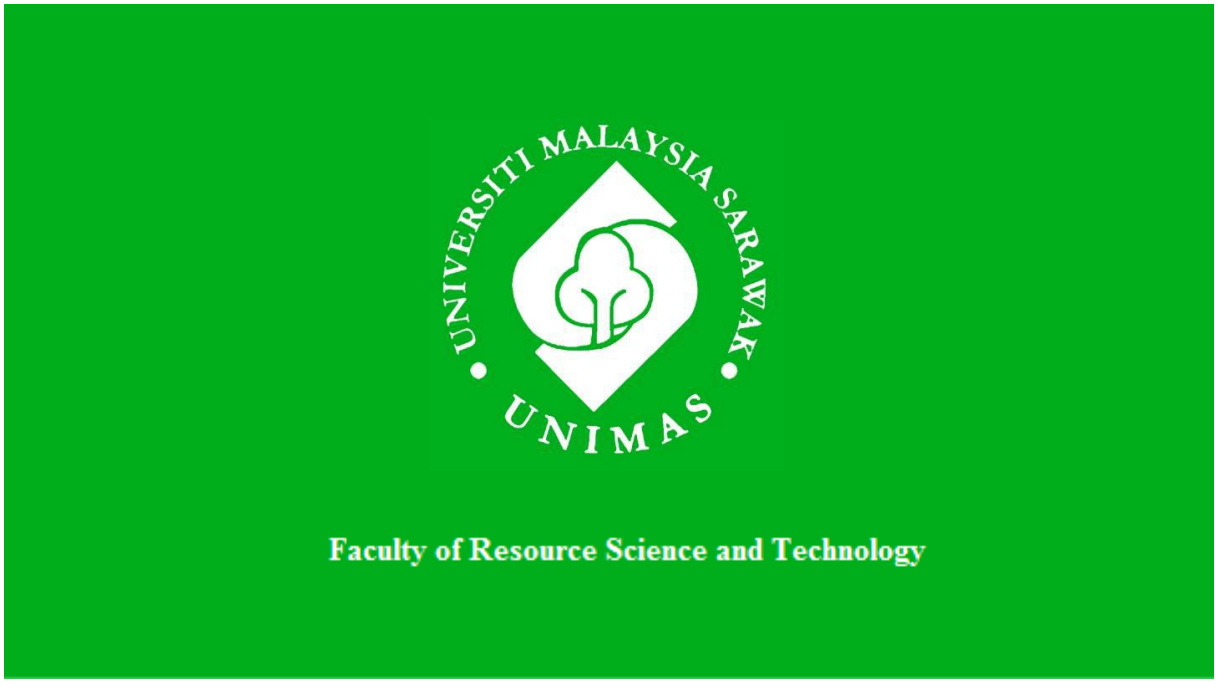

Preliminary study of antibacterial properties in *Mapania*

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This project is submitted in partial fulfilment of the requirement for degree of Bachelor of Science with Honours

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Declaration

I declare that no portion of this research work had been submitted to support the application of other degree or qualification at any other universities or institutions of higher learning

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LIST OF ABBREVIATIONS

2-D paper chromatography	Two dimensional paper chromatography
µg	Micro gram
AA	Acetic acid
BAW	n-Butanol, Acetic acid, Water
D+HUS	Diarrhea-associated haemolytic uremic syndrome
Nm	Nano meter
MIC	Minimum inhibitory concentration
mL	Milli litter
NH_3	Ammonia
Rf value	Distance move by the spot / distance move by solvent
SPSS	Statistical package for social science

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Preliminary study of antibacterial properties in *Mapania*

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ABSTRACT

The aim of this research was to study the antibacterial properties from the leaves and rhizomes of five different *Mapania* species (*M. cuspidata* var *petiolata, M. meditensis, M. lorea, M. walichii* and *M. hispida*). Ten group flavonoids compound were identified which are 5-deoxyisoflavones, isoflavones, chalcone, flavanones, anthocyanins, flavone glycoside, flavones, flavonols, glycosylflavones and aurones by using 2-D chromatography paper. Antibacterial activities of ethanolic flavonoids extract from leaves and rhizomes of each species were carried out against *E. coli* 0157:H7 and *B. cereus* ATTC 33019. In disc diffusion test methods, it was found that *M. cuspidata* var *petiolata* has showed high inhibition zone (1.4 cm) among all the species with the MIC of 0.125 mg/ml in both *E. coli* 0157:H7 and *B. cereus* ATTC 33019. The leaves have the highly inhibition zone than the rhizomes in both *E.coli* and *B. cereus.*

Key words: antibacterial, *Bacillus cereus, Escherichia coli*, flavonoids, *Mapania*, MIC

ABSTRAK

Tujuan kajian ini dikaji adalah untuk mengkaji ciri-ciri antibakteria yang ada pada daun dan akar daripada lima spesis *Mapania* yang berlainan (*M. cuspidata* var *petiolata, M. meditensis, M. lorea, M. walichii* dan *M. hispida*). Sepuluh kumpulan flavonoids di kesan iaitu adalah 5-deoxyisoflavones, isoflavones, chalcone, flavanones, anthocyanins, flavone glycoside, flavones, flavonols, glycosylflavones dan aurones dengan menggunakan 2-D paper kromatografi. Aktiviti antibacteria daripada flavonoids yang telah ekstrak dengan ethanol daripada daun dan akar dari setiap spesis diuji dengan *E. coli* 0157:H7 and *B. cereus* ATTC 33019. Dalam kaedah difusi disk, didapati *M. cuspidata* var *petiolata* mempunyai zon perencatan paling tinggi (1.4 cm) diantara kelima – lima spesis dengan MIC 0.125 mg/ml apabila diuji dengan kedua *E. coli* 0157:H7 dan *B. cereus* ATTC 33019. Bahagian daun mempunyai zon perencatan bacteria yang tinggi berbanding bahagian akar apabila diuji pada . *coli* 0157:H7 dan *B. cereus* ATTC 33019.

Kata kunci: antibakteria, *Bacillus cereus, Escherichia coli*, flavonoids, *Mapania*, MIC

Chapter 1

Introduction

Cyperaceae is the other grass family besides Poaceae but with triangular stem (Bryson & Carter, 2008). With a large family they have, Cyperaceae become the third largest family in monocotyledon. Cyperaceae has been neglected as weeds but, some research found that most species in Cyperaceae has considerable important in both economic and conservation of nature (Simpson et al, 2003). This includes *Mapania*, which some of its species contributes a lot to local people. The local name *Mapania* is 'pandan tikus' or also known as 'rumput rusiga'. Most of the studies regarding *Mapania* are about the morphology, molecular, evolution and the uses, phylogeny, and phytochemical properties. Somehow, it is getting more interesting with *Mapania* when some of the local use it as traditional medicines. The 'pandan tikus' (*Mapania cuspidata*) especially are the most frequent species used in postnatal treatment. The rhizomes or leaves were boiled for drinking. There is no scientific evidence on the *Mapania* antibacterial properties. Thus, five selected *Mapania* were collected at Sungai Mutut and Sematan to be tested for the antibacteria properties. The selected *Mapania* were *M. walichii, M. hispida, M. cuspidata, M. lorea* and *M. meditensis.* The compound used for testing was the flavonoids. This is due to its known properties of defending the plant itself from infections and some potentially benefits to human.

The bacteria selected for testing were *Escherichia coli* 0157:H7 and *Baccillus cereus* ATCC 33019. These bacteria were chosen for its pathogenicity towards human. *Escherichia coli* can be easily found in human and animal intestines (Public Health Agency of Canada, 2014). Most of *Escherichia coli* is harmless but the strain *Escherichia*

coli 0157:H7 is pathogenic. It produces some toxin known as Shiga toxin and can cause serious infection which may lead to death. The *Escherichia coli* 0157:H7 can cause serious kidney damage and severe diarrhea (Delaware Health and Social Service, 2011).

Baccillus cereus ATCC 33019 can cause serious food-borne and anthrax disease. These bacteria strain produce toxin called emetic toxin and enterotoxin. B*accillus cereus* can be easily found in environment and also in food. These bacteria are spore forming bacterium and the spore is very tough, it can survive even in harsh environment. Due to their tough physical nature and metabolic dormancy, the *Bacillus cereus* spore becomes resistance to environmental stress than vegetative cells (Shangkuan et al, 2000) .

1.1 Problem statement:

The existing knowledge of *Mapania* are on its morphology, molecular, phylogeny, evolution and the uses, phylogeny, and phytochemical contain. There are few studies however, on antibacterial properties of *Mapania*. Thus this research aimed to a preliminary study of the antibacterial properties from the leaves and rhizomes of five different *Mapania* species.

1.2 Objectives:

1. To determine the inhibition zone of bacteria between leaves and rhizomes of five different *Mapania* species.

2. To determine the inhibition zone of bacteria between the five different species of *Mapania*

Chapter 2

Literature Review

2.1. *Mapania* morphology and distribution

After Orchidaeae and Poaceae, the Cyperaceae are the third largest family in monocotyledon with 104 genera and 5000 species. Figure 2.1.1 show the anatomy of grass family. The family become an economic prominent as they are used in many way such as for weaving, fuel, construction, in providing food, medicine, perfumery materials and also in agriculture weeds. Additionally, Cyperaceae also needed as dependable indicator for declination of habitat in wet land ecosystem and also the major component in wet land ecosystem (Simpson, Furness, Hodkinson, Muasya & Chase, 2003).

Cyperaceae is divided into two subfamilies which are, Cyperoideae and Mapanioideae based on molecular data. Cyperoideae, predominantly discover in grassland environments of temperate and tropical regions. Mapanioideae family are related to the tropical forest environment (Silva, Alves & Coan, 2014).

In Mapanioideae, there are two tribes further divided which are Hypolytreae and Chrysitricheae. Hypolytreae is distributed throughout the tropical regions of the earth; these tribes become the point of diversity in the Asia-Pacific area. In tropical wet forest and their side, the species are sturdy, big plants of the herb layer. There are nine genera under Hypolytreae tribes. Chrysitricheae consist of four genera. It is mainly distributed in Southern Hemisphere. Scoping from the swampy forest and tropical wet forest, the species are found in a diverse of habitats (Simpson et al, 2003).

Figure 2.1.1: Illustration of *Mapania* sp (Clarke, 1909)

The general morphology for *Mapania* is: "The roots surround the rhizome is stilt like and thick, ascending on it. Culms (stems) is posited at the central or lateral, (sub)scapose. Lateral culms few to many, with several basal leaves reduced to a sheath. Leaves eligulate, sometimes pseudopetiolate, rarely all leaves reduced to a sheath. Inflorescence rarely pseudolateral, paniculate, corymbiform, subanthelate or capitate , when on lateral culms often reduced to few spikelets. Primary bracts small (on most lateral culms) or large and leaflike (on most central culms). Spikelets with few to numerous spirally arranged persistent glumes, each subtending a flower. Flowers bisexual. Scales 4-6, basal pair lateral, ± ciliate-keeled, each with a single stamen, of the remaining 2-4 scales 0-2 with a stamen, very rarely only 1 stamen per flower. Style 2-3-fid, base not distinct, not or slightly thickened, persistent or not. Achene thick lenticular or rounded trigonous, sometimes 3-ribbed, sometimes with a fleshy exocarp". (eMonocot Cyperaceae, 2013)

Table 2.1.1 shows the classification of *Mapania*. *Mapania* is under Hypolytreae tribes of Mapanioideae family. Because of the uncommon characters in vegetative and reproductive organs among *Mapania*, it is becoming a disputed family member in Cyperaceae. In the equatorial regions of South America, Asia and Africa, the genus *Mapania* is under the Hypolytreae tribes with 84 species (Silva, Alves & Coan, 2014). There are 176 species under *Mapania* but there are still new species to be discovered.

Table 2.1.1: Taxonomic classification of *Mapania*

Kingdom	Plantae
Class	Liliopsida
Phylum	Streptophyta
Order	Poales
Family	Cyperaceae
Genus	*Mapania*
Species	*Mapania sp.*

Source: (Global biodiversity information facility, 2014)

The most common *Mapania* that well known by the local is *Mapania cuspidata* which there are few vernacular name used which are ‘serapat’, ‘siak-siak rimba’, ‘sempit’ and also ‘penawar Fatimah’ (Priyadi et al., 2010).

Mapania a group of mostly live in a deep forest sedges are broadly allocated all the way through tropics, Peninsular Malaysia and Borneo are becoming the point variation of *Mapania* to be pondered with 16 and 25 species recorded accordingly. However, the research about *Mapania* is still ongoing to found the new species. Though, *Mapania* is endemic by the

fifty percent in Borneo. Because of the shortage of good stopping information about its morphological characteristic, *Mapania* is hard to be identified. Especially in the Asia, *Mapania* species show a broad range in its morphological diversity. The change in its inflorescence stands to put in different appearance as its mature even the individual structures remain consistent in size and shape (Shabdin, Culham, Simpson & Meekiong, 2013).

2.2 The uses of *Mapania*

Since for ages, people use *Mapania* in many purposes. In Peninsular Malaysia and Brunei, mostly use *M. palustris* and *M. sumaterana* for making mats and basket (eMonocot Cyperaceae, 2013). In Liberia, West Africa, the old native use the leaf of *M. linderi* for thatching to avoid water or rain from coming into the house. But the important used of this plant is fading from generation to generation because of the technology and also this species is threatened with genetic erosion (Brink & Achigan-Dako, 2012).

According to Shabdin (2012), most of its purpose is to use as medicinal treatment among the local. *M. cuspidata* is widely use for traditional medicine; the rhizome is treated in a post-natal treatment while the leaf used for diarrhoea. Both rhizome and leaf are boiled to be drunk (Morad, 2012). In The antifungal properties is isolated from the herbal medicinal plant in Peninsular Malaysia, this is including *Mapania* species. There is one of the species in *Mapania* at Africa, which is *M. microcephala* that is believed to have antifungal properties the same as *Mapania* species in Peninsular Malaysia (William, 2013).

There are a few test antibacterial on *Mapania* by previous study. In Pahang forest, some test of antibacterial is tested among the herbs found. *Mapania patiolale* shows active reaction towards *Escherichia coli* (Liliwirianis, Wan Mohd Zain, Kassim & Abdul Karim, 2011). Most of the test is take the local *Mapania* in Peninsular Malaysia. In United State of America, *Mapania* had used to test for treating on Aids. The process is still in progress for immune dysfunction study. The *Mapania* is mixed with other plant herb in the test (Innovation, and intellectual property research group, 2004).

The leaf and rhizomes is commonly using in traditional medicine purposes. Mainly, the boiled water from most of the rhizomes of *Mapania cuspidata* is used for post natal treatment. The leaves also give much savour such as for beauty treatment and also for

woman health (Wannura, 2010). It is believed that the leaf also brings many goods towards the beauty of a woman, but not proven scientifically yet how effective are the uses of *Mapania* in traditional treatment purposes.

Leaf, bark and roots of *M. cuspidata* were extracted to do the antifungal and antimicrobial screening against five bacteria (*B. cereus, B. subtilis, E. coli, S. aureus* and *Pseudomonas aeruginosa*) and two fungi (*Candida albicans*). *Mapania cuspidata* is ethno botanically believe to cure the dysentery disease in this research. The fungi chose to be tested along because of the harm infection in oral and genital in humans. The result reveals the inhibition zone (7 mm) at the most when tested with *B. cereus* for the whole vegetative parts. In *P. aeruginosa* shows inhibitions zone only at bark and leaf part. While, there were no inhibition zone observed in the rest of the bacteria and fungi (Wiart et al, 2004).

2.3 Flavonoids in *Mapania*

Secondary metabolites are derived from the first metabolites. The main classes of secondary metabolites consist of phenolics compound. The phenolics compound plays an important role to the major organoleptic characteristic in plant-derived food and drink. Besides food and drink, they also provide the qualities of nutritional from vegetables and fruits especially for properties of taste and colour. They sure show a wide range of texture (Tapas, Sakarkar & Kakde, 2008). The statement state also by Tapas et al (2008), Flavonoids are the most universal and abundance group among the phenolic acids. Flavonoids can be found in every plant and there are records stately, there are more than 8000 types of flavonoids that have been found so far.

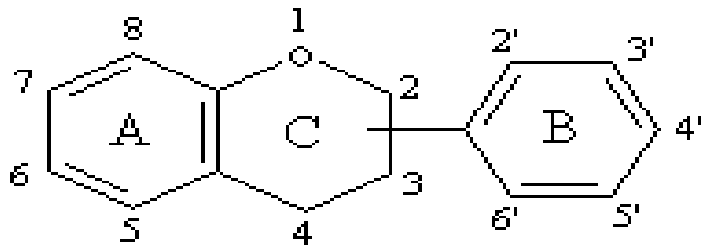

Figure 2.3.1: Basic structure of flavonoids (Tapas et al, 2008)

Flavonoids (Figure 2.3.1) are any compound that consist of C_6-C_3-C_6 carbon chain or can be recognized any of natural products that consist of phenylbenzopyran function. Flavonoids can be mainly divided into three group assorting to their position to the aromatic ring attachment to the benzopyrano (chromano) moiety. The classes (figure 3) are **1.** The flavonoids (2-phenylbenzopyrans), **2.** The isoflavonoids (3-benzopyrans) and **3.** The neoflavonoids (4-benzopyrans) (Grotewold, 2006).

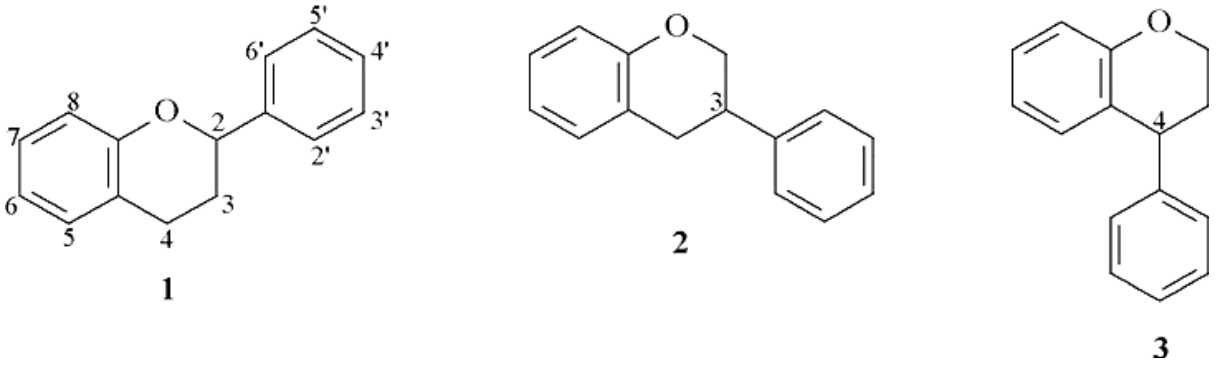

Figure 2.3.2: Three main groups in Flavanoids (Tapas et al, 2008)

Flavonoids are the secondary products produce by the polypropanoid pathway with phenylalanine as a start up molecule (Janićijević, Tošić & Mitrović, 2007). Flavonoids produced by plants as they feel threatened by surrounding. The product may vary in colour, aroma and also flavour (Hari Babu & Savithramma, 2014). The purpose is to protect them. For the plants themselves, every part of flavonoids have their own function in protecting plant. The most obviously reaction are as UV protector by filtering the only wanted light in the range of 280-315 nm region. This UV protector is usually done by anthocyanins. The other flavonoids such as flavanones, isoflavones and flavones act as anti-fungal agent while there are some groups in flavonoids give protection herbivore mammal and insect (Janićijević, Tošić & Mitrović, 2008) .

Flavonoids do give benefit to human after been analyzed and studied by many scientists. As cited by Janićijević, Tošić and Mitrović (2008), flavonoids have characteristic of anti-inflammatory (kaempferol, quercetin, myricetin), antioxidant (flavones and catechins), antimicrobial (flavonoids and esters of phenolic acid), antibacterial activity (quercetin), antithrombotic (flavonol) and anticarcinogenic activities. Based on this research, it can be shown that flavonoids contain major active nutraceutical ingredients.

Noori, Jafari and Zakeri (2015) had been studied on the member of Cyperaceae which the selected species are *Carex divisa, Carex melanostachya* and *Carex stenophylla*. Within this studied, they manage to extract the flavonoids compound mainly from its areal parts and roots. The flavonoids obtain from these three species are flavonoid sulphate, myricetin, narigenin, rhamnetin, apigenin, ehrysin, quercetin, luteulin, morin, kaempferol, aglycones flavonoids and flavones C- and C-/O-glucoside. Previous study by, Hari Babu and Savithramma (2014), found that the natural product from Cyperaceae are glycosides, alkaloids, flavonoids, lignins, phenols, quinones, coumarins, saponins, steroids, tannins and terpenoids

Mapania under Cyperaceae family is predicted to have more or less the same secondary compound as the family has. Not much previous research studies about phytochemical in *Mapania*. Chemical compound on *Mapania* had been studied by Shabdin (2012), two phenolic acid and six flavonoids are found. There are some proanthocyanidins in only two varieties of *M.cuspidata*.

2.4 *Escherichia coli* 0157: H7

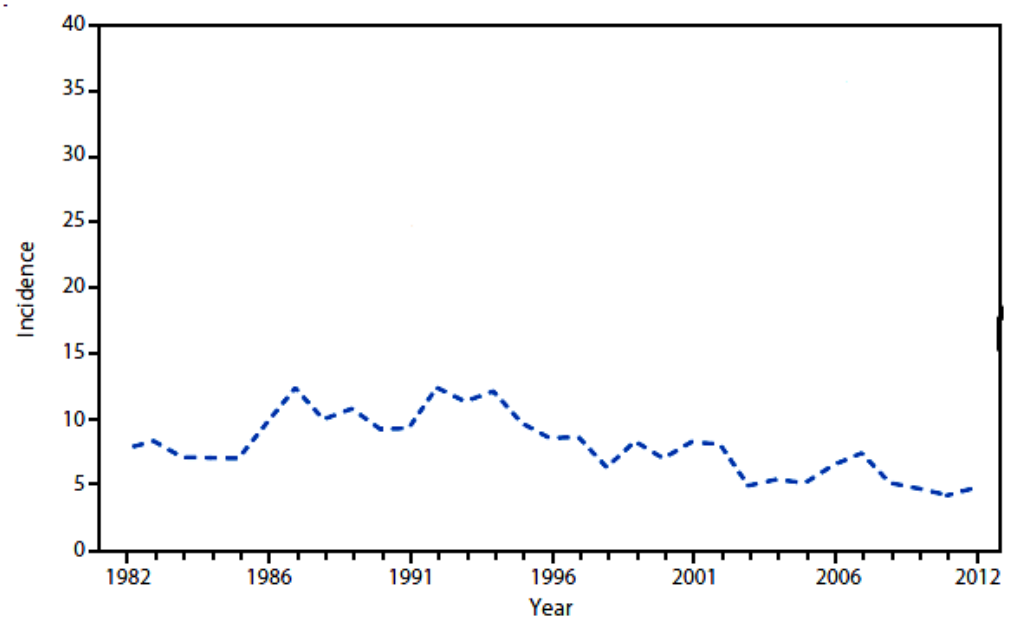

Figure 2.4.1: Shiga toxin-produce *Escherichia coli* incidence reported in United State (Adams, 2014)

Escherichia coli strain 0157:H7 is producing Shiga toxin and can cause severe disease to kidneys and vast symptom of the central nervous system. Common infection caused by these bacteria is diarrhea-associated haemolytic uremic syndrome (D+HUS). The syndromes are acute renal failure, thrombocytopenia, and microangiopathic haemolytic anaemia (Obrig, 2010).

Diagnose that have been done within six years (1982 – 2012), Shiga toxin-producing *E. coli* causes infection more than 96 000 illness (Adams, 2014). The cases of this infection are some leading to death, as the young children is more susceptible to the infection due to acute renal failure. There infection can be spread by person to person, laboratory-related, waterborne, animal contact and also foodborne (Rangel et al., 2005).

2.5 *Baccillus cereus* ATCC 33019

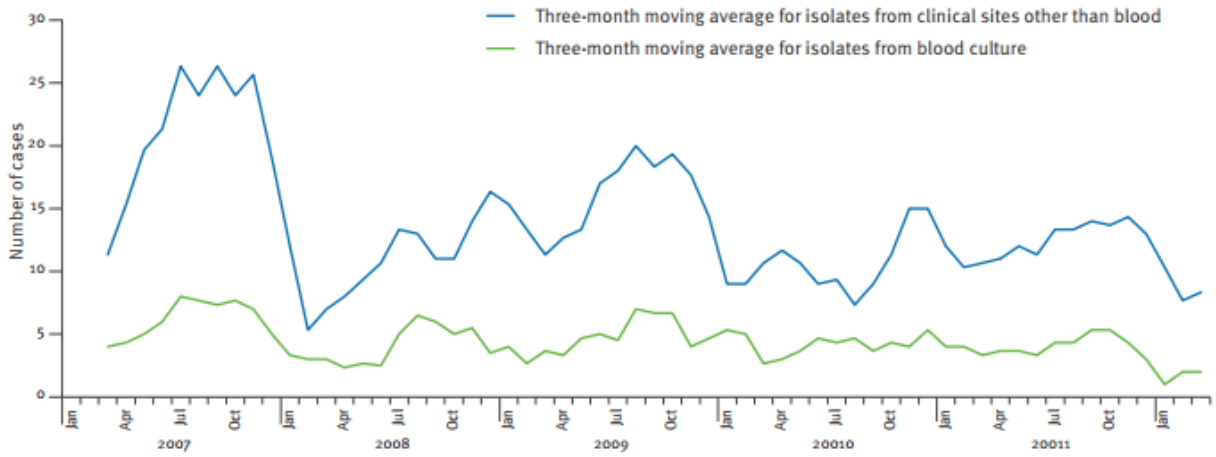

Figure 2.5.1: Isolated Bacillus cereus from clinical sites and blood culture (Brown et al, 2012)

Bacillus cereus can cause the food poisoning; somehow it gave serious invasive disease such as brain abscess, pneumonia, bacteraemia, endocarditic and osteomyelitis (Brown et al, 2012). *Baccillus cereus* ATCC 33019 can produce enterotoxin and emetic toxin (Shangkuan et al, 2000). *Baccillus cereus* can be found in any environment, the spore of the *B. cereus* can survive harsh environment.

There are two types of foodborne disease that cause by *B. cereus* which are diarrhoeal syndrome and emetic. The diarrhoeal syndrome is happening when the *B. cereus* is inside the host and they emit the enterotoxin. The symptom shows by the people who get the infection are nausea, abdominal cramps and watery diarrhoea. While the emetic syndrome, is because of the cereulide which is the ingestion of a cyclic peptide toxin. The symptom is similar as the diarrhoeal syndrome but happen in shorter time than the diarrhoeal syndrome (Jenson & Moir, 2003).