CHAPTER 2 :LITERATURE REVIEW

2.1 Gastroenteritis

2.1.1 Definition

Gastroenteritis is a broadly defined term originating from the Greek language, where 'gastron' means 'stomach', and 'enteron' means 'small intestine'. Therefore, the final meaning of this term is 'inflammation of the small intestines and the stomach' (Al Jassas et al., 2018). Gastroenteritis normally refers to acute infectious diarrhoea or acute gastroenteritis. The World Health Organisation (WHO) defines acute gastroenteritis as a clinical syndrome characterised by increased stool frequency, where three or more loose or watery stools in 24 hours or several loose/watery bowel movements exceed the regular amount of daily bowel movements by two or more, with or without vomiting or fever (WHO, 2017).

2.1.2 Epidemiology

Gastroenteritis is the second common infection, which causes death worldwide as a result of lower respiratory infection, with estimated 4.6 billion episodes each year. In 2015, approximately two billion cases of gastroenteritis resulted in 1.3 million deaths (Haidong Wang et al., 2016). Furthermore, gastroenteritis cases mostly affect the developing countries (Bennett et al., 2014), as proven through the death cases occurring to children under five years old from the world's poorest nation (Tate et al., 2012). Fatal gastroenteritis cases mostly occur in low- and middle-income countries (LMIC). Although fatal cases rarely occur in high-income countries (HIC), they are the leading

causes of emergency department visits and hospitalisation (Schnadower et al., 2015). Diarrheal disease constantly affects developing countries, such as Asia and Africa, where access to clean water, sanitation, and urgent medical care may be limited (WHO, 2017). In Malaysia, there were 13.5 million cases of acute diarrhoea each year which resulted in 234,000 outpatient visits, 70,000 hospitalizations, and 61 deaths in under-fives annually (Gurpreet et al., 2011; Loganathan et al., 2016).

Children are a major group affected by gastroenteritis, especially in developing countries (Webber, 2009), as proven through the children of under five years old, who suffer from two to four diarrheal episodes per year. The prevalence of diarrhoea among children under five in Malaysia was 4.4% (61 cases) annually (Aziz et al., 2018). However, the mortality cases significantly decrease each year (Barret & Fhogartaigh, 2017), while in developed countries, lesser than 0.3 episodes of diarrhoea occur on each person, although it is still associated with more than five million cases in the US each year. To be specific, death occurs to almost two million children annually due to diarrheal disease. Moreover, the majority of these children is poor and living in a resource-poor area (Regina LaRocque et al., 2013), and their immunity is a significant factor of this disease compared to older children and adults (J Eckardt & C Baumgart, 2011; Dennehy, 2019).

Gastroenteritis could be caused by many organisms, with viral gastroenteritis being the most common cause of diarrheal disease, followed by bacterial, parasites, and fungus. Viral gastroenteritis is the most common among children under five years old compared to older children or adults. This order is commonly transmitted through the faecal-oral route and starts from 12 hours to five days after exposure to the organism. Meanwhile, bacterial enteritis affects adults and children of older than two years old and occurs through oral-faecal contamination, poultry exposure, or contaminated meat.

(Bányai et al., 2018; Dennehy, 2019). Bacteria also account for 80% of cases of diarrhoea among travellers, while *E. coli* accounts for the majority case of cases, followed by *Campylobacter, Salmonella,* and *Shigella* (Bruzzese et al., 2018). In Malaysia, although the public health achievement is substantial, management of cholera outbreak could still pose a challenge especially in Sabah state (Jikal et al., 2019).

The parasitic causes of gastroenteritis are uncommon among healthy children in developed countries, which also account for 1% to 8% of gastroenteritis cases. Parasitic infections occur more frequently among the recent immigrants, travellers, backcountry campers, individuals who are exposed to farm animals, and immune-compromised patients. Notably, *Giardia lamblia* and *Cryptosporidium parvum* infections are the most common causes of parasitic disease in developed countries (Dennehy, 2019).

2.1.3 Sign and Symptoms

The main symptom of gastroenteritis is diarrhoea. Although diarrhoea is the primary symptom of gastroenteritis, others typical symptoms are including nausea and vomiting, abdominal pain and cramping, mild fever and chills, and loss of appetite.

The symptom for acute viral gastroenteritis and bacterial gastroenteritis are including non-bloody diarrhoea, vomiting, and fever. They are almost similar and clinically indistinguishable. However, clinical features suggest that bacterial gastroenteritis, indicated through blood and mucus in the stool, high fever (> 40ºC), associated seizures, tenesmus, severe abdominal pain, and smaller volume stools, occurs among children ageing older than two years old (Dennehy, 2019).

Bacterial gastroenteritis diarrhoea can be classified as noninflammatory, inflammatory, or invasive based on the effect of the enteric pathogen on the intestinal

mucosa. The noninflammatory or secretory diarrhoeas are characterized by low-grade or no fever and diffuse watery non-bloody stools. Secretory diarrhoea is caused by enterotoxin-producing organisms such as *Vibrio cholerae* and enterotoxigenic *E. coli*. While, inflammatory diarrhoea is often characterized by high fevers (greater than 40 °C), dysentery, severe abdominal pain, and smaller volume stools. Dysentery is intestinal infection of the intestine resulting in diarrhoeal with blood or mucus (Willian & Berkley, 2018)

Parasitic infection normally leads to abdominal cramping, watery diarrhoea, vomiting, and low-grade fever (Dennehy, 2019), while fungus gastroenteritis comprises symptoms of diarrhoea, vomiting, abdominal pain, melena, haemorrhage, and fever (Lamps et al., 2014).

Acute gastroenteritis normally occurs for less than 14 day-duration, while persistence gastroenteritis occurs for more than 14 days, although shorter than 30 day-duration. Chronic gastroenteritis occurs for more than 30 day-duration (Sattar & Singh, 2019).

2.1.4 Aetiology of Gastroenteritis

Viruses, bacteria, parasite, and fungus are the organisms, which lead to gastroenteritis. Specifically, viruses are the common causes of gastroenteritis among children in developed and developing countries, with rotaviruses and noroviruses being the most common (Phavichitr & Catto-Smith, 2003; Elliott, 2007; Dennehy, 2019). Bacterial gastroenteritis cases depend on the geographical area. In developing countries, death occurs to more than 500, 000 infants and young children due to acute gastroenteritis with the most common bacterial agents, namely *Shigella* and *Vibrio cholerae* (Kotloff, 2017). Notably, the most common organisms in Europe include

Campylobacter, *Salmonella spp.*, Enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC) (European Centre for Disease, 2012; Spina et al., 2015). Meanwhile, in sub-Saharan Africa and South Asia, *Shigella* is the main agent (J. Liu et al., 2016).

2.1.4.1 Viruses

Viruses are the most common causes of gastroenteritis in developed and developing countries (Webber, 2009). Among all the major enteric viruses, rotaviruses tend to occur among young children, whereas noroviruses affect people of all ages (Bányai et al., 2018). Rotavirus primarily affects young children and leads to severe gastroenteritis and dehydration (Al Jassas et al., 2018). The infection of this virus will alter the function of small intestine epithelial, which results in diarrhoea (Ramig, 2004). Furthermore, it is normally transmitted through faecal-oral transmission, hand and respiratory system, or contaminated surface (Dennehy, 2000). Meanwhile, Norovirus commonly infects all ages, which is also the leading case of foodborne disease and gastroenteritis outbreak worldwide. This virus is normally transmitted in crowded places, such as prisons, school, cruise ship, nursing homes, and similar setting places (Al Jassas et al., 2018). Other viral pathogens leading to gastroenteritis are Astrovirus, Adenovirus, and Sapovirus.

2.1.4.2 Bacteria

Bacterial gastroenteritis is more prevalent compared to virus gastroenteritis in developing countries and the peak of summer seasons (Farthing et al., 2013). The most common bacterial enteric pathogens in developing countries include *Escherichia coli*, *Salmonella spp*, *Shigella* spp, *Vibrio sp.*, including *Vibrio cholerae* and non-01

cholerae, and *Campylobacter* (Elliott, 2007; Galanis, 2007; Farthing et al., 2013; Barrett & Fhogartaigh, 2017). In developed countries, bacterial pathogens only account for 2% to 10% of cases of gastroenteritis. Notably, *Campylobacter* is the most common cause of bacterial gastroenteritis in developed countries (Elliott, 2007; Galanis, 2007;Barrett & Fhogartaigh, 2017).

2.1.4.3 Parasites

Parasites are most common in developing countries (Farthing et al., 2013). Parasite gastroenteritis is mainly caused by a protozoan (e.g., *Cryptosporidium*, *Giardias*, *Entamoeba histolyca*, and *Blastocystis hominis*) and helminth (e.g., *Ascaris lumbricoids*, *Trichuris trichiura*, and *Necator amiranus*) (Farthing et al., 2013; Al Jassas et al., 2018). *Cryptosporidium* is one of the main causes of the diarrheal outbreak, which mainly originates from contaminated supplies of water and swimming pool. It also leads to an outbreak due to the production of resistant oocytes, which could resist disinfectant (Krones & Högenauer, 2012; Al Jassas et al., 2018).

Although *Cryptosporidium* is common in children, they are frequently asymptomatic (Farthing et al., 2013). Although parasites leading to gastroenteritis are uncommon in developed countries, they only account for 1% to 8% cases of gastroenteritis with *Giardia lambia* in the USA, while *Cryptosporidium parvum* infections are the most common (Dennehy, 2019). Although parasites are less common in gastroenteritis, parasites can cause chronic gastroenteritis. Moreover, *Giardias* are the significant cause of chronic gastroenteritis, which are normally found in the contaminated water source, swimming pools, and day-care centre (Al Jassas et al., 2018).

2.1.5 Pathogenesis of Bacterial Gastroenteritis

The pathogenesis of bacterial gastroenteritis is related to the bacteria ability to overcome host defence. Although small intestines have an important function in the absorption of fluids, the enteric pathogen could alter the function of small intestines, which leads to diarrhoea. Different factors and mechanisms are associated with bacteria in causing diarrhoea (see **Figure 2.2**). These mechanisms include including the bacteria inoculum size, adherence, invasion, and production of toxin (Regina LaRocque et al., 2013; Al Jassas et al., 2018). To perform the management of gastroenteritis, the understanding of the pathophysiology of the disease is important (Al Jassas et al., 2018).

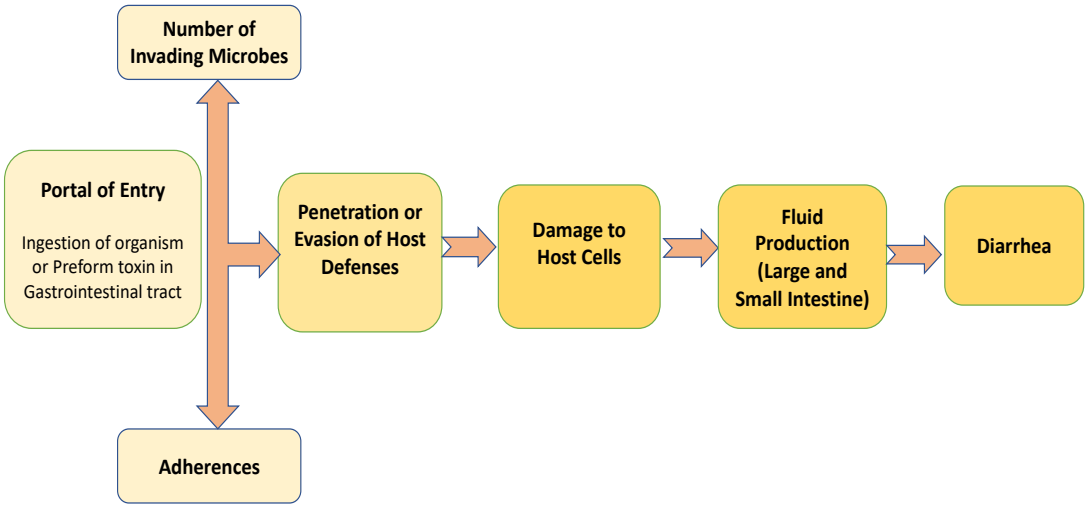

Figure 2.1: Microbial mechanism of pathogenicity

2.1.5.1 Inoculum size

One of the most critical factors of the pathology severity is the inoculum size. This size refers to the numbers of microorganisms, which ingestion leads to a variety of diseases for every species, such as Enterohemorrhagic *Escherichia coli* (EHEC) and *Shigella*. To illustrate, 10 to 100 bacteria could cause infection, while the ingestion of one million organisms for *V. cholerae* leads to disease (Regina LaRocque et al., 2013). Therefore, it is concluded that the inoculum size differs based on the bacteria (Richards & Douglas, 1978; Al Jassas et al., 2018).

2.1.5.2 Adherence

In the initial step for the pathogenic process, some organisms must adhere to gastrointestinal mucosa and compete with normal intestinal flora to cause disease. Adherence is also one of the significant virulence factors related to gastroenteritis. These bacteria adhere to intestinal epithelium either through pili/fimbriae, adhesins, or invasive strategies. Furthermore, specific cell-surface protein is involved in the attachment of bacteria to intestine cells, which is important as pathogenesis determinant. Different bacteria have a different mechanism in establishing this adherence, in which they produce several adhesive factors and protein. These elements assist the attachment of bacteria to the intestine wall and promote bacterial colonization and/or propagation (Pizarro-Cerdá & Cossart, 2006; Pan et al., 2014; Al Jassas et al., 2018).

V. cholerae will adhere to the brush border of small-intestinal enterocytes via specific surface adhesins (Al Jassas et al., 2018). The pili *E. coli* facilitates its adherence to the mucosal epithelial in a host-specific manner, while *E. coli* produces an adherence protein known as colonisation factor antigen, which is essential for the colonisation of

the upper small intestine by the organism before the production of enterotoxin. This is followed by the development of watery diarrhoea (Strömberg et al., 1990; Babai et al., 2000; Ron, 2006; Regina LaRocque et al., 2013). Major adhesins FbBP-A of *S. aureus* bind the ECM-associated fibronectin, cause the clustering of it, and trigger intracellular signalling (Ozeri et al., 2001). *Shigella*s have actin comet tail, which allows the formation of bacteria-containing extension at cell membrane invading the adjacent cell (Carayol & Tran Van Nhieu, 2013).

2.1.5.3 Toxin production

One of the most important virulence factors in the development of gastroenteritis is the production of toxins. Bacteria toxin could be grouped into two general classes, namely exotoxin and endotoxin (Edae & Wabalo, 2019). Specifically, enterotoxin leads to watery diarrhoea through its secretary effects on the mucosa of the small intestine (Dennehy, 2019). Furthermore, secretory diarrhoea could occur through enterotoxin-producing bacteria, such as *V. cholerae* and Enterotoxigenic *E. coli.* Cholera toxin produced by *V. cholerae* changes the electrolyte transport in the gut and alters the ion secretion and absorption, which leads to cholera (watery diarrhoea) (Dennehy, 2019).

Several strains of *E. coli* produce heat-labile enterotoxin(LT) and heat-stable enterotoxin(ST) (Regina LaRocque et al., 2013). Enterotoxigenic *E. coli* produces heat-labile enterotoxin similar to cholera toxin and causes secretory diarrhoea by the same mechanism. It also leads to heat-stable enterotoxin and diarrhoea by stimulating the intestinal mucosa for the release of inflammatory mediators. Notably, the production of one or more exotoxins is important in the pathogenesis of numerous enteric organisms, such as *Bacillus anthracis* and *Staphylococcus aureus.* These bacteria produce exotoxin

or pre-formed toxin, which leads to vomiting and abdominal cramp within a few hours after ingestion (Barrett & Fhogartaigh, 2017).

2.1.5.4 Invasion

Some bacteria, such as *Shigella* and enteroinvasive *E. coli* can invade the mucosal epithelial cells of the intestine. Following that, the bacteria tend to multiply intraepithelial and spread to adjacent cells. These organisms also lead to the destruction of intestinal mucosa and dysentery, in which the passage of small volume stools containing blood, mucus, and pus is often associated with fever, abdominal pain, and tenesmus(Regina LaRocque et al., 2013; Barrett & Fhogartaigh, 2017). However, although Salmonella causes inflammatory diarrheal through the invasion of the bowel mucosa, it is not associated with the destruction of enterocytes or the full clinical syndrome of dysentery (Regina LaRocque et al., 2013). Some invasive organisms, such as *E. coli* could produce cytotoxins as they invade the intestinal mucosa to induce an acute inflammatory reaction. In this case, the activation of cytokines and inflammatory mediators is involved (Dennehy, 2019). Some invasive organisms like *E. coli* also can produce cytotoxins; they invade the intestinal mucosa to induce an acute inflammatory reaction, involving the activation of cytokines and inflammatory mediators (Dennehy, 2019).

2.1.6 Common Bacteria Causing Gastroenteritis

2.1.6.1 *Shigella* spp

Shigella spp is a Gram-negative, non-motile, facultatively anaerobic, and non-spore-forming rod. The genus is divided into four serogroups with multiple serotypes, namely A (*S. dysenteri*, 12 serotypes), B (*S. flexneri*, six serotypes), C (*S. boydii*, 18 serotypes), and D (*S. sonnei*, one serotype). The majority of Shigella cases occur among children in underdeveloped regions, with *S. flexneri* causing the most endemic disease in the developing country. The highly virulent Shiga-toxin producing *S. dysenteri*, leads to an epidemic in developing countries and high rates of mortality if untreated (Barrett & Fhogartaigh, 2017).

Shigella is the cause of shigellosis. The systemic symptoms of shigellosis include abdominal pain, headache, watery diarrhoea, and/or dysentery (bloody and scanty mucoid stools). Other signs of shigellosis may include abdominal tenderness, vomiting, fever, and dehydration (Keusch., 1996; Humphries & Linscott, 2015). The infection is initiated by ingestion of *Shigellae* via faecal-oral, while the bacteria will invade the colonic epithelial and cause inflammatory colitis. These are the interdependent processes amplified by the local release of cytokines and the infiltration of inflammatory elements. Colitis in the rectosigmoid mucosa and concomitant malabsorption leads to the characteristic signs of bacillary dysentery, including scanty, unformed stools tinged with blood and mucus (Keusch., 1996).

Appropriate antibacterial treatment will shorten the duration of diarrhoea, fever, and reduce intestinal protein loss and pathogen excretion within one to two days. In the case of severely ill patients, antibacterial therapy could be administrated intravenously. Severe dysentery could be treated with ampicillin, trimethoprim-sulfamethoxazole, and

4-fluoroquinolone, such as ciprofloxacin for the in-patients of over 17 years old. Although vaccines for *Shigella* spp are not currently available, some promising candidates are developed.

2.1.6.2 *Salmonella* spp

Salmonella spp is a Gram-negative and flagellated facultative anaerobic bacillus, which is characterised by O, H, and Vi antigens. Over 2,500 serovars are currently classified as separate species (Giannella., 1996; Brenner et al., 2000; Popoff et al., 2000). Because of the diversity of the genus, several isolates may be challenging to identify due to atypical biochemical reactions. *S. enterica var. Typhimurium* and *S. enteritidis* are common gastrointestinal pathogens worldwide, with approximately 94 million cases of non-typhoid *Salmonella* gastroenteritis and 115,500 deaths (Majowicz et al., 2010). In developing countries, particularly the Indian subcontinent, typhoidal isolation leads to the majority of disease with an estimation of 21.6 million cases yearly and 216,500 deaths (Crump et al., 2004).

Salmonellosis ranges clinically from the common *Salmonella* gastroenteritis (diarrhoea, abdominal cramps, and fever) to enteric fevers (including typhoid fever). These types of fever are life-threatening febrile systemic illness, which requires prompt antibiotic therapy and causes focal infections and asymptomatic carrier state. The most common form of salmonellosis is the self-limited, uncomplicated gastroenteritis (P. H. Black et al., 1960; Giannella., 1996; Shimoni et al., 1999; Humphries & Linscott, 2015).

Pathogenic *Salmonellae* ingested in food survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. The invasion of epithelial cells stimulates the release of pro-inflammatory cytokines, which causes an inflammatory reaction (Finlay et al., 1989). The acute inflammatory

response leads to diarrhoea, ulceration, and destruction of the mucosa (Giannella., 1996), while the spreading of bacteria from the intestines leads to systemic disease.

Provided that there was no evidence that antimicrobial shortens the duration of disease, antimicrobial therapy is normally not implied for non-typhoidal *Salmonella* gastroenteritis (Ashkenazi & Cleary, 1991; Fasano, 2000). However, antibiotics are recommended for high risk of invasive disease among patients, including children of younger than three months, and patients with immunodeficiency, hemoglobinopathies, HIV infection, and chronic/severe colitis (Ashkenazi & Cleary, 1991; Fasano, 2000; Armon et al., 2001).

2.1.6.3 Diarrheagenic *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram-negative bacillus of the family Enterobacteriaceae. *E. coli* is a typical member of normal flora in the large intestine when these bacteria do not acquire element encoding for virulence factor. Virulent strains differ from nonvirulent *E. coli* only in terms of the genetic elements for virulence factors (Doyle & Dolores,1996). Furthermore, *E. coli* possesses a broad range of virulence factor (see **Table 2.2**), which could be categorised into adhesins and exotoxin. For gastroenteritis, six major groups of *E. coli* are present, namely *enterotoxigenic (ETEC)*, *enteropathogenic (EPEC)*, *enteroaggregative (EAEC)*, *enterohaemorrhagic (EHEC)*, and *enteroinvasive (EIEC) E. coli*, and *Shiga toxin-producing E. coli (STEC)* (Murray et al., 2015).

While *EPEC* constantly leads to watery diarrhoea, particularly among infants in developing countries, *ETEC* causes this disease among young children and becomes the most common cause of traveller's diarrhoea. Furthermore, although *EIEC* epidemiology is almost similar to *EPEC*, the symptom may range from watery diarrhoea to invasive

dysentery. Furthermore, *EHEH*, especially *E. coli* 0157, is the most common and important serogroup, which leads to a major outbreak in developed countries. This infection is associated with the life-threatening haemolytic uremic syndrome (HUS), which manifests as acute renal failure, microangiopathic haemolytic anaemia, and thrombocytopenia among infants. While *EAEC* cause chronic diarrhoea in children in developing countries. *STEC* is primarily caused by ruminants (e.g., cow); inadequate sanitization, faecal contamination in the river and stream, and insufficient control in meat and food industries lead to watery diarrhoea.

E. coli transmission occurs through the faecal-oral route. Pili/fimbriae allows bacteria to colonise the ileal mucosa, while cytotonic enterotoxins (encoded on a plasmid or bacteriophage DNA) induce watery diarrhoea. Plasmid-encoded invasion factors lead to the invasion of the mucosa, while the plasmid or bacteriophage-encoded cytotoxic enterotoxins lead to tissue damage. The presence of either of these factors leads to host inflammatory reaction and an influx of lymphocytes, which lead to dysentery (Doyle & Dolores, 1996). This enteric pathogen is treated symptomatically unless when disseminated disease occurs. The *in-vitro* susceptibility test guides antibiotic therapy. Additionally, an appropriate infection-control practice is used to reduce transmission (Doyle & Dolores, 1996; Murray et al., 2015).

Table 2.1: Specialise virulence factors associated with *Escherichia coli*

Bacteria	Adhesins	Exotoxins
ETEC	Colonisation factor antigen (CFA/I, CFA/II, CFA/III)	Heat labile toxin (LT-1); heat-stable toxin (STa)
EPEC	BFP; intimin	
EAEC	Aggregative adherence fimbriae (AFF/I, AAF/II, AAF/III)	Enteroaggregative heat-stable toxin; plasmid encode toxin
EHEC	BFP; intimin	Shiga toxin (Stx-1. Stx-2)
EIEC	Invasive plasmid antigen	Haemolysin (HlyA)
Uropahatogens	P pili; Dr fimbriae	

BFP, Bundle-forming pili; ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli.*

Source:(Murray et al., 2015)

2.1.6.4 *Campylobacter spp*

Campylobacter species are gram-negative, thin and curved, motile, and microaerobic. This species requires an atmosphere with reduced oxygen and increased carbon dioxide levels for aerobic growth. Therefore, special culture conditions are required for the recovery of these organisms from a clinical sample (Murray et al., 2015). Furthermore, *Campylobacter* causes acute gastroenteritis with diarrhoea, fever, nausea, abdominal pain, and vomiting (Blaser., 1996), and mainly leads to the most common cause of bacterial gastroenteritis in the United States (Murray et al., 2015).

The bacteria colonise the small and large intestines, causing inflammatory diarrhoea with fever, as indicated through the stools containing leukocytes and blood. However, the role of toxins in pathogenesis is unclear. *Campylobacter* enteritis is normally a self-limited illness, where antibiotic therapy is not required. Antibiotic only reserved for a patient will lead to severe illness or high-risk clinical circumstances

(Freeman & Wilcox, 1999; Fasano, 2000). Therefore, prevention and control of the illness remain fundamentally important.

2.1.6.5 *Vibrio cholerae*

Vibrio cholerae (V. cholerae) is a curved, motile, gram-negative rod with the single polar flagellum. It is subdivided into more than 200 serogroups, with serogroup 01 being further subdivided into serotype (Inaba, Ogawa, Hikojima) and biotype (classical, El-Tor) (Murray et al., 2009).

Cholera is endemic in developing countries, particularly Asia, Africa, and Central and South America. According to WHO's estimation, over 1.4 billion individuals worldwide are faced with the risk of developing cholera each year, with approximately 2.86 million and over 95,000 deaths occurring annually (M. Ali et al., 2015). The most endemic cholera in Asia and Africa is caused by El Tor (01) biotypes.

Although *V. cholerae* is alkaline-tolerant, it is sensitive to acid and is eliminated in the stomach. However, virulent survival organisms have adhesion, colonise the small bowel, and secrete the potent cholera enterotoxin. This toxin binds to the plasma membrane of intestinal epithelial cells and releases an enzymatically active subunit, which leads to a rise in cyclic adenosine 5^1-monophosphate (cAMP) production. The high intracellular cAMP leads to the high secretion of electrolytes and water into the intestinal lumen (Finkelstein, 1996).

The infection of *V. cholerae* leads to cholera disease, which is indicated through profuse watery diarrhoea without abdominal cramp. This symptom leads to severe dehydration and electrolyte imbalance. Furthermore, the hypovolemic shock could occur in four to 12 hours unless if a rehydration therapy is provided (Pickering, 2000). Notably, oral and parenteral rehydration is highly important for the restoration of fluid

and electrolyte abnormalities. Antibiotic therapy has been shown to eradicate vibrio's and reduce the duration of diarrhoea and the amount of fluid loss (Fasano, 2000; Pickering, 2000).

2.1.6.6 *Staphylococcus aureus*

Staphylococcus aureus (S. aureus) is a gram-positive, facultative anaerobe, and coccal (round shape) appearing in a grape-like cluster. Although *S. aureus* consists of common commensals of skin, it is one of the primary modes of transmission of *S. aureus* food poisoning enteritis. Provided that human is the primary source, the organism is present in food through cross-contamination. The presence of *S. aureus* in food will produce stable heat enterotoxin.

S. aureus produce varieties of toxin (e.g.; Staphylococcal enterotoxins and staphylococcal-like protein) which demonstrated emetic and non-emetic activity. Food poisoning and intoxication cause by the ingestion of preformed, heat-stable enterotoxin. Symptoms are rapid onset including sudden start of nausea, vomiting, stomach cramps, and with or without diarrhoea (Argudín et. al., 2010). If the toxin remain in the small intestine, it leading to inflammation and swelling, including abdominal pain, cramping, diarrhoea, dehydration, and fever (Murray et al., 2015). Provided that *S. aureus* enteritis is mainly caused by toxin production, proper hygiene and sanitisation are crucial in avoiding food contamination. Treatment for *S. aureus* is by supportive treatment, which based on symptoms, including fluid and electrolyte replacement (Vesterlund et al., 2006).

2.1.7 Management of Gastroenteritis

Bacterial gastroenteritis is rarely required for hospitalisation, except for significant dehydration, severe disease, and specific co-morbidities, which will increase the disease complication. Meanwhile, mortality is commonly caused by dehydration among individuals of young ages or the elderlies. The current support for the treatment of acute infectious gastroenteritis includes a symptomatic treatment, which consists of oral rehydration and early reintroduction of food. Anti-infective drug options, such as antibiotics and antiparasitic are available for the treatment of bacterial and parasitic causes of gastroenteritis. However, the use of this drug is not always highlighted, often unnecessary, and should be avoided in some cases.

2.1.7.1 Rehydration Therapy

Rehydration is the most prioritised treatment for gastroenteritis. World Health Organisation (WHO), The American Academy of Paediatrics (AAP), and the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommend oral rehydration solution (ORT) as the treatment of choice for children with mild-to-moderate gastroenteritis in developed and developing countries. This recommendation is based on the results of many randomised and controlled trials, including several large meta-analyses (Gastroenteritis, 1996; Bresee et al., 2003). Notably, ORT is the most cost-effective method to reduce the hospitalisation requirement.

However, ORT is contraindicated in the initial management of severe dehydration, children with paralytic ileus, frequent and persistent vomiting (more than four episodes per hour), and painful oral conditions, such as moderate to severe thrush. Therefore, intravenous rehydration is required (Anigilaje, 2018). Breast-feeding and

formula-feeding should be provided for infants, while the recommence of regular unrestricted diet should be recommended in all cases when dehydration is corrected (Fhogartaigh & Edgeworth, 2009). The lactose-free formula may be required for an infant with severe gastroenteritis until mucosal recovery after two weeks.

2.1.7.2 Nutrition

Normal diet is recommended when dehydration is corrected. Luminal contents are the known growth factor for enterocytes, which also assist in mucosal repair after an infection (Sandhu, 2001). The introduction of a normal diet within a few hours of rehydration has been shown to shorten the duration of disease. Children could consume a regular age-appropriate diet, which especially focuses on food with complex carbohydrates (e.g., rice, wheat, potatoes, bread, and cereals), lean meats, fruits, and vegetables while avoiding fatty food and simple carbohydrate.

2.1.7.3 Empirical Antibiotic Therapy

The antibiotic should not be routinely provided for patients with uncomplicated gastroenteritis. However, in determining the clinical or epidemiological clues to a causative pathogen, such as those in an outbreak setting, antibiotic could be provided for severely unwell patients with suspected invasive disease or sepsis. Furthermore, high-risk groups demonstrate that dehydration has serious consequences, which include traveller's diarrhoea (Fhogartaigh & Edgeworth, 2009; Shah et al., 2009; Barrett & Fhogartaigh, 2017). Antibiotics treatment can also be considered for premature infants, infants younger than six months old, immunocompromised individuals, children with chronic illnesses, patients with *Clostridium* difficile-associated pseudomembranous

enterocolitis, dysenteric shigellosis, or cholera (National Collaborating Centre for & Children's, 2009).

Fluoroquinolone, such as ciprofloxacin, is a preferred option for empirical therapy in the most setting. However, the increase in resistance, specifically in *Campylobacter*, non-typhi *Salmonella*, and *Shigella* spp leads to the use of macrolide, which includes azithromycin (Marcos & DuPont, 2007; Fhogartaigh & Edgeworth, 2009). With the lower association with the drug compared to quinolones, azithromycin shows better safety profile for children and pregnant women (Fhogartaigh & Edgeworth, 2009). The present data suggested that patients with haemorrhagic colitis secondary to EHEC, including *E. coli* O157: H7 had a higher possibility to develop the complication of haemolytic uremic syndrome upon treatments with the antibiotic. Therefore, antibiotic use should generally be avoided in these patients unless when toxicity or secondary bacteraemia is present during colon inflammation.

2.1.8 Adjunctive Management

Antimotility (i.e., loperamide) and antidiarrheal (i.e., kaolin-pectin) may be used in non-inflammatory diarrhoea in adult. However, this treatment is contraindicated for the treatment of acute gastroenteritis in children because it increases the risk of adverse effects, including ileus, drowsiness, and nausea. The anti-nausea and antiemetic drug (e.g., ondansetron) may be provided to facilitate the tolerance of oral rehydration in children of older than four years old and control nausea and vomiting, which are associated with acute gastroenteritis in adolescents and adults. Moreover, the supply of zinc, multivitamin, probiotic, and complementary and alternative medicine (CAM) assists in the management of gastroenteritis, especially among children.

2.1.8.1 Supplementation Zinc Therapy and Multivitamins in Children

As an adjunct to ORT, routine zinc therapy is beneficial for a moderate reduction of severe gastroenteritis and diarrheal episode among children in developing countries. The supplementation of zinc sulphate in recommended doses reduced the risk of diarrhoea in the following three months and non-accidental death by 50%. Therefore, the management of diarrhoea in malnourished children and persistent diarrhoea is important (Farthing et al., 2013).

Children with persistent diarrhoea should receive supplementary multivitamin and minerals. These supplements offer a broad range of vitamins and minerals, including a minimum of two recommended daily allowances (RDAs) of folate, vitamin A, zinc, magnesium, and copper (WHO, 2005). Vitamin A has been proven to shorten the duration and severity of diarrhoea in children (Villamor & Fawzi, 2000; Kheirkhah et al., 2016).

2.1.8.2 Probiotic

Probiotics are supplements containing live microbes, which normally include bacteria or yeast. In some cases, these microbes are used to prevent or treat symptoms of infectious diarrhoea. Several published meta-analyses of controlled clinical trials suggested the use of probiotic (especially *Lactobacillus*) in the treatment of acute infectious diarrhoea in children. The mechanism of actions is strain-specific, as proven through the efficacy of some strains of lactobacilli (e.g., *Lactobacillus casei GG* and *Lactobacillus reuteri* ATCC 55730) and *Saccharomyces boulardii*. These probiotics

are useful in reducing the severity and duration of acute infectious diarrhoea among children (Allen et al., 2003; Szajewska et al., 2014).

The oral administration of probiotics shortens the duration of acute diarrheal illness among children by approximately one day. Possible mechanisms of action for probiotics include synthesis of antimicrobial substances, competition with pathogens for nutrients, modification of toxins, and/or stimulation of nonspecific immune responses to pathogens, which facilitate their clearance (Allen et al., 2003; Szajewska et al., 2014). A recent meta-analysis recorded that probiotics might be effective for the prevention of *Clostridium difficile*-associated diarrhoea among patients who received antibiotics (Johnston et al., 2012). Despite the observation that probiotics assisted in the prevention of *C. difficile*-associated diarrhoea (CDAD), it was not proven that the probiotics were effective in treating CDAD not provided any added benefits as adjunctive therapy when combined with standard antibiotic treatment regimens.

2.1.8.3 Complementary and Alternative Medicine

Complementary and alternative treatment (CAM) for the preservation of health, disease treatment, and prevention is currently more prevalent among healthy individuals and patients with chronic disease. In gastroenteritis cases, the available treatments could be ineffective or lead to undesirable impacts, or less effective but safe for patients. However, this feature might lead to conflicting advice, different prescribed treatments, and high direct and indirect costs (Lane, Dalton Iii, et al., 2009; Lane, Weidler, et al., 2009; Hoekman et al., 2015). In this context, the highly prevalent use of complementary and alternative medicine (CAM) could be observed among patients suffering from gastrointestinal disorders.

Approximately two-thirds of the population in developed and developing countries were reported by WHO, including the important role of CAM and its function in the prevention and promotion of health for a large proportion of the population especially in developing countries (World Health, 2001). The use of CAM in gastroenteritis normally involves homoeopathy, nutritional therapy, Ayurvedic medicine, herbal medicine, and Meditation. A systematic review found that *Potentilla erecta*, carob bean juice, and herbal compound preparation used *Matricaria chamomilla* in the treatment of diarrhoea. It was also proven that peppermint oil reduced the duration, frequency, and severity of pain among children suffering from undifferentiated functional abdominal pain, although no serious adverse impact was reported (Anheyer et al., 2017).

2.2 Plant as an Alternative Medicine

Humans have been using a plant as a medicine since the middle Paleolithic ages of approximately 60,000 years ago (Fabricant & Farnsworth, 2001). In ancient times, leaves, flowers, stems, berries, and roots are used in health care for therapy and medicine. These plant-based medicines appear in the form of crude drugs such as teas, powder, poultices, and other herbal formulations (Balick & Cox, 1996; Samuelsson, 2004). Notably, medicinal plants remain the dominant form of medicine in most countries, with over three fourth of the world population primarily depending on the plant-origin products for their daily healthcare (Barrett & Kieffer, 2001).

Bearberry and cranberry juice were reported as the threats to urinary tract infection (Raz et al., 2004), while lemon, garlic, and tea tree were identified as the broad-spectrum antimicrobial agents (Heindrich et al., 2004). Some plant extracts with high medicinal value include the leaves of *Glyphea brevis*, which are used to treat

hepatitis and intestinal diseases (Noumi & Yomi, 2001). The extracts of roots, seed, and stem barks of *Monodora myistica* are used to treat scabies, malaria, helminthiasis, and dysenteric syndromes (Okpekon et al., 2004). Date fruits (*Phoenix dactylifera)* are consumed for dietary and medicinal use and the treatment of various ailments (antimicrobial, anti-inflammatory, gastroprotective, anti-cancer, and antioxidant) in a wide range of traditional systems of medicine (Baliga et al., 2011).

Many plants used as traditional medicine have now been validated through numerous scientific researches. The bioactive compounds of the plant are isolated for direct use in medicine. Drug discovery from medicinal plant leads to the isolation of early drugs, such as morphine from cocaine, codeine, quinine, and opium (Samuelsson, 2004; Balunas & Kinghorn, 2005).

2.3 Ethno-medicinal of Selected Plant Mention in The Holy Quran and Hadith

Treatment using medicinal plants has been performed since the early stage of human civilization (Malik.2001). In Islam, diseases are cured in two ways, namely the cure of soul through prayers and the cure of ailments through medicine (Saheb et al., 2019). Currently, natural remedies are used in 80% of the world population, mostly developing countries for primary healthcare due to cultural acceptability, ease of access, and affordability (El-Seedi et al., 2013). Therefore, natural products mentioned in the Holy Qur'an and Hadith have received attention from botanists, biochemists, and pharmacologists, leading to further research (Wani et al., 2011).

Various plants are mentioned in the Holy Quran, such as date palm, onion, garlic, pomegranate, olives, barley, figs, squash, and grape among others. The Prophet (PBUH) used certain herbs, recommended various plants as a cure for common diseases, and frequently commented on the nature and value of various food and spices.

He also recommended some food, such as dates, olives, fig, pomegranate, black seeds, grapes, and many others for alleviating several ailments.

2.3.1 Date Fruits as Mention in Quran and Hadith

The Prophet Muhammad (PBUH) stated, “Stomach is the home of disease. Diet is the main medicine” (Surah Al-A'raaf, verse 31). There has been a long history of the use of plant-based food for human ailments. In contrast to conventional medicine, which is mainly based on worldly acquired knowledge (Aqli), the Prophet Muhammad practices are more focused on holistic medicine, specifically the knowledge of religion or revealing knowledge. The Prophet recommended certain food, such as dates, olives, pomegranate, black seeds, grape, and figs among others as treatment of several ailments. Well known for their prescience, these food originated centuries before modern research explored on their benefits for human health (Syed, 2003; Deuraseh, 2006).

Sunnah (prophetic tradition) advocates the pre-date use of food as medicines for many centuries, which also serve as the precursors to the principles of modern treatment. Accordingly, Prophet Muhammad (PBUH) stated, “The one who sent down the disease sent down the remedy and for each disease, Almighty has given a cure.” People are encouraged to seek remedies and use them with skill and kindness (Saad et al., 2005). The following statement from the Prophet (PBUH) was narrated in the Sahih:

"Whoever ate seven Ajwa dates in the morning, will not be harmed by poison or magic the rest of that day."

Among all the food mentioned by the Prophet Muhammad (PBUH), dates are the most preferred food, which is also frequently mentioned in the Quran and hadith.

Dates are constantly described as “Al-Atyaban”, which means wholesome, nourishing, beneficial, ultimate, and complete food for survival. From the Islamic perspective, dates are mentioned 20 times in the Quran through several terms, such as *nakhil*, *al-nakhl*, *rutob*, and *nakhlah*, which indicate the best food for humans, as stated in Surah al-An‘am verse 141:

> *"It is He Who produce the gardens, with trellises and without, and dates, and tilth with produce of all kinds, and olives and pomegranates, similar (in-kind) and different (in variety): eat of their fruit in their season but render the dues that are proper on the day that the harvest is gathered. Nevertheless, waste not by excess: for Allah loveth not the wasters."*

As a fruit known for its nutrient value, the date is recommended, as shown through Rasulullah S.A.W’s statements through the narration of Hazrat Abdullah bin Umar R.A,

> *"There is a tree among the trees, which is similar to a Muslim (in goodness). Its leaves do not fall. What is that tree?” The Prophet S.A.W himself said, “that is the date palm tree.” (Farooqi, 1998)*

From the perspective of the Prophetic tradition, more than a few authentic texts of the Prophet regarding the consumption of dates are present, which reveal that dates are classified as the leading food of fruits chosen by the Prophet daily.

2.4 Date Fruits (*Phoenix dactylifera L.)*

2.4.1 Background

Date palm is a plant belonging to the genus Phoenix. The botanical name for the dates (*Phoenix dactylifera*) was derived from the Phoenician word "*Phoenix,"* which means trees. Following that, the word "*dactylifera*" originates from the Greek word "daktulos", which means the radius reflective of the fruit (Biglari, 2009). The Arabic word "nakhl" refers to the date palm tree, while the word "tamr" refers to the fruit. Notably, the date palm (*Phoenix dactylifera* L.*,* Arecaceae) is one of the oldest plants cultivated on Earth and widely planted in hot and dry climates of Asia, Middle East, Africa, and Arabian Peninsula. This plant is an important food resource for the day-to-day lives of people from the aforementioned regions (Al-Farsi et al., 2005; Al-Farsi et al., 2007). Fruits of the date palm are the main sources of staple food in the kingdom of Saudi Arabia, the Middle East, and South Asian countries. Similarly, dates constantly play an important role in the economic and social lives of people from these regions.

2.4.2 Stages of Growth and Maturity of Date Palm Fruit

The development of date comprises several stages, namely Hababouk, Kimri, Khalal, Rutab, and Tamar (Al-Farsi* & Lee, 2008). The appearances of date fruit in the first four stages are illustrated in **Figure 2.2.** Many studies characterised the physical and chemical changes that occurred in dates as they passed through these stages (Al-Shahib & Marshall, 2002).

Hababouk is the first stage of the date development, where the unripe dates are of a pea size with a weight of approximately one gram (Baliga et al., 2011). At the Kimri stage, the moisture content of fruit is considerably high (85%), while the size, weight**,** and sugar content of the date increase. Furthermore, the fruit colour begins to turn

yellow or red at the end of this stage based on the data variety. At the Khalal stage, dates are red, pink, or yellow with a hard texture. As the moisture content of fruit decreases gradually, the sucrose starts to be converted to reduced sugars (glucose and fructose). Notably, the sucrose conversion occurs at a high rate in some varieties, while the fruit is palatable (Al-Farsi* & Lee, 2008). The moisture content of fruit at the Rutab stage is 35%. The fruit tip begins to turn brown as the date loses its weight due to moisture loss. Moreover, tissue softening and skin browning also take place, followed by further conversion of sucrose into glucose and fructose. It is noteworthy that dates are sold fresh. At the Tamar stage, dates are thoroughly dried with 20% moisture content and entirely ripped (El-Sharnouby & Al-Eid, 2009).

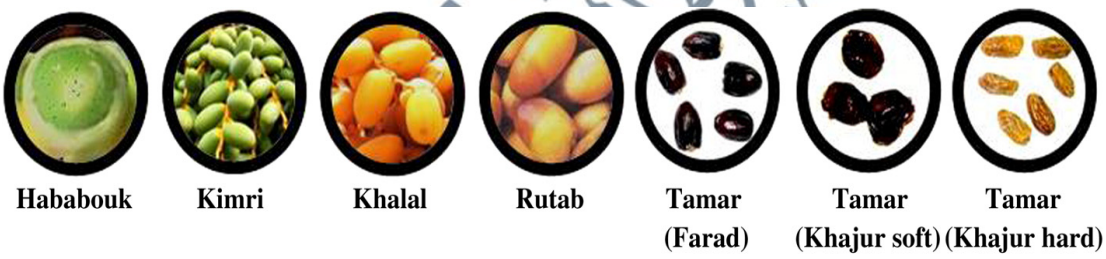

Figure 2.2 : Stages of growth and maturity of date palm fruit.

Source: (Baliga et al., 2011)

2.4.3 Nutritional Composition of Date fruits

Dates are good sources of energy, vitamins, and a group of elements, including phosphorus, iron, potassium, and a significant amount of calcium (Hafiz et al., 1980; Anwar-Shinwari, 1987). The chemical composition of the date demonstrates that the date flesh is an important source of sugar (81% - 88%; mainly fructose, glucose, and sucrose), dietary fibre (5% - 8.5%) and a small amount of protein, fat, ash, and polyphenol (AlHooti et al., 1995; Al-Shahib & Marshall, 2002; Al-Farsi et al., 2007).

2.4.3.1 Carbohydrate Content

The major nutritional constituents in dates are carbohydrate, including simple and complex carbohydrate mainly consisting of sucrose, glucose, fructose, and a small number of polysaccharides, such as starch and cellulose (Al-Farsi* & Lee, 2008). Sucrose is completely inverted into glucose and fructose at the Tamar stage (Manickavasagan et al., 2012). The total sugar in date palm fruit increases as the fruit ripening progresses from the Kimri to Tamar stage, followed by its increase from 3.4% to 7.7% in the Kimri stage and from 43.40% to 87.54% in the Tamar stage (Al-Shahib & Marshall, 2002; Manickavasagan et al., 2012).

2.4.3.2 Fatty acids Content

Date flesh contains fatty acids, which are mainly concentrated in the skin (Manickavasagan et al., 2012). The fatty acid content in the date flesh from the varieties in different regions was studied by many researchers (AlHooti et al., 1995; Al-Farsi et al., 2005; Al-Farsi et al., 2007) who found that the oil content in flesh was low and varied from 0.1% to 3.25% of the fresh weight. The major free fatty acids identified in

date flesh oil in order of abundance were caprylic acid, linoleic acid, lauric acid, pelargonic acid, and myristic acid (Manickavasagan et al., 2012).

2.4.3.3 Vitamin Content

The flesh of date palm is an important source of water-soluble vitamins. Date contains a minimum of six vitamins, such as C, B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin), and vitamin A, which was found in date palm fruit (Al-Shahib & Marshall, 2002). Vitamins A and C were found in relatively low concentrations in dried dates (Manickavasagan et al., 2012).

2.4.3.4 Mineral Content

Date palm fruit is rich in minerals and has a proportion of potassium of 25% higher than the proportion found in banana (Sulieman et al., 2012). Based on a range of studies observing the mineral content of date palm flesh, it was found that potassium was the most abundant mineral with significant quantities of calcium, sodium, phosphorus, magnesium, iron, zinc, copper, and manganese (Ahmed et al., 1995; Al-Farsi et al., 2005; Ismail et al., 2008; Khan et al., 2008; Chaira et al., 2009). The mineral content of date palm fruit-based on these studies is presented in **Table 2.3**.

Table 2.2: Range of mineral composition of a date fruit from different cultivars

Mineral	Composition (mg/100 g)
Potassium	400–1160
Calcium	15–90
Sodium	3–290
Phosphorus	60–100
Magnesium	50–90
Iron	0.3–6
Zinc	0.2–2.4
Copper	0.1–2.3

Data based on Ahmed et al., (1995); AlFarsi et al., (2005); Ismail et al., (2006); Khan et al., (2008).

2.4.4 Phytochemical Composition of Date Fruits

Phytochemicals are secondary plant metabolites or integral cellular components, which also have gained increased interest among several investigators, including clinicians due to their antioxidant activity, cholesterol-lowering properties, and other potential health benefits, such as chemoprevention of cancer, prevention of diabetes, and cardiovascular diseases. Phytochemical analysis of the date plant showed the presence of alkaloids, steroids, flavonoids, sterols, and tannins.

Date fruits are renowned for the presence of many classes of bioactive components, such as carotenoids, polyphenols mainly phenolic acids, isoflavones, lignans, and flavonoids, tannins, and sterols (J. A. Duke & Beckstrom-Sternberg, 2007; Al-Farsi* & Lee, 2008; James A. Duke, 2017). Several published studies are related to the chemical composition of different varieties of dates grown in different parts of the world (Al-Farsi et al., 2005; Liolios et al., 2009).

The quantity and composition of the phytochemicals in date fruits vary widely depending on the date varieties, including the stage of maturation, storage, postharvest processing, the extent of hydration, experimental conditions used for the analysis, and

the geographical origin of the dates (Al-Laith, 2009; Amorós et al., 2009; Al-Turki et al., 2010). It was reported that date experienced the loss of total carotenoids (up to 30%) and anthocyanins (93%), including the increase in total phenolics (22% - 153%) and phenolic acids (64% - 107%) upon significant sun-drying (Al-Farsi et al., 2005).

2.4.4.1 Phenolic acids

Phenolic acids constitute one of the main classes of secondary metabolites and have been a subject in studies in recent years. These acids contain hydroxylated benzene ring with one or more carboxyl groups attached directly or indirectly to them. Similar to other phenolics, these acids are believed to assist in plant defence against a variety of pests and pathogens. Based on Mansouri *et al.*'s (2005) analysis of the phenolic profile of seven Algerian varieties of date, it was found that the acids consisted of p-coumaric, ferulic and sinapic acids, some cinnamic acid derivatives, and three different isomers of 5-o-caffeoyl shikimic acid.

According to Al-Farsi *et al.* (2005), studies with three varieties of Omani dates demonstrated the presence of both free (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and bound phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and o-coumaric acid). Comparative studies with fresh and dried Fard dates demonstrated that a significant increase in phenolic content led to drying due to the degradation of tannins and maturation of degradative enzymes at higher temperatures (Al-Farsi et al., 2005). Chaira *et al.* (2009) recently observed that among the common date cultivars of Tunisian, the Mermella variety was the lowest (5.73 mg/100 g fresh weight), while the Korkobbi variety showed the highest phenolic content (54.66 mg/100 g fresh weight) (Chaira et al., 2009).

2.4.4.2 Sterols

Sterols or steroid alcohols are the subgroups of steroids with a hydroxyl group at the three-position of the A-ring, which are also amphipathic lipids. Known as phytosterols, the sterols of plants possess myriad health benefits (Liolios et al., 2009). Based on Kikuchi and Miki's (1978) analysis of the sterols of date fruit, it was observed that the sterols comprised cholesterol, campesterol, stigmasterol, β-sitosterol, and isofucosterol.

2.4.4.3 Carotenoids

Carotenoids is a class of natural fat-soluble pigments and imparts bright colouration to the plants. Notably, it is an important source of vitamin A, which protects the cell from the deleterious effects of free radicals by functioning as antioxidants (Di Mascio et al., 1991). Studies also demonstrated that certain dates contained carotenoids, lutein, β-carotene, and neoxanthin (Boudries et al., 2007).

A significant decline in the carotenoid levels occurred during the transition from the Khalal through Tamar stage. During the ripening process, the levels of provitamin A increased slightly in the Deglet-Noor variety and decreased in Tantebougte and Hamraya (Boudries et al., 2007). The analysis of the total carotenoid contents in both fresh and dried varieties of Fard, Khasab, and Khalas suggested the loss of carotenoids during sun-drying (Al-Farsi et al., 2005).

2.4.4.4 Procyanidins (Condensed tannin)

Procyanidins are condensed tannins and the main precursors of blue-violet and red pigments in fruits, vegetables, nuts, seeds, flowers, and barks (Fine, 2000). Using the acetone–water–acetic acid solvent extraction method, Hong et al. (2006) extracted procyanidins from Deglet Noor variety of dates in the Khalal stage of maturity. The chemical analysis suggested that the procyanidin was present as higher molecular weight polymers, undecamers through heptadecamers, and decamers (Hong et al., 2006).

Tannins may prevent herbivores from predation (Feeny, 1970) and microorganisms either by increasing the resistance against pathogens (Brownlee et al., 1990) or protecting vital tissues against decay, such as wood. Notably, the toxicity of tannins towards microorganisms is well documented. Toxicity studies incorporated various fields of research, such as food science, wood science, soil science, plant pathology, pharmacology, and human and animal nutrition. Furthermore, toxicity is normally estimated by measuring the reduction of the *in-vitro* growth of the mycelium for filamentous fungi. This estimation was also performed using plate count methods, disk diffusion methods, nephelometry, or respirometry for bacteria and yeasts. Some biochemical parameters, characteristics of the metabolism of certain microorganisms, were employed, which included cellulose degradation, glucan synthesis, nitrate, methane, or ethanol production.

2.4.4.5 Flavonoids

Flavonoids in plants possess various health benefits, which include antioxidant and radical scavenging activities, reduction of certain chronic diseases, and prevention of several cardiovascular disorders and specific types of cancerous processes (Tapas et al., 2008). Based on Hong et al.'s (2006) assessment of the flavonoid content in the Deglet Noor variety during the Khalal stage of maturity, 13 flavonoid glycosides of luteolin, quercetin, and apigenin were identified. It was also observed that both methylated and sulfated forms of luteolin and quercetin were present as mono-, di-, and triglycosylated conjugates, while apigenin was present only as of the glycoside. Quercetin and luteolin mainly formed O-glycosidic linkages, while apigenin was present as the C-glycoside. Currently, dates were also the only distinct food containing flavonoid sulfates (Hong et al., 2006). Chaira et al. (2009) recently reported that among the famous Tunisian dates, the highest content of flavonoids was present in the Korkobbi variety (54.46 mg quercetin equivalents/100 g fresh weight).

2.4.4.6 Anthocyanins

Anthocyanins are water-soluble vacuolar pigments, which may appear in red, purple, or blue. Widely distributed in many fruits, vegetables, cereal grains, and flowers, they show potential health benefits (Hong Wang et al., 1997). Studies by Al Farsi et al. (2005) recorded that among the analysed fresh date varieties, the highest amount of anthocyanins was identified in Khasab (1.5 mg/100 g), followed by Fard (0.9 mg/100 g) and Khalas (0.87 mg/100 g) varieties. Moreover, a direct correlation was found between the levels of anthocyanin and fruit colour. Anthocyanins were

detected only in fresh dates, indicating that they were possibly destroyed during sun drying (Al-Farsi et al., 2005).

2.4.5 The Nutraceutical Benefit of Date Fruits

Date fruits are claimed to offer essential nutrients and benefits in human health (Manickavasagan et al., 2012). These fruits are also high in antioxidants, which alleviate oxidative stress associated with various disease (Baliga et al., 2011; Benmeddour et al., 2013; Daoud et al., 2019). Date fruits are highly nutritious and show antimicrobial activity (Al-Daihan & Bhat, 2012; Bhat & Al-Daihan, 2012; Aamir et al., 2013; El Sohaimy et al., 2015; Samad et al., 2016), anticancer activity (Zhang et al., 2013; Eid et al., 2014), antioxidant potential (Al-Turki et al., 2010; Martín-Sánchez et al., 2014; El Sohaimy et al., 2015), antidiabetic activity (Hasan & Mohieldein, 2016; Khalid et al., 2017), neuroprotective (Pujari et al., 2011; Souli et al., 2014), anti-inflammatory activities (El Arem et al., 2014; Bouhlali et al., 2016), and hepatoprotective potential (Al-Qarawi et al., 2008; El Arem et al., 2014).

2.4.5.1 Traditional Nutraceutical Practises

Dates have been widely used in traditional medicine preparation for many centuries, especially in the Middle-east and Indian subcontinents, including Egypt, India, Morocco, Iran, and Iraq (Krentz & Bailey, 2005; Khare, 2007; Hussain et al., 2020). The dates are used traditionally as diabetes remedy in Morocco (Tahraoui et al., 2007), while date-palm pollen and male flower are used in ancient Egypt as aphrodisiac and increase in fertility (Khare, 2007). Dates have also been used as anti-hypertension for centuries. Moreover, the phytochemicals present in dates are associated with the

reduction of hypertension, hypercholesterolemia, and lipoprotein oxidation (Vayalil, 2002).

Although few scientific and clinical evidence were available regarding the medicinal properties of dates, many recent studies proved the importance and benefit of dates. To illustrate, date kernel showed anti-ageing properties and reduced skin wrinkling in women (Bauza et al., 2002). Beneficial effects were found from the date pulp boiled in milk on pregnant and lactating mothers (Puri et al., 2000), including their benefits as expectorant, laxative, diuretic and demulcent (Khare, 2007; Barh & Mazumdar, 2008).

2.4.5.2 Antioxidant Potential

Date fruits are rich in phytochemicals, such as polyphenol, flavonoids, carotenoids, sterols, and tannins, which have potent antioxidant properties (Martín-Sánchez et al., 2014). The antioxidant obtains free radicals, assists in the prevention of chronic diseases, including heart attack, cancer, and Alzheimer's and Parkinson's disease (G. H. Kim et al., 2015). Furthermore, carotenoids in date fruits are acting as a strong antioxidant, which functions as the precursor of vitamin A and protects cells from free radicals (Julia et al., 2015). Dates are also rich in phytosterols, such as campesterol, stigmasterol, and isofucosterol, with the phytosterol being bound to the estrogen receptor and causing antiestrogenic effects (Al-Turki et al., 2010). Moreover, phenolic compound demonstrates antioxidant properties (Rahmani et al., 2014), while the polyphenols in date fruits help reduce oxygen species (ROS) for cancer prevention (Borek et al., 1986).

2.4.5.3 Anti-diabetic Activity

Active compounds in dates, such as flavonoids, steroids, phenolic, and saponin are anti-diabetic agents. These compounds possess free-radical scavenging ability, as proven in diabetic rat model studies (Zhang et al., 2015; Hasan & Mohieldein, 2016). Furthermore, date extracts also enhance the pathological indicator of diabetic neuropathy in diabetic rats (Zangiabadi et al., 2011). The anti-diabetic impact may be related to phenolic, which inhibits α-glucosidase and affects glucose absorption in small intestine and kidneys (Khalid et al., 2017). Diosmetin glycoside in dates also increase insulin excretion and stimulate glycogen synthase, which maintains blood glucose stability (Singh et al., 2012). Diabetic male rats treated with diosmetin glycoside significantly increases serum testosterone level and decrease the total prostatic phosphate activity (Singh et al., 2012).

2.4.5.4 Neuroprotective activity

Date fruits demonstrated a neuroprotective effect against bilateral common carotid artery occlusion that induced oxidative stress and neuronal damage (Pujari et al., 2011).

2.4.5.5 Anti-inflammatory Activities

Anti-inflammatory properties and impacts against the inflammatory associate disorder of date fruits were studied by many researchers (Ferrero-Miliani et al., 2007; Al-Qarawi et al., 2008; Vayalil, 2012; Rahmani et al., 2014). The *in-vitro* study by Taleb et al. (2016) found that polyphenol in date syrup exhibited anti-inflammatory activity by reducing angiogenic responses, such as cell migration, tube formation, and matrix

metalloproteinase (Taleb et al., 2016). Based on another study by Das et al. (2015) on embryonic kidney cell line (HEK) and murine RAW macrophages-induced inflammation, it was found that the date extract could therapeutically inhibit intracellular oxidative stress (Das et al., 2015).

The anti-inflammatory activity of dates is mainly regulated through phenolic compounds, such as ferulic acid, caffeic acids, and syringic acid (Jung et al., 2007; Lin et al., 2010) and flavonoids (Das et al., 2015). Date fruits exhibited good anti-inflammatory activity from a mice model with carrageenan-induced through a noticeable reduction of paw volume (Taleb et al., 2016). Date sap also exhibited good anti-inflammatory as it enhanced wound healing in Wistar rats (Abdennabi et al., 2016).

2.4.5.6 Hepatoprotective Potential

A study by Al-Qawari et al. (2014) was performed on Wistar rats with carbon tetrachloride (CCl4)-induced hepatotoxicity. The date fruits extracts were administered orally to the Wistar rat, which then led to a significant reduction of $CC1_4$-induced elevation in plasma enzyme and bilirubin concentration. This was followed by improvement in the morphological and histological liver damage in rats (Al-Qarawi et al., 2008). Furthermore, the significant hepaprotective impact of the date extracts was observed on Wistar rats with di-chloroacetic acid (DCA) induced liver damage. Based on the oral administration of date extracts to male Wistar rat at 0.5 and 2 g/l for two months, the hepatic marker enzyme levels and conjugated bilirubin were reduced (El Arem et al., 2014).

2.4.5.7 Dates as Antimicrobial

The use of a natural derived antimicrobial agent is considered an alternative and solution for antibiotic-resistance bacteria due to their cost-effectiveness and fewer side effects (Bhat & Al-Daihan, 2012). Previous studies demonstrated the benefits of dates as anti-helminthic (Abdel-Ghaffar et al., 2011; Klimpel et al., 2011), anti-fungal (Daoud et al., 2019), anti-viral (Jassim & Naji, 2010), and antibacterial (Ayachi et al., 2009; Al-Daihan & Bhat, 2012; Kchaou et al., 2013; Al Qroom & Al Momani, 2014; Bouhlali et al., 2016; Samad et al., 2016).

Notably, although date fruits are most commonly used for the testing of antibacterial activity, different parts of the date palm, such as the leaf, fruits, seed, bark, and spathe are used to determine their antibacterial activity against pathogenic bacteria. Many researchers reported on antibacterial properties in different date cultivars, such as Medjool, Sukkari, and Deglet-nour. The antibacterial activity of leaf, spathe, and bark of *Phoenix dactylifera* was also reviewed (Al-Daihan & Bhat, 2012; Al-zoreky & Al-Taher, 2015). Different extracts (aqueous, methanol, petroleum ether, and acetone) were tested against standard gram-positive (*S. aureus, S. saprophyticus, L. monocytogenes,* and *S. pyogenes*) and gram-negative strain (*E. coli, P. aeruginosa,* and *Salmonella sp.*). It was found that all parts of the plant showed antibacterial potential in all extracts, while the aqueous extracts of leaf and bark were more effective compared to methanol and acetone extracts. Moreover, methanol extracts of spathe were more effective compared to methanol and petroleum ether. *Phoenix dactylifera* pits were also found to inhibit the activity of *E. coli*, *B. subtilis*, *S. aureus*, *P.aeruginosa, S. pyogenes*, *S. flexneri*, and *Klebsiella pneumonia* (Al-Daihan & Bhat, 2012; Perveen et al., 2012).

The study by Al-Daihan and Bhat (2012) on Mosaifah cultivar recorded that antibacterial activity against *S. aureus, E. coli, S. pyogenes*, and *P. aeruginosa* was present in date extracts. Ajwa dates fruits also exhibited good antibacterial activity against gram-positive and gram-negative bacteria (Al-Judaibi et al., 2014; Samad et al., 2016). Other in vitro studies performed on other date varieties (e.g., Deglet-nour, Medjool, Nabtet ali, Sukkari, and Mabroom) demonstrated antibacterial activity against *Salmonella sp*, *Klebsiella sp*, *Micrococcus sp*, *Shigella spp*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *S. pyogenes*, *Serratia marcescens*, and *L. monocytogene* (Ayachi et al., 2009; Kchaou et al., 2013; Al Qroom & Al Momani, 2014; Bouhlali et al., 2016; Samad et al., 2016). Based on these antibacterial studies, methanol extracts were found to exhibit the highest antibacterial activity. Gram-positive bacteria were more sensitive to date fruits extracts compared to gram-negative bacteria (Farhana et al., 2017).

The *in vivo* study on date fruits showed the neutralisation of the haemolytic activity of the streptococcal exotoxin, streptolysin O. Although resistance to haemolytic activity of streptolysin O was found from the erythrocytes obtained from date fruits fed to the volunteers, date intake did not affect the titer of antistreptolysin O antibodies. It was suggested that the inhibitory substance might be steroidal, while the neutralisation property occurred through erythrocyte membrane stabilisation and inhibition of streptolysin O enzyme (Abuharfeil et al., 1999). It was proposed that phytochemical in date fruits (i.e., phenolic, flavonoids, and tannin) had a role in the antimicrobial activity.

However, the studies performed on the underlying mechanism of the active compound for the antibacterial activity of date fruits were lacking. Therefore, this subject, including the antibacterial activity of date fruits, will be highlighted and

investigated in this study. Despite the numerous works of research performed on the antibacterial activity, there was an inadequate study performed on the anti-adhesive properties of date fruits, which are important in the prevention of gastroenteritis.