendix 1 - 3**), 11 compounds in Mariami (see **Appendix 4 - 6**), and 12 compounds in Medjool dates (see **Appendix 7 - 9**). Other compounds were also characterised using cross-referencing with literature and internal database. A total of 12 phytochemical compounds were tentatively proposed in Ajwa dates (see **Appendix 1 - 3**), followed by four compounds in Mariami dates (see **Appendix 4 - 6**), and two compounds in Medjool dates (see **Appendix 7 - 9**). These compounds were classified into a group consisting of phenolic and its derivatives, flavonoids, fatty acid, organic acid, and terpenoids. However, other compounds peaks were unknown.

6.2.5.1 Identification of Phytochemical compound in Ajwa dates Hot aqueous extract.

Figure 7.1 presents the full chromatograph of Ajwa dates hot aqueous extract, while **Appendix 1** presents the list of phytochemicals detected in Ajwa dates hot aqueous extract. Out of the 39 compound peaks detected in these extracts, 16 compound peaks were identified, specifically the flavonoid and it is derivative, including apigenin (peak 13), isoquercitrin isomer (peak 24), Hexosyl luteolin sulfate (peak 27), and chrysoeriol hexadecyl sulfate (peak 28). Phenolic and its derivatives, such as punicalagin isomer (peak 21 and 23) also identified in these extracts. Other compounds, such as organic acids, fatty acids, and sugar, were identified in this extract, although other peaks were unknown.

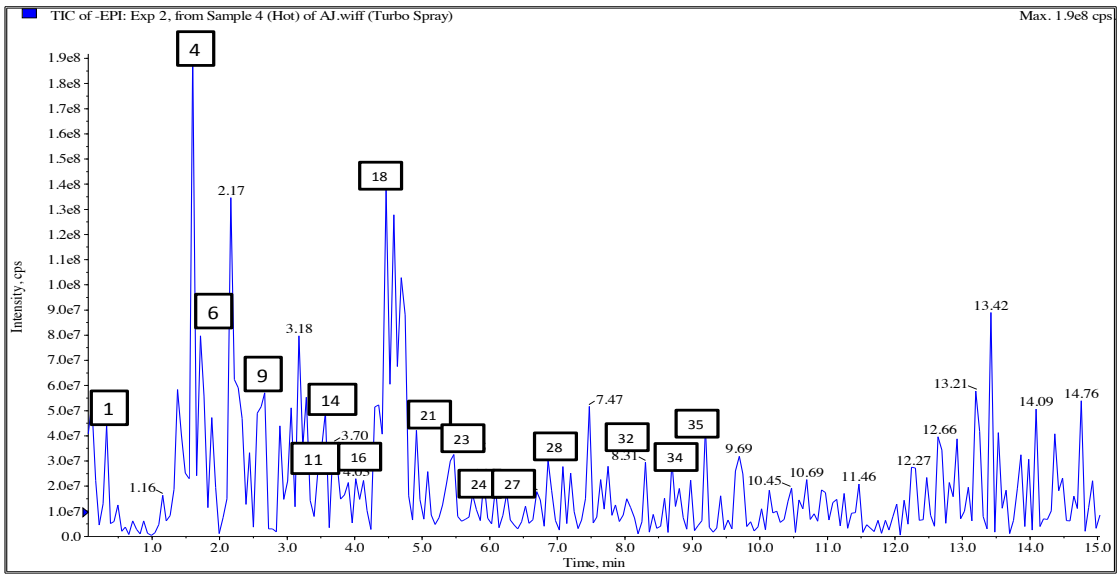

Figure 6.10: Full chromatogram of phytochemical compounds detected in Ajwa dates cold aqueous extract by UHPLC-ESI-QTOF-MS/MS

6.2.5.2 Identification of Phytochemical Compound in Ajwa Dates Cold aqueous extract

Figure 7.2 illustrates the full chromatograph of Ajwa dates cold aqueous extract, while **Appendix 2** presents the list of phytochemicals detected in Ajwa dates cold aqueous extract. Out of the 29 compound peaks detected in these extracts, 14 compound peaks were identified, which included phenolic acids and derivatives, such as caffeic acid derivative (peak 3, 6, 9, and 10). Meanwhile, punicalagin (peak 14) identified flavonoid and derivatives, such as chrysoeriol hexadecyl sulfate (peak 18) identified in this extract. Other compounds, such as fatty acids, were also identified in this extract, although other compound peaks were unknown.

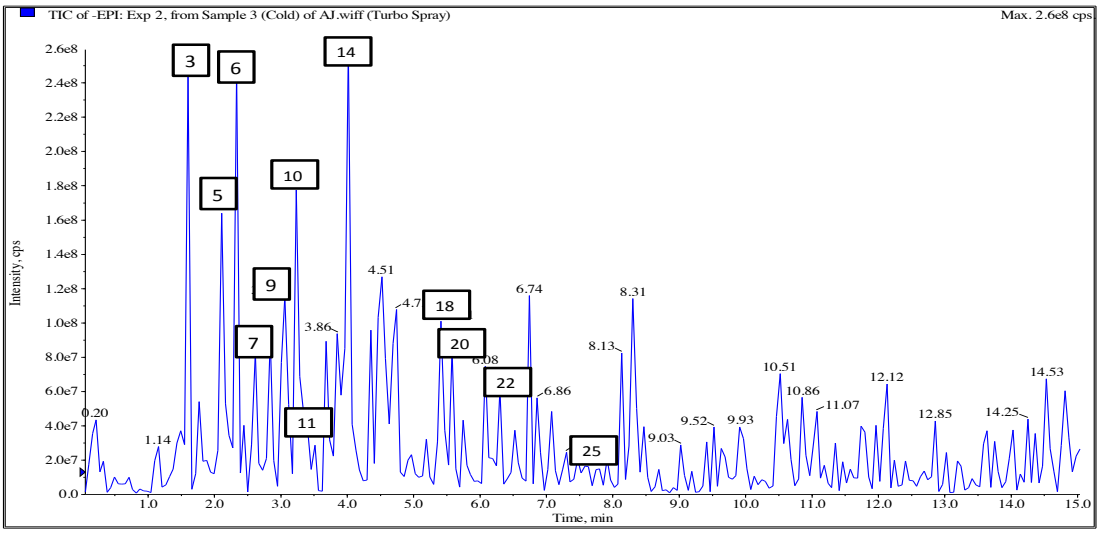

Figure 6.11: Full chromatogram of phytochemical compounds detected in Ajwa dates hot aqueous extract by UHPLC-ESI-QTOF-MS/MS

6.2.5.3 Identification of Phytochemical Compound in Ajwa Dates Methanol extract

Figure 7.3 illustrates the full chromatograph of Ajwa dates methanol extract, with **Appendix 3** presenting the list of phytochemicals detected in Ajwa dates methanol extract. Out of the 26 compounds peaks detected in these extracts, eight compound peaks were identified, including flavonoid and its derivatives, such as apigenin derivative (peak 3), chrysoeriol hexadecyl sulfate (peak 7), and myricetin-3-galactoside (peak 17). This was followed by the identification of phenolic and derivatives, such as 3,30-di-O-methyl ellagic acid (peak 8) and fatty acids. Other compound peaks were unknown.

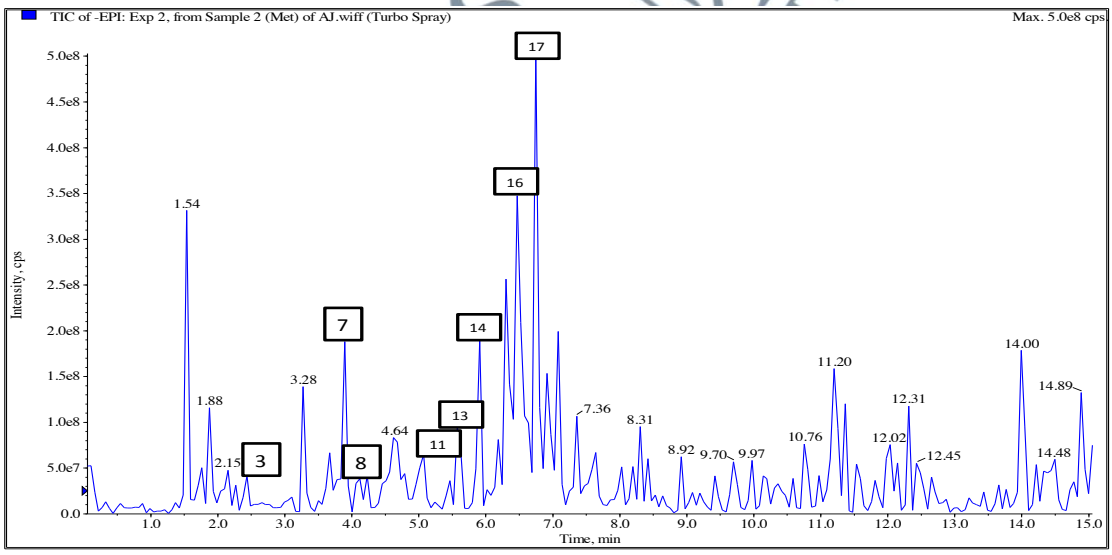

Figure 6.12: Full chromatogram of phytochemical compounds detected in Ajwa dates methanol extract by UHPLC-ESI-QTOF-MS/MS

6.2.5.4 Identification of Phytochemical Compound in Mariami Dates Hot Aqueous Extract

Figure 7.4 presents the full chromatograph of Mariami date hot aqueous extract, while **Appendix 4** illustrates the list of phytochemicals detected in the hot aqueous extract. A total of 31 compound peaks were detected in this extract. Within 31 compound peaks, 18 compound peaks were identified. Furthermore, some compounds (e.g., caffeic acid derivative) with the same m/z appeared repeatedly at different retention times. Phenolic acids and derivatives, such as caffeic acid derivative (peak 1 to 6, 12, 22, 23, 26, 27, 28, 30, and 31), ellagic acid hexoside (peak 8), rosmarinic acid (peak 19), and rosmarinic acid derivative (peak 24 was identified in this extract. Flavonoid and derivative, such as apigenin derivative (peak 17) and organic acids were also identified in this extract although other compounds peaks were unknown.

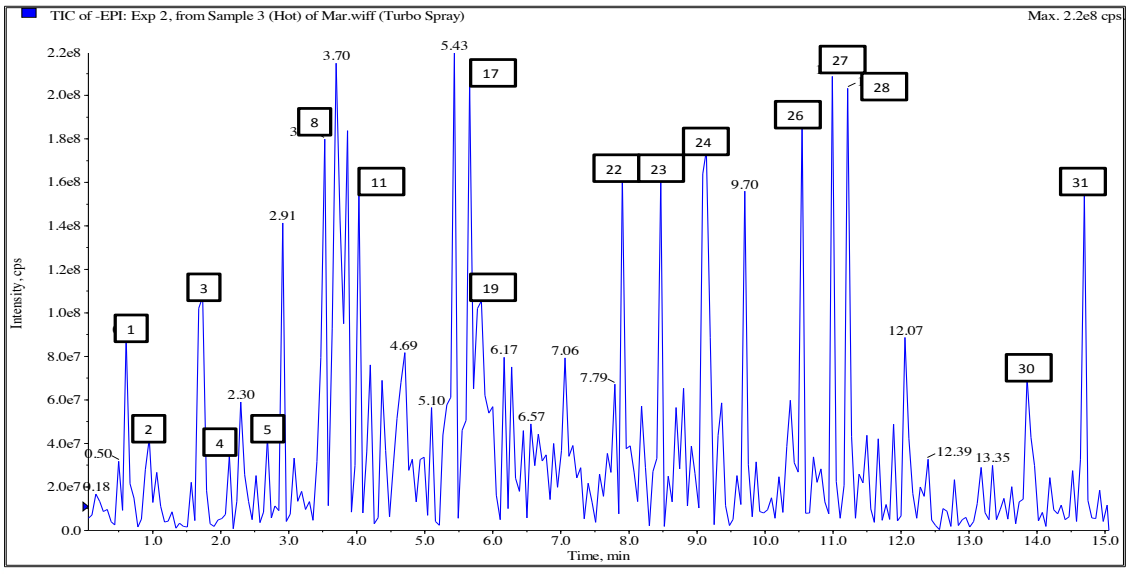

Figure 6.13: Full chromatogram of phytochemical compounds detected in Mariami dates hot aqueous extract by UHPLC-ESI-QTOF-MS/MS

6.2.5.5 Identification of Phytochemical Compound in Mariami Dates Cold Aqueous Extract

Figure 7.5 presents the full chromatograph of Mariami dates cold aqueous extract, while **Appendix 5** demonstrates the list of phytochemicals detected in Mariami dates cold aqueous extract. Out of the eight compound peaks detected in this extract, five compound peaks were identified, which included phenolic and derivatives, such as caffeic acid derivative (peak 2, 4, 6, and 7) and 3,30-di-O-methyl ellagic acid (peak 3). Caffeic acid was repeatedly detected at different retention time. Other compound peaks were unknown.

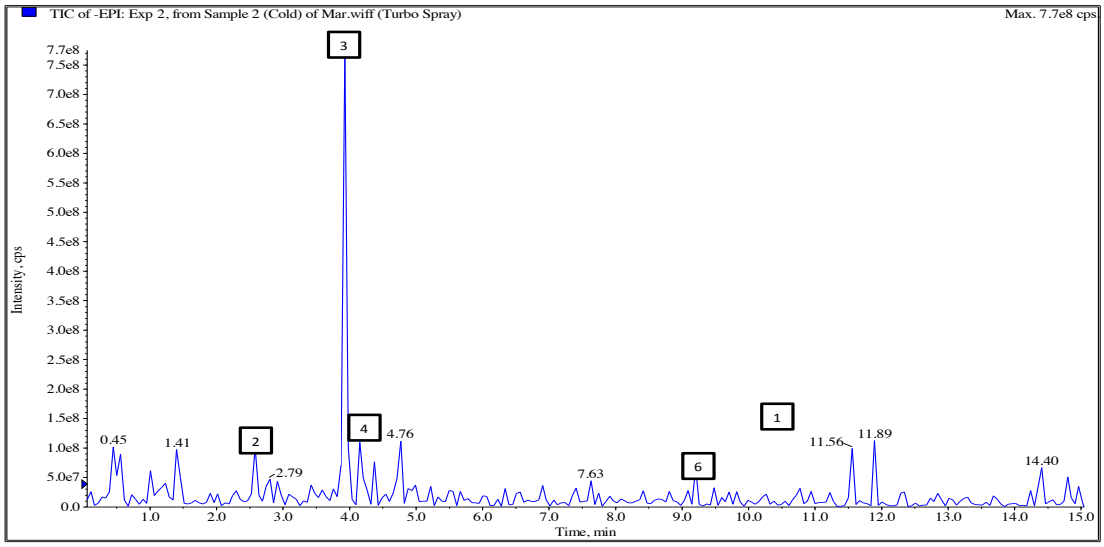

Figure 6.14: Full chromatogram of phytochemical compounds detected in Mariami dates cold aqueous extract by UHPLC-ESI-QTOF-MS/MS

6.2.5.6 Identification of Phytochemical Compound in Mariami Dates Methanol Extract

Figure 7.6 presents the full chromatograph of Mariami dates methanol extract, while **Appendix 6** illustrates the list of phytochemicals detected in Mariami dates methanol extract. Out of the 39 compound peaks detected in this extract, 15 compound peaks were identified, including phenolic acids and derivatives, such as caffeic acid derivative (peak 1, 4, 33, and 37), caffeic acid (peak 16), punicalagin (peak 9), and rosmarinic acid (peak 26). This was followed by the identification of flavonoid and derivatives, such as apigenin derivative (peak 19) and luteolin (peak 24). Other compound peaks were unknown.

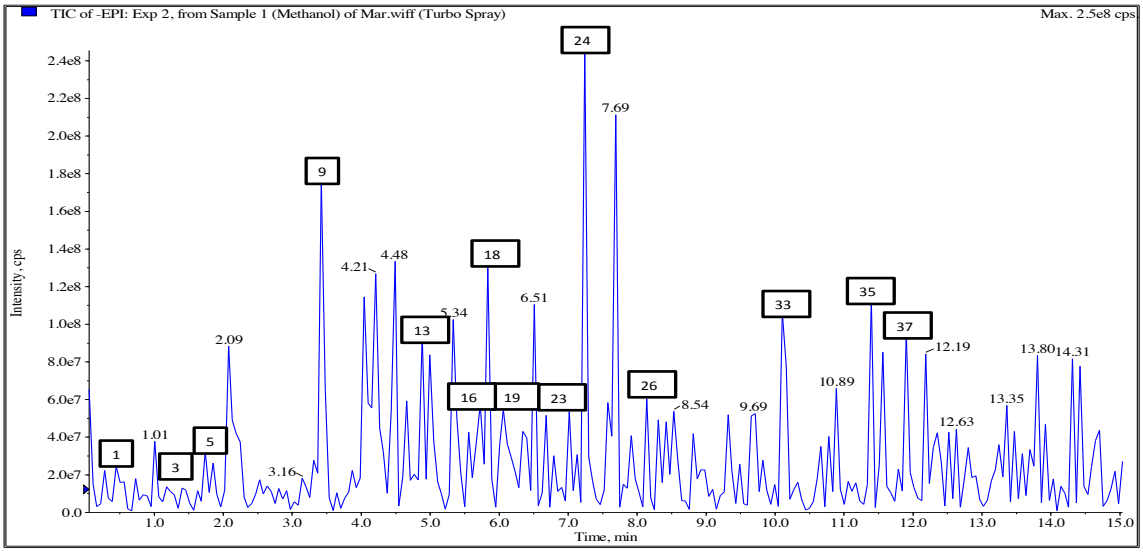

Figure 6.15: Full chromatogram of phytochemical compounds detected in Mariami dates methanol extract UHPLC-ESI-QTOF-MS/MS

6.2.5.7 Identification of Phytochemical Compound in Medjool Dates Hot Aqueous Extract

Figure 7.7 presents the full chromatograph of Medjool date hot aqueous extract, while **Appendix 7** illustrates the list of phytochemicals detected in the aqueous extract. Out of the 38 compound peaks detected in this extract, 22 compound peaks were identified. Several compounds, such as caffeic acid derivative and caffeoylquinic acid isomer were repeatedly detected at different retention times. Phenolic acid and derivatives, such as caffeic acid derivative (peak 1 to 6, 11, 23, 29, and 31), ellagic acid hexoside derivative (peak 12), punicalagin (peak 14), rosmarinic acid (peak 18), caffeoylquinic acid isomer (peak 21), apigenin derivative (peak 28), apigenin isomer (peak 28), were identified in this extract. Flavonoid and derivatives, such as diomestin (peak 13) and organic acids were also detected in this extract. Other compound peaks were unknown.

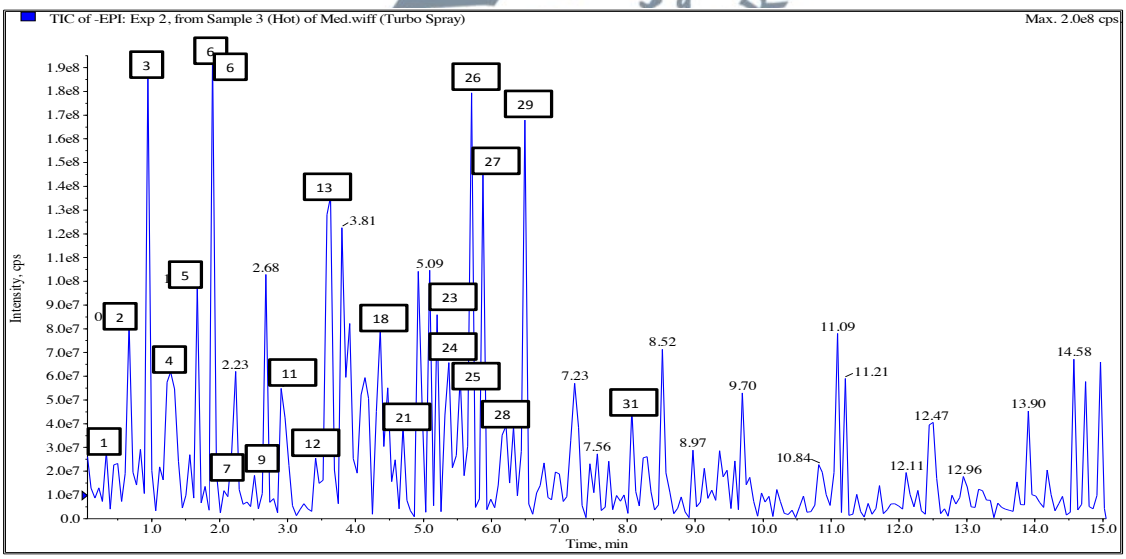

Figure 6.16: Full chromatogram of phytochemical detected in Medjool dates hot aqueous extract by UHPLC-ESI-QTOF-MS/MS.

6.2.5.8 Identification of Phytochemical Compound in Medjool Dates Cold Aqueous Extract

Figure 7.8 presents the full chromatograph of Medjool dates cold aqueous extract, while **Appendix 8** illustrates the list of phytochemicals detected in the aqueous extract. Out of 26 compound peaks detected in this extract, 20 compound peaks were identified, with caffeic acid derivative and punicalagin repeatedly appearing at different retention time. Phenolic acids and derivatives, including caffeic acid derivative (peak 1, 3, 5, 6, 10,13, 14, 18, 21, 22, 23, 24, 25, and 26), caffeic acid (peak 2), punicalagin (peak 4 and 8), ellagic acid hexoside (peak 7), and fatty acids were identified in this extract. Other compound peaks were unknown.

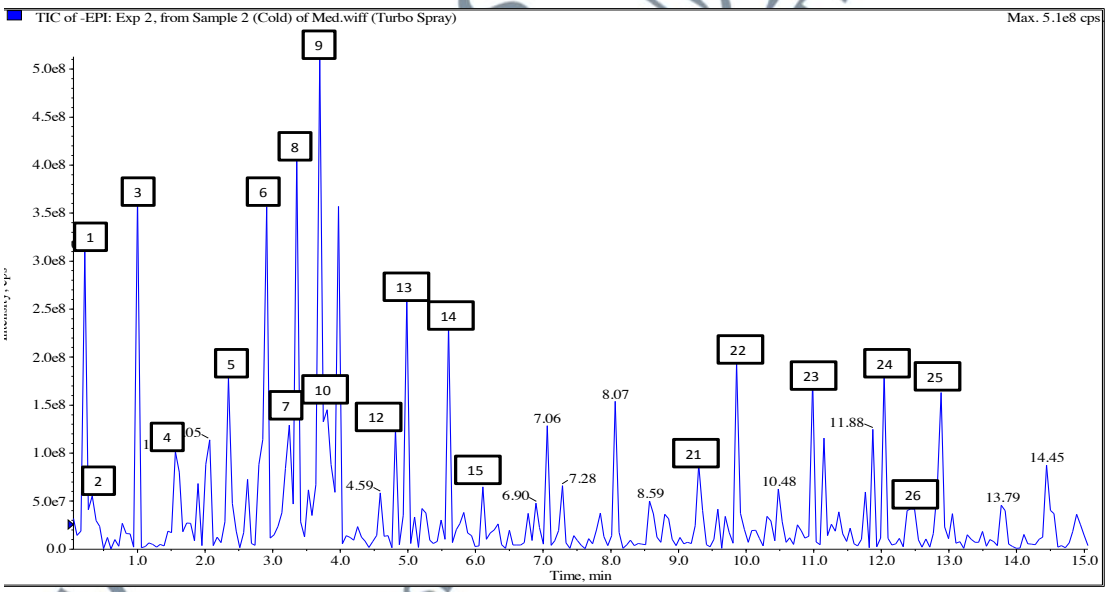

Figure 6.17: Full chromatogram of phytochemical detected in Medjool dates cold aqueous extract by UHPLC-ESI-QTOF-MS/MS

6.2.5.9 Identification of Phytochemical Compound in Medjool Dates Methanol extract

Figure 7.9 illustrates the full chromatograph of Medjool dates methanol extract, while **Appendix 9** presents the list of phytochemicals detected the methanol extract. Out of 19 compound peaks detected in this extract, 12 compound peaks were identified, with caffeic acid derivative repeatedly appearing at different retention time. Phenolic acids and derivatives, including punicalagin (peak 2) and caffeic acid derivative (peak 10, 11, 12, 13, 16, 17, 18, 19) were identified in this extract. This was followed by the detection of flavonoids and its derivatives, such as diosmetin (peak 3), apigenin isomer (peak 7), and apigenin derivative (peak 8). Other compound peaks were unknown.

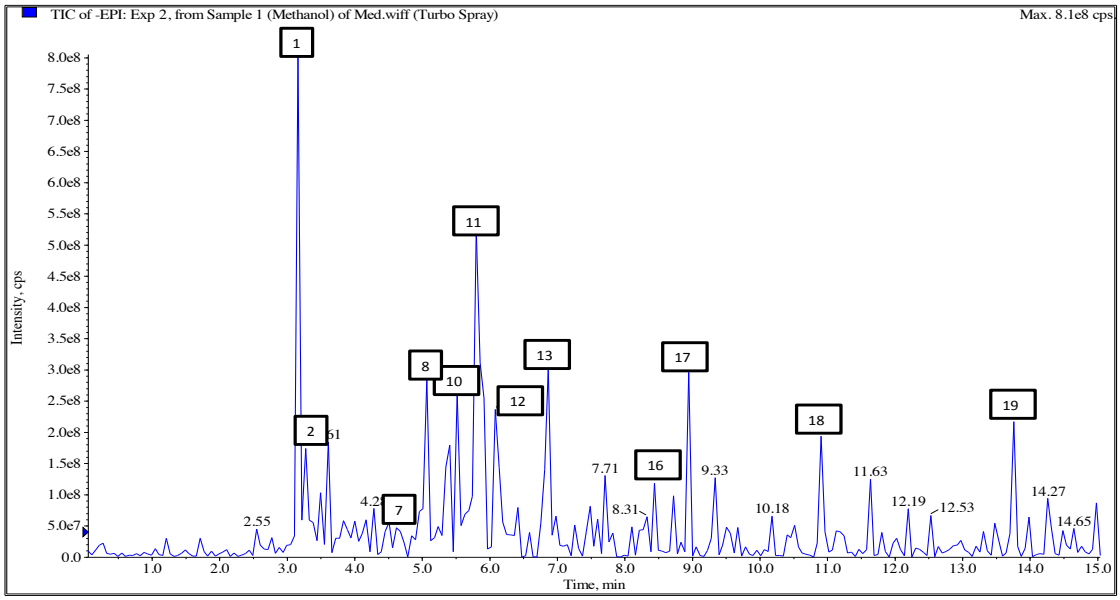

Figure 6.18: Full chromatogram of phytochemical detected in Medjool dates methanol extract by UHPLC-ESI-QTOF-MS/MS.

6.2.6 Classification of Phytochemical Compound in Dates Fruits

In summary, a total of 28 compounds in Ajwa dates, 16 compounds in Mariami dates, and 14 compounds in Medjool dates were detected. These compounds were then classified into several groups, namely phenolic compound, flavonoid, fatty acid, organic acid, and terpenoids. The data are summarised in **Table 7.1.**

6.2.6.1 Organic acids

Gluconic acid was detected in Ajwa and Mariami dates, while penylvaleric acids and tartaric acid were detected in Ajwa dates. While p-hydroxybenzoic acid derivative and quinic acid were identified in Mariami dates, Malic acid was detected in Medjool dates.

6.2.6.2 Phenolic acids and derivatives

Caffeic acid, caffeic acid derivative, and punicalagin were detected in Ajwa, Medjool, and Mariami dates, while 3,30-di-O-methyl ellagic acid and punicalagin isomer were identified in Ajwa dates. This was followed by the detection of 3,30-di-O-methyl ellagic acid, ellagic acid hexoside derivative, rosmarinic acid, and rosmarinic acid derivative in Mariami dates, including the identification of caffeoylquinic acid isomer, ellagic acid hexoside derivative, and rosmarinic acid in Medjool dates.

6.2.6.3 Flavonoid derivatives

Flavonoids were mostly detected in Ajwa dates, while apigenin derivative was identified in Ajwa, Mariami, and Medjool dates. Chrysoeriol hexadecyl sulfate, isoquercitrin acetate, hexosyl luteolin sulfate, and myricetin-3-galactoside were detected in Ajwa dates. Catechin was identified in Mariami dates, while apigenin isomer and diosmetin were identified in Medjool dates.

6.2.6.4 Terpenoids

Terpenoids were only identified in Ajwa dates dihydroxyl dimethyl 19-[(Dglucopyranosyl)oxy] -19-oxo-ent-labda-8(17),13-dien-16,15-olide derivative, while oleanolic acid was identified in Ajwa dates.

6.2.6.5 Fatty acids

Six fatty acids were detected in Ajwa dates, namely 15,16-dihydroxy- 9Z,12Z-octadecadienoic acid, 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzenepropanoic acid, sphingolipid conjugate III, stearic acid, and trihydroxy-9, 14-octadecadienoic acid. However, Mariami dates only identified linoleic acid, while Medjool dates only identified decanoic acid derivative.

6.2.6.6 Others compound

Other compounds, such as lignan (e.g., hydroxypinoresinol hexoside and unknown lignin) and thiamine monophosphate (vitamin B) were detected in Ajwa dates although no lignin and vitamin were detected in Mariami and Medjool dates.

Table 6.9: Classification of the phytochemical compound in Ajwa, Mariami, and Medjool dates

Compounds	Ajwa dates	Mariami dates	Medjool dates
Phenolic acids and derivatives	Caffeic acid	Caffeic acid	Caffeic acid
	Caffeic acid derivative	Caffeic acid derivative	Caffeic acid derivative
	Punicalagin	Punicalagin	Punicalagin
	3,30-di-O-methyl ellagic acid	3,30-di-O-methyl ellagic acid	Caffeoylquinic acid isomer
	Punicalagin isomer	Ellagic acid hexoside derivative	Ellagic acid hexoside derivative
	-	Rosmarinic acid	Rosmarinic acid
	-	Rosmarinic acid derivative	
Flavonoid derivatives	Apigenin derivative	Apigenin derivative	Apigenin derivative
	Chrysoeriol hexadecyl sulfate,	Catechin	Apigenin isomer
	Isoquercetrin acetate	-	Diosmetin
	Hexosyl luteolin sulfate	-	-
	Myricetin-3-galactoside	-	-
Organic acids	Gluconic acid	Gluconic acid	Malic acid
	Penylvaleric acids	P-hydroxybenzoic acid derivative	Syringic acid
	Tartaric acid	Quinic acid	-
	-	Caffeoyl glucose derivatives	-
	-	Sinapic acid hexoside	-
Fatty acids	Decanoic acid derivative	Linoleic acid	Decanoic acid derivative
	15,16-dihydroxy-9Z,12Z-octadecadienoic acid	-	-
	2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzenepropanoic acid	-	-
	Sphingolipid conjugate III	-	-
	Stearic acid	-	-
	Trihydroxy-9, 14-octadecadienoic acid	-	-

Terpenoids	Dihydroxyl dimethyl 19[(Dglucopyranosyl)oxy]-19-oxo-ent-labda-8(17),13-dien-16,15-olide derivative	-	-
	Oleanolic acid	-	-
Others	Hydroxypinoresinol hexoside		-
	Unknown lignan		-
	Thiamine monophosphate	-	-
	Acyl Sucrose	-	-

6.2.7 Quantification of Phytochemical Compound Using Calorimetric Assay

6.2.7.1 Total Phenolic Content (TPC)

The TPC was determined from the standard calibration curve using Gallic acid standard, while the values were expressed as mg Gallic acid equivalence per 100 g of dry weight (mg GAE/100g DW). The TPC of all date fruit extracts is presented in **Table 7.11** and **Figure 7.4**.

As for the cold aqueous extract, Ajwa dates contained higher TPC with 379.84 ± 7.20 mg GAE/100 g DW compared to Medjool dates, with 297.98 ± 6.71 mg GAE/100 g DW. This value was higher compared to Mariami dates with 277.48 ± 22.80 mg GAE/100 g DW ($p < 0.05$). There was no significant difference between the TPC of Mariami date and Medjool dates.

In the case of hot aqueous extract, Ajwa dates contained higher TPC of 379.84 ± 7.20 mg GAE/100 g DW compared to Medjool with 1053.47 ± 22.10 mg GAE/100 g DW ($p < 0.05$). Meanwhile, Mariami dates contained higher TPC with 1446.11 ± 22.11 mg GAE/100 g DW compared to Medjool dates ($p < 0.05$). Although Ajwa and Mariami

comprised higher TPC compared to Medjool, there was no significant difference between the TPC of each date variety.

As for the methanol extracts, Medjool dates contained significantly higher TPC with 1464.85 ± 18.33 mg GAE/100 g DW compared to Ajwa dates (715.72 ± 1.06 mg GAE/100 g DW) and Mariami dates (505.51 ± 19.93 GAE/100 g DW) ($p < 0.0001$). However, no significant difference was present between the TPC of Ajwa date and Mariami dates.

Table 6.10: Total phenolic content (TPC) of all dates varieties extracts. The values are expressed as mean ± Standard Deviation. GAE/100 g DW - Gallic acid equivalence / 100 gram of dry weight

	Total Phenolic Content (mg GAE/ 240ml samples)		
Extraction type / Date fruits variety	Ajwa dates	Medjool dates	Mariami dates
Cold aqueous	379.84 (±7.20)	297.98 (±6.71)	277.48 (±22.80)
Hot aqueous	1445.31(±19.22)	1053.47 (±22.10)	1446.11 (±22.11)
Methanol	715.72 (±1.06)	1464.85 (±18.33)	505.51 (±19.93)

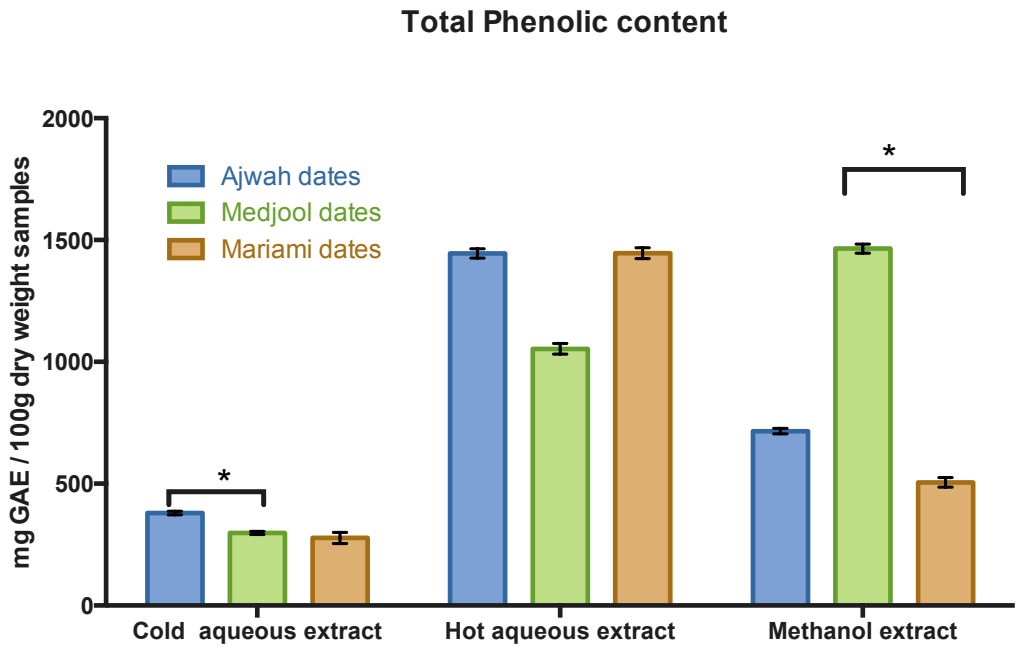

Figure 6.19: Total phenolic content (TPC) of all dates varieties extracts. Asterisk* represents significant value (*$p < 0.05$) between the different dates

6.2.8 Total Tannin Content (TTC)

The TTC was determined from the standard calibration curve using gallic acid standard (0.01 - 0.2 mg/ml and TTC in the extract was expressed as mg Gallic acid equivalence/100g dry weight (mg GAE/100g DW). The TTC all date extracts are illustrated in **Table 7.12** and **Figure 7.5**.

In the case of cold aqueous extract, Ajwa dates contained higher TTC with 68 ± 9.5 GAE/100 g compared to Medjool and Mariami, although there was no significant difference between the TTCs of all date varieties.

As for hot aqueous extract, Medjool dates contained higher TTC with 197.13 ± 7.04 mg GAE/100 g DW compared to Ajwa and Mariami dates, while no significant difference was found between the TTCs of all date varieties.

For methanol extract, Medjool dates contained higher TTC with 214.07 ± 2.56 mg GAE/100 g DW compared to Ajwa dates (p = 0.219), although no significant difference was recorded between the TTCs of Ajwa and Mariami date, including the TTCs of Medjool and Mariami dates.

Table 6.11: Total Tannin Content (TTC) of all date varieties extracts. Values are expressed as mean ± Standard Deviation. GAE/100 g DW denotes gallic acid equivalence/100 g of dry weight

	Total Tannin Content (mg GAE/ 240ml samples)		
Extraction type / Date fruits variety	Ajwa dates	Medjool dates	Mariami dates
Cold aqueous	68 (±9.5)	54.60 (±9.32)	64.61 (±2.13)
Hot aqueous	164.36 (±10)	197.13 (±7.04)	155.59 (±7.41)
Methanol	11.81 (±4.41)	214.07 (±2.56)	56.79 (±6.88)

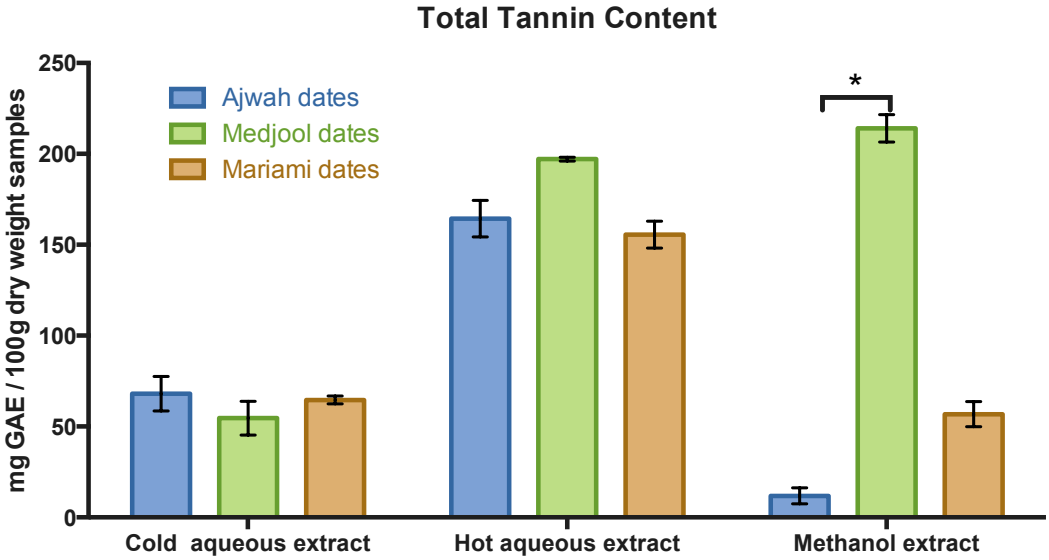

Figure 6.20: Total Tannin Content (TPC) of all date extracts. Asterisk represents significant value (*p < 0.05) between the different dates.

6.2.9 Total Flavonoid Content (TFC)

The TFC was measured using the aluminium chloride colorimetric assay and expressed as mg rutin equivalence per 100 g of dry weights (mg RE/100 g DW). The TFC of all date extracts is presented in **Table 7.13** and **Figure 7.6**.

In the case of cold aqueous extract, Ajwa dates contained higher TFC with 77.43 ± 1.28 RE/100 g DW compared to Medjool dates with 65.27 ± 0.49 mg RE/100 g DW ($p = 0.0211$). Ajwa dates also contained higher TFC compared to Mariami dates with 73.45 ± 0.07 mg RE/100 g DW, although there was no significant difference between the TTCs of Ajwa and Medjool dates, including the TTCs of Medjool and Mariami dates.

In the case of hot aqueous extract, Mariami dates contained significantly higher TFC with 455.14 ±1.62 mg RE/100 g DW compared to Medjool dates with 145.47 ± 1.62 mg RE/100 g DW ($p < 0.005$). Moreover, Ajwa dates contained higher TFC compared to Medjool dates. However, there was no significant difference between the TTCs of Ajwa and Medjool dates, including the TTCs of Ajwa and Mariami dates.

As for methanol extract, Ajwa dates contained significantly higher TFC with 216.93 ± 4.30 RE/100 g DW compared to Mariami dates 106.13 ± 6.42 mg RE/100 g DW ($p < 0.005$). Similarly, Ajwa dates contained higher TFC compared to Medjool dates, although there was no significant difference between Mariami and Medjool dates.

Table 6.12: Total flavonoid content (TFC) of all date varieties extracts. Values are expressed as mean ± standard deviation. RE/100 g DW denotes rutin equivalence/100g of dry weight

	Total Tannin Content (mg RE/ 240ml samples)		
Extraction type / Date fruits variety	Ajwa date	Medjool date	Mariami date
Cold aqueous extract	77.43 (±1.28)	65.27 (±0.49)	73.45(±0.07)
Hot aqueous extract	207.41 (±3.71)	145.47 (±1.62)	455.14(±1.62)
Methanol extract	216.93 (±4.30)	117.50 (±1.04)	106.13(±6.42)

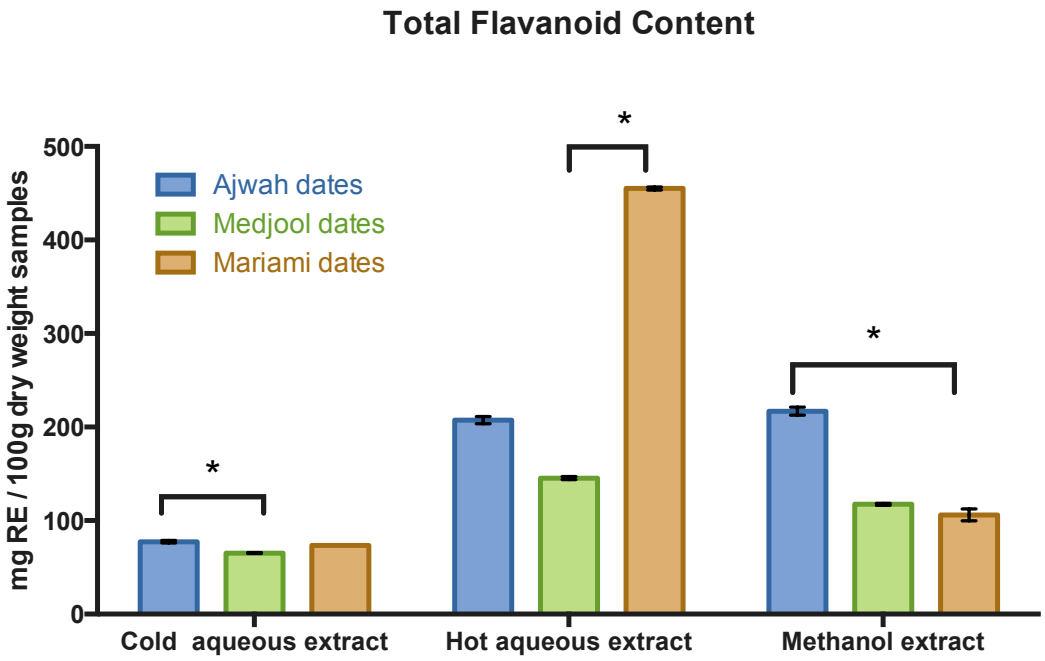

Figure 6.21: Total Flavonoid Content (TFC) of all date extracts. Asterisk represents significant value (*$p < 0.05$) between the different dates.

6.3 Conclusion

Ajwa, Medjool, and Mariami date extracts showed antibacterial and anti-adhesion activities against all tested bacteria, while the hot aqueous extracts of Ajwa dates were recorded with higher inhibition zone compare to Medjool and Mariami dates. Ajwa dates also showed significantly higher anti-adhesion activity compared to Medjool against all tested bacteria and Mariami date extracts against 5 0f 6 tested bacteria.

Ajwa date extracts contained the highest phytochemical content, which 78 compound peaks detected compared to Mariami and Medjool dates. Phytochemical analysis of date fruits extracts identified the varieties of phytochemicals, including phenolic acid, flavonoids, organic acids, fatty acids, and terpenoids. Phytochemical quantification recorded that Medjool hot aqueous extract contains highest TPC, Medjool methanol extracts contains highest TTC, and Mariami hot aqueous extracts contains highest TFC.