EHEC	*Enterohaemorrhagic Escherichia coli*
EIEC	*Enteroinvasive Escherichia coli*
mm	millimetre
nm	nanometre
cm	centimetre
g	gram
mg	milligram
ml	millilitre
µL	Microliter
DMEM	Dulbecco's Modified Eagle medium
DMSO	Dimethyl Sulfoxide
FBS	Fetal Bovine Serum
CLSI	Clinical and Laboratory Standard Institute
CPD	Critical Point Machine
CCl_4	*carbon tetrachloride*
MH	Mueller-Hinton

CHAPTER 1 : INTRODUCTION

1.1 Background of Study

Three to five billion cases of acute gastroenteritis are present worldwide. The admission rate for acute gastroenteritis increased significantly during the past decade (WHO, 2017), with nearly 1.5 to 2.5 million deaths worldwide per year (Sattar & Singh, 2019). Notably, children especially from developing countries received the most impact (Webber, 2009; Curns et al., 2010; Sattar & Singh, 2019). The diarrhoeal disease in children under five years was the second most common cause of death by infectious disease after pneumonia, which also affected over three to five billion children each year worldwide (Sattar & Singh, 2019)

Gastroenteritis is primarily caused by viruses and bacteria, followed by parasites and fungus (Harlem, 1999; Helms & Quan, 2006; Webber, 2009). Although viral gastroenteritis cases are the highest, no specific antimicrobial agent for viral gastroenteritis is present (Farthing et al., 2013). The infection is normally self-limited and only requires supporting treatments, such as appropriate fluid, electrolyte therapy, and constant availability of good nutrient (Phavichitr & Catto-Smith, 2003). Meanwhile, although the cases of bacterial gastroenteritis are more severe, anti-infective options (e.g., antibiotics) are available for treatment to reduce the symptom severity and the excretion of pathogen to prevent infection and extraintestinal complications (Eslick, 2019). However, the use of antibiotic are often unnecessary and not always suggested in the treatment of bacterial gastroenteritis (Dennehy, 2019). To illustrate, the widespread use of antibiotic has led to resistance of the enteric pathogen,

resulting in the necessity to find alternative ways of treating bacterial gastroenteritis, such as breastfeeding for infant, supplementation micronutrient, complementary and alternative medicine (CAM), zinc, and probiotics (Ashkenazi & Cleary, 1991; Sazawal et al., 1995; Goossens & Sprenger, 1998; Davies & Davies, 2010; Farthing et al., 2013). Furthermore, CAM could be defined as various therapies, which are not considered parts of conventional medicine, and are mainly described into biological therapies (e.g., natural product, probiotic, dietary supplement, diet-based therapy, vitamin, and mineral), body manipulation (e.g., acupuncture and chiropractic massage), and mind-body therapies (e.g., meditation and yoga). There has been a high prevalence of CAM used among patients suffering from gastrointestinal disorder (Serpico et al., 2016; Nousiainen et al., 2014). Notably, herbal medicine is the most favourable among parents for their children as it is believed to be natural and safe (Pike et al., 2013).

Numerous *Quranic* verses and *Hadith* (the narrations of Prophet Muhammad PBUH) have recommended certain food with healing properties and benefits to human health, such as honey, barley dates, figs, goat milk, black seeds (*habbatussauda)*, and olive oil (Syed, 2003; Deuraseh, 2006). Specifically, dates are the favourable food by Prophet Muhammad (PBUH), as stated in an authentic narration, "Whoever eats seven date fruits in the morning, no poison will harm him until it is the evening" (Muslim, 1990). Date fruits are rich in carbohydrate, protein, unsaturated fatty acids, vitamins, fibre, minerals, and antioxidant (Tenover, 2006; Usda, 2009; Ogungbenle, 2011; Amira et al., 2012). This fruit is also considered an emerging and potential candidate for the development of health-promoting foods due to high nutritive values and potential health-promoting activities.

Numerous studies have proved that date palm has medicinal effects such as antioxidant, anti-inflammatory, anti-cancer antimicrobial, and anti-mutagen (Vayalil,

2002; Al-Farsi et al., 2007; Al-Daihan & Bhat, 2012). Even though many antimicrobial activities of date fruits have been studied, however studies focusing on bacterial causing gastroenteritis are lacking. The study on the anti-adhesion properties of date fruits and the antibacterial mechanism of action underlying of bioactive phytochemicals was not well understood. Additionally, maintaining health status by applying a prophetic diet in daily life is considered *Sunnah* (Prophetic Tradition), which advocates the anticipated use of food as a medicine for many centuries. *Sunnah* has also become a precursor to the principle of modern treatment.

1.2 Problem Statement

The impact and burden of acute gastroenteritis is frequently underestimated, where the morbidity and mortality which associated with the enteric pathogen, including bacteria, have been seriously underestimated (World Health, 2015). In Malaysia, the true burden of acute gastroenteritis is largely unknown, since many patients do not seek medical attention for mild episode (Lim, 2007). The most significant impact of this disease is mainly present in underdeveloped and developing countries, where endemic diarrhoea is common, however they are often ignored.

In the last two decades, many new pathogens have been associated with diarrheal disease. It includes *Campylobacter* sp., *Escherichia coli* O157:H7, *Vibrio cholerae* 0139, *Vibrio vulnificus*, and *Nitzschia pungens* (cause amnesic shellfish poisoning). These agents are transmitted through food water and cause severe disease in immunocompromised person. Some can lead to long term sequela.

Bacterial gastroenteritis will lead to sequelae, s renal failure following haemolytic-uraemic syndrome due to *Escherichia coli* 0157:H7, irritable bowel syndrome, inflammatory bowel disease, reactive arthritis, and Guillain-Barre syndrome

(Gibney et al., 2014; Lackner et al., 2019). This post-infection and disease burden would increase the disability-adjusted life years (DALYs), which is a measure involving an estimate of years of lost life and years spent with disability due to the disease (Eslick, 2019).

The treatment for bacterial gastroenteritis is currently a challenge due to the increase in the resistance to gastrointestinal pathogens. This antimicrobial resistance comprises the implications of the treatment option. World's Health Organization (WHO) reported that approximately two-thirds of the population in developed and developing countries used traditional and complementary alternative medicine (CAM). Furthermore, WHO recognized the important role of CAM in the prevention and promotion of health for a large proportion of the population, especially in developing countries, and advocated the functionality of CAM (World Health, 2017).

Systematic review revealed that dates fruits showed good antibacterial activity against pathogenic bacteria (Farhana et al., 2017). Given the scientific proof regarding the antibacterial activity of date fruits, there is a high potential for date fruits to be used as an alternative treatment and prophylaxis for bacterial gastroenteritis. However, studies focusing on bacterial causing gastroenteritis are lacking. Additionally, the study on the anti-adhesion properties of date fruits also lacking. Therefore, more studies are needed to develop an understanding of the mechanism underlying the bioactive compound in date fruits, which has a role in antibacterial and ant-adhesion activities.

1.3 General objective

To investigate the antibacterial and anti-adhesion effects and the antibacterial mechanism of date fruits (*Phoenix dactylifera)* against bacteria causing gastroenteritis.

1.3.1.1 Specific Objective

1. To determine the inhibitory effect of Ajwa dates (*Phoenix dactylifera*) extracts and thermo-stability of Ajwa date on their antibacterial activity.
2. To determine the antibacterial activity of Ajwa dates-infused water, which followed the preparation conducted by the Prophet Muhammad (PBUH) against bacterial gastroenteritis.
3. To compare the inhibitory effect (inhibition/killing and anti-adhesion active and the phytochemical analysis (identification and quantification of active phytochemical) of three varieties of dates (Ajwa, Mariami, and Medjool).
4. To evaluate the mechanism of action of Ajwa dates by isolating the bioactive phytochemical that responsible for antibacterial activity, and to examine the mode of action of Ajwa date extracts and isolated flavonoids from Ajwa dates against the selected bacteria using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

1.4 Hypotheses

1. The Ajwa dates exhibit antibacterial activity against all tested bacteria and antibacterial properties in Ajwa date fruit is thermo-stable.
2. Ajwa date-infused water following the preparation by Prophet Muhammad (PBUH) exhibit antibacterial activity against bacterial gastroenteritis.
3. Ajwa dates exhibit significant inhibitory effects (inhibition/killing and anti-adhesion) compared other date varieties. Date fruits contain bioactive phytochemicals, such as polyphenol, flavonoid, tannin, and date extracts comprise of high phenolic, flavonoid, and tannin contents.
4. Bioactive compounds, such as phenolic, tannin, and flavonoids are responsible for the antibacterial activity in the date. SEM and TEM reveal damage on the morphology and intercellular of bacterial cells after the treatment with Ajwa date extract and isolated bioactive compound.

1.5 Overview of Thesis

This thesis consists of two parts, namely the determining the antibacterial activity in the date fruits and the understanding of the mechanism underlying the antibacterial activity in the date fruits. Chapter 2 of the thesis comprises a literature review of a topic related to this study, while Chapter 3 outlines the methods implemented in this study. The data and analysis in the first part of the study are presented in Chapter 4, Chapter 5, and Chapter 6, which present the data regarding the extraction of date fruits by aqueous and methanol extracts, followed the testing of antimicrobial and anti-adhesion activities. Data and analysis in the second part of the study are presented in Chapter 7. Chapter 8 presents a thorough discussion of this study followed by a conclusion and future recommendation in Chapter 9.

To determining the antibacterial activity of date fruits, testing was performed on the Ajwa date including optimization of extraction, followed by testing the antimicrobial activity by well diffusion assay and Minimum Inhibitory concentration (MIC) assay, and Minimum Bactericidal Concentration (MBC) assay. Furthermore, Ajwa dates soaked in water at room temperature to imitate the preparation by the Prophet Muhammad (PBUH) and the infused water was conducted. The antimicrobial activities of different varieties of data, namely Ajwa, Medjool, and Mariami, were determined using well-diffusion agar assay , Minimum Inhibitory concentration (MIC) assay, and Minimum Bactericidal Concentration (MBC) assay, while the anti-adhesion activities were identified using Caco-2 cells model. Furthermore, the phytochemical contents in date fruit extracts were screened and identified using UHPLC-ESI_QTOF-MS/MS, followed by the quantification of the total phenolic, total tannin, and total flavonoid using a colourimetric assay. The mechanism underlying the antibacterial activity of date fruit was explored further, while the active phytochemical responsible

for antimicrobial activity was isolated using preparative-HPLC. All isolated fractions were tested in terms of their antimicrobial and anti-adhesion activity, while the mechanisms of killing were identified using electron microscopy.