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Antibacterial Activity of Methanolic Crude Extracts from Selected Plant Against *Bacillus cereus*

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ABSTRACT

Bacillus cereus is a well-known food-poisoning bacterium. In this study, six methanolic crude extracts, from *Azadirachta indica*, *Choromolaena odorata*, *Justicia gendarussa*, *Mangifera odorata*, *Strobilanthes crispus* and *Tinospora crispa*, were investigated for their antibacterial activities against *B. cereus*. For this purpose, different concentrations of the methanol solvent crude extract from selected plants were used (1, 2, 4, 6, 8, 10, 15, and 20 mg/ml) and the diameter of *B. cereus* growth inhibition zone was measured at every 24 hours for 5 days. The antibacterial assay for all the crude extracts showed the inhibition of *B. cereus* growth by concentrations ranging from 2 mg/ml to 20 mg/ml, with a significant correlation between the extract concentrations and degrees of antibacterial activity. Rapid formation of inhibition zones within 24 hours of incubation was obtained, before a slight reduction in the inhibition of the diameter of zone was observed after 120 hours of incubation. The Minimal Inhibition Concentration (MIC) value for *J. gendarussa*, *M. odorata* and *S. crispus* crude extracts were at 2 mg/ml, while *A. indica*, *C. odorata* and *T. crispa* were at 6 mg/ml, 8mg/ml and 10 mg/ml, respectively. However, the Minimal Bactericidal Concentration (MBC) for all the crude extracts were at much higher concentration with the crude extract of *J. gendarussa*, *M. odorata* and *S. crispus* obtained the MBC values at 6mg/ml, whereas *A. indica*, *C. odorata* and *T. crispa* were at 10 mg/ml.

Keywords: Growth inhibition, diffusion assay, methanolic extract, Minimal Inhibition Concentration, Minimal Bactericidal Concentration

ABBREVIATIONS

ANOVA	:	one-way analysis of variance
mg/ml	:	milligram per milliliters
SEM	:	standard error of mean

INTRODUCTION

An interest in the study of medicinal plants, as a source of pharmacologically active compounds, has increased worldwide. Disadvantages of the modern medicines such as its high cost, unavailability in remote areas and the emergence of multidrug-resistant pathogens have become the driving force behind the pursuit of the 'green' medicines (Bandow *et al*., 2003). In addition, multiple resistances in human pathogenic micro-organisms have developed due to the indiscriminate use of commercial antimicrobial

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drugs commonly employed in the treatment of infectious diseases (Zampini *et al*., 2005). The undesirable side-effects of certain antibiotics and the emergence of the previously uncommon infections have forced scientists into looking for new antimicrobial substances from various sources like medicinal plants. Latif (1997) reported that approximately 2000 medicinal plant species from Malaysia have the potential pharmaceutical value and most of them have been widely used as herbal medicines. Generally, the whole plant or its parts such as leaves, roots, flowers or fruits were used to obtain the extract for the preparation of medicinal remedies.

Over the years, medicinal plants have been exploited in traditional medicine for various treatments due to their anti-microbial properties (Kelmanson *et al*., 2000; Srinivasan *et al*., 2000). A number of compounds have been isolated from plants, and their chemical structures were fully elucidated and many of them were tested for possible biological activities (Crombie *et al*., 1990). Edeoga *et al*. (2005) stated that the most important bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. Moreover, a study by Saadabi *et al.* (2006) revealed that alkaloid, flavanoids, tannins, sterols as well as triterpenes were present in the plants which exhibited a remarkable antibacterial activity against some pathogenic bacteria.

Among other, *Bacillus cereus* is identified as one of food-borne bacteria causing food-borne diseases with known aetiology (Gasaluk *et al*., 1996; Pan *et al*., 1997). However, the number of cases is likely to be underestimated as a consequence of the short duration and relative mildness of the illness (Granum, 1997). *Bacillus cereus* causes two distinct types of food poisoning, characterized either by diarrhoea and abdominal pain, or by nausea and vomiting (Jensen *et al*., 2003). More importantly, their spores are resistant to many of the heat-treatments used in the food industry such as pasteurisation, and some of the spores are able to germinate and grow at food storage temperatures (Andersson *et al*., 1995). Carlin *et al*. (2000) found that the spore forming and psychrotrophic properties of *Bacillus* species are the main deteriorating factors which would spoil food products and shorten their shelf life.

Even though certain plants have been demonstrated for their effects against pathogenic bacteria, a number of them have not been investigated for their antibacterial activities. In this report, the screening of the antibacterial activities of methanolic crude extracts from six selected plants against common food borne pathogenic bacterium, *Bacillus cereus* is described. Six selected medicinal plants are *Strobilanthes crispus, Azadirachta indica, Justicia gendarussa, Tinospora crispa, Chromolaena odorata* and *Mangifera odorata.* The effectiveness of each plant extract has been determined in the form of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values.

MATERIALS AND METHODS

Plant Material and the Preparation of Plant Extracts

The leaves of *A. indica*, *C. odorata*, *J. gendarussa*, *M. odorata*, *S. crispus* and *T. crispa* were collected from the Agricultural Park, Universiti Putra Malaysia (UPM), in Serdang, Malaysia. All the leaves were picked 30 cm from the aerial part, and wrapped in newspaper to maintain their freshness. The surface sterilization was carried out by washing all the samples with water, followed by 10% (v/v) sodium hypochlorite solution, before they were rinsed with sterile distilled water and air dried at room temperature. Dried samples were then ground into powder using a grinder before they were sieved and fine powder of plant materials was therefore collected.

The preparation of the methanolic crude extract was carried out according to the method proposed by Chandrasekaran and Venkatesalu (2003). Twenty grams of dried samples of each plant were soaked in 50 ml of 80% (w/v) methanol for 24 hours at 30°C, and then filtered using Whatman No.1 filter paper. Finally, the filtrate was evaporated using a rotary evaporator at 60˚C until it was fully dried.

The Preparation of Test Extract Solutions

The preparation of the methanolic crude extract was carried out as suggested by Chandrasekaran and Venkatesalu (2004). A series of different methanolic crude extract concentrations (1mg/ml, 2 mg/ml, 4 mg/ml, 6mg/ml, 8mg/ml, 10mg/ml, 15mg/ml and 20 mg/ml) were prepared by dissolving a known weight of the plant crude extract in 5% (v/v) dimethyl sulphoxide (DMSO), which acted as a solvent.

The Preparation of the Bacteria Culture

Bacillus cereus ATCC 10876 was obtained from the Plant Systematic and Microbial Laboratory, Biology Department, Universiti Putra Malaysia, and maintained on the Nutrient Agar slant. The preparation of the test strain culture was carried out by isolating a single colony of *B. cereus* from the nutrient agar and transferred into 250ml Nutrient Broth, before it was shaken at 180rpm for 24 hours.

Agar Well Diffusion Assay

Nutrient agar was seeded with 1×10^5 cell/ml of *B. cereus* before a series of 0.6cm in diameters wells were made using sterile cork borer. Twenty µL of the methanolic crude extract solutions, in various concentrations, were then transferred into each well and allowed to set. All the plates were incubated at 37°C and the diameters of the clear zone, surrounding each well were measured as the inhibition zone to the nearest millimetre for every 24 hours interval up to 120h. Sets of 5 replicates were used for each type of the extract.

Determination of the Minimum Inhibitory Concentration (MIC)

The same series of the methanolic crude extract, as previously mentioned in the agar well diffusion, were used for the MIC determination. Twenty microlites of methanolic crude extract was mixed into 80µl sterile Nutrient Broth before 1×10^5 cells/ml *B. cereus* culture was added into the mixture. The mixture was then pipetted into the microtiter plate and incubated for 24h at 37˚C. The lowest methanolic crude extract concentration that did not show any growth of *B. cereus* was considered as the MIC value.

The Determination of the Minimum Bactericidal Concentration (MBC)

Ten µL of the test mixture in the microtiter well which did not show any growth of *B. cereus* in MIC determination was sub-cultured onto fresh Nutrient Agar plates before further incubation was carried out at 37˚C for 24 hours. The least concentration, with no *B. cereus* growth, was considered as being the MBC value.

Statistical Analysis

The results obtained were expressed as mean ± SEM. Significant differences among the treatment groups were tested using the one-way analysis of variance (ANOVA), and the comparison of the mean values was made using the Tukey test at 5% significance level. All the statistical analyses were performed using the software program SPSS.

RESULTS AND DISCUSSION

The methanolic crude extracts of the 6 medicinal plants were tested for their antibacterial activities against *Bacillus cereus* using agar well diffusion method. The overall diameter of inhibition for all the methanolic extract concentrations, for all the plant species against *B. cereus*, are shown in Table 1. 80% (v/v) methanol and 5% (v/v) of DMSO were also tested as a control experiment and no activity for these control solutions was observed (data not shown). However, all the methanolic crude extracts from different plant species showed some antibacterial activities towards the growth of *B. cereus,* indicating that the antibacterial activity observed was due to the activity of the bioactive compound present in the plant crude extract and not caused by the solvent used in the extract preparation. The bioactive compound activities in all the methanolic crude extracts seemed to show a similar trend towards the growth of *B. cereus*.

TABLE 1
Antibacterial activity of selected methanolic extracts against *B. cereus*

Extract	Concentration (mg/ml)	Diameter of inhibition zone (cm ± SD)*						MIC**	MBC***
		Incubation Period (h)							
		0	24	48	72	96	120		
Strobilanthes crisaus	Control	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	1	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	2	0.60±0.00^{a}	0.75±0.01^{b}	0.72±0.02^{b}	0.73±0.01^{b}	0.70±0.02^{b}	0.70±0.01^{b}	+	√
	4	0.60±0.00^{a}	0.84±0.01^{c}	0.80±0.01^{c}	0.81±0.01^{c}	0.80±0.02^{c}	0.82±0.01^{c}	+	√
	6	0.60±0.00^{a}	0.90±0.02^{d}	0.89±0.02^{d}	0.90±0.01^{d}	0.88±0.01^{d}	0.90±0.01^{d}	+	X
	8	0.60±0.00^{a}	0.95±0.01^{e}	0.96±0.01^{e}	0.97±0.01^{e}	0.98±0.02^{e}	0.99±0.01^{e}	+	X
	10	0.60±0.00^{a}	0.97±0.01^{e}	1.01±0.01^{f}	1.01±0.01^{f}	1.00±0.01^{ef}	1.04±0.01^{f}	+	X
	15	0.60±0.00^{a}	1.00±0.01^{f}	1.03±0.01^{f}	1.03±0.01^{f}	1.02±0.01^{f}	1.04±0.01^{f}	+	X
	20	0.60±0.00^{a}	1.08±0.02^{g}	1.07±0.02^{g}	1.07±0.02^{g}	1.07±0.02	1.09±0.01^{g}	+	X
Justicica gendarusa	Control	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	1	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	2	0.60±0.00^{a}	0.75±0.01^{b}	0.73±0.01^{b}	0.71±0.01^{b}	0.72±0.01^{b}	0.72±0.02^{b}	+	√
	4	0.60±0.00^{a}	0.80±0.01^{c}	0.79±0.01^{c}	0.77±0.02^{c}	0.76±0.02^{c}	0.76±0.02^{bc}	+	√
	6	0.60±0.00^{a}	0.83±0.02^{cd}	0.82±0.02^{cd}	0.79±0.02^{cd}	0.80±0.01^{d}	0.79±0.01^{cd}	+	X
	8	0.60±0.00^{a}	0.86±0.01^{de}	0.84±0.01^{de}	0.83±0.02^{de}	0.84±0.02^{e}	0.83±0.02^{de}	+	X
	10	0.60±0.00^{a}	0.90±0.02^{ef}	0.88±0.01^{e}	0.86±0.01^{e}	0.87±0.02^{ef}	0.86±0.02^{ef}	+	X
	15	0.60±0.00^{a}	0.92±0.02^{g}	0.93±0.02^{f}	0.91±0.01^{f}	0.89±0.02^{f}	0.89±0.02^{g}	+	X
	20	0.60±0.00^{a}	1.03±0.03^{h}	1.02±0.03^{g}	1.00±0.02^{g}	1.00±0.01^{g}	0.98±0.03^{h}	+	X
Mangifera odorata	Control	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	1	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	2	0.60±0.00^{a}	0.73±0.01^{b}	0.73±0.01^{b}	0.73±0.01^{b}	0.72±0.01^{b}	0.74±0.01^{b}	+	√
	4	0.60±0.00^{a}	0.81±0.01^{c}	0.81±0.01^{c}	0.80±0.01^{c}	0.80±0.01^{c}	0.80±0.01^{c}	+	√
	6	0.60±0.00^{a}	0.84±0.01^{c}	0.85±0.01^{d}	0.83±0.01^{d}	0.82±0.01^{c}	0.83±0.01^{d}	+	X
	8	0.60±0.00^{a}	0.87±0.02^{d}	0.88±0.01^{e}	0.88±0.01^{e}	0.88±0.01^{d}	0.88±0.01^{e}	+	X
	10	0.60±0.00^{a}	0.89±0.01^{d}	0.89±0.01^{ef}	0.88±0.01^{e}	0.88±0.01^{d}	0.88±0.01^{e}	+	X
	15	0.60±0.00^{a}	0.92±0.01^{e}	0.92±0.01^{f}	0.91±0.01^{f}	0.90±0.01^{d}	0.91±0.01^{f}	+	X
	20	0.60±0.00^{a}	0.98±0.01^{f}	0.97±0.10^{g}	0.95±0.01^{g}	0.95±0.01^{e}	0.95±0.01^{g}	+	X

Table 1 (*continued*)

Azadirachta indica	Control	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	1	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	2	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	4	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	6	0.60±0.00^{a}	0.68±0.01^{b}	0.68±0.02^{b}	0.69±0.02^{b}	0.69±0.03^{b}	0.69±0.02^{b}	+	√
	8	0.60±0.00^{a}	0.72±0.01^{bc}	0.71±0.01^{bc}	0.72±0.01^{bc}	0.71±0.01^{bc}	0.72±0.01^{bc}	+	√
	10	0.60±0.00^{a}	0.74±0.03^{c}	0.74±0.02^{c}	0.75±0.02^{c}	0.75±0.02^{c}	0.76±0.03^{c}	+	X
	15	0.60±0.00^{a}	0.80±0.04^{d}	0.81±0.01^{d}	0.81±0.02^{d}	0.81±0.02^{d}	0.81±0.03^{d}	+	X
	20	0.60±0.00^{a}	0.90±0.02^{e}	0.90±0.02^{e}	0.91±0.04^{e}	0.91±0.02^{e}	0.91±0.02^{e}	+	X
Chromalaena odorata	Control	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	1	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	2	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	4	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	6	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	8	0.60±0.00^{a}	0.69±0.02^{b}	0.67±0.02^{b}	0.67±0.02^{b}	0.67±0.02^{b}	0.67±0.02^{b}	+	√
	10	0.60±0.00^{a}	0.72±0.01^{c}	0.72±0.01^{c}	0.72±0.01^{c}	0.07±0.01^{c}	0.72±0.01^{c}	+	X
	15	0.60±0.00^{a}	0.78±0.01^{d}	0.79±0.04^{d}	0.77±0.01^{d}	0.77±0.01^{d}	0.77±0.01^{d}	+	X
	20	0.60±0.00^{a}	0.95±0.01^{e}	0.87±0.01^{e}	0.86±0.01^{e}	0.85±0.01^{e}	0.85±0.01^{e}	+	X
Tinosaora crisaa	Control	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	1	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	2	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	4	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	6	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	8	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	0.60±0.00^{a}	-	√
	10	0.60±0.00^{a}	0.71±0.01^{b}	0.70±0.01^{b}	0.71±0.01^{b}	0.07±0.01^{b}	0.71±0.01^{b}	+	X
	15	0.60±0.00^{a}	0.75±0.02^{c}	0.74±0.01^{c}	0.74±0.01^{c}	0.74±0.01^{c}	0.74±0.01^{c}	+	X
	20	0.60±0.00^{a}	0.85±0.02^{d}	0.80±0.03^{d}	0.81±0.02^{d}	0.81±0.02^{d}	0.82±0.02^{d}	+	X

Means in each column with the same superscript letter are not significantly different amongst themselves when the Tukey tests were used at 5% significance level.

* Diameter of the inhibition zone was included 0.6cm of the well diameter and expressed as the mean ± SD; (N = 5).

** Minimum Inhibition Concentration: (-) no inhibition zone observed; (+) inhibition zone observed.

*** Minimum Bactericidal Concentration: √: Presence of bacteria growth; X: No bacteria growth observed.

After 24 hours of incubation, the growth of *B. cereus* was drastically inhibited by all the crude extracts for all the concentrations. However, after 48 hours, a slight reduction on the inhibition activity against *B. cereus* was observed, before it was steadily maintained until 120 hours. After 24 hours, drastic increases in the diameter of the inhibition zones might directly be caused by immature inocula being less resistant to antibacterial activities. However, after 48 hour of growth, antibacterial activities were diminished; this was probably due to the enzyme denaturation and the bacteria becoming much more dominant (Beukinga *et al.*, 2004).

To evaluate the effectiveness of the concentration of the methanolic crude extracts on the growth of *B. cereus*, the MIC test was conducted using a series of methanolic crude extract concentrations. The MIC values for all the plant crude extracts are shown in Table 1. At a concentration of 2mg/ml, the methanolic crude extracts of *J. gendarussa*, *S. crispus* and *M. odorata* started to show inhibition on the growth of *B. cereus,* with average inhibition rates of 6.18µm/h, 5.83µm/h and 4.63µm/h respectively for *S. crispus, J. gendarussa* and *M. odorata*. All the plant methanolic crude extracts showed the highest mean of bacterial growth inhibition diameter at the concentration of 20mg/ml, with the *S. crispus* methanolic extract as the best inhibitor against *B. cereus* growth, at the rate of 29.51µm/h. In addition, there were strong correlations between the extract concentrations and the diameters of the inhibition zones at this stage, with the correlation coefficients ranging from 0.843 to 0.930 (data not shown). A similar result was also derived by Boer *et al.* (2004) and Sawangjoroen *et al.* (2004). A high antibacterial activity of *S. crispus* leaf extract against the growth of *B. cereus* was expected since several chemical compounds, such as polyphenols, catechins, caffeine, alkaloids, tannins, β-sitosterol and stigmasterol (which could be obtained from the *S. crispus* leaf extract) have a significant antibacterial activity against some pathogenic bacteria (Maznah *et al*., 2000; Endrini, 2003; Abdah *et al*., 2004). *Justicia* species was known as medicinal herbs in the South East Asia region and has a huge potential benefit for diverse biological activities such as lowering the risk of cardiovascular diseases (Lucas *et al.,* 2004) and cancer risk (Marchand, 2002). Although *J. gendarussa* is lignan-free (Lorenza *et al*., 1999), their methanolic crude extracts were still found to be able to inhibit *B. cereus* growth at lower concentrations, due to the presence of other compounds such as β- sitosterol, 2-amino benzyl alcohol and 2-(2'amino-benzylamino) benzyl alcohol (Chakravarty *et al*., 1982).

Azadirachta indica, *C. odorata* and *T. crispa* methanolic extracts were only able to show inhibition on the bacterial growth at higher concentration. The methanolic crude extract of *A. indica* started to inhibit bacterial growth at 6 mg/ml, while this was at 8 mg/ml and 10 mg/ml respectively for *C. odorata* and *T. crispa*. A similar result of the moderate antibacterial activity for these three plant extracts was also reported previously. The alcoholic crude extracts of *A. indica* and *C. odorata* were found to be ineffective as antibacterial; this might be due to the low extractable of phenolic (Apori *et al*., 2000). However, a significant effect was found as larvicidal extract against *Plasmodium falciparum* (Hout *et al*., 2006) and the larvae of *Aedes aegypti* and *Anopheles stephensi* (Kiran *et al*., 2005). On the other hand, the methanolis crude extract of *T. crispa* was not effective in inhibiting the growth of *B. cereus* with only 6.16µm/h, as compared to the control at the concentrations of 10mg/ml. This corresponds with the report by Zakaria *et al.* (2005).

In spite of the MIC results, at 6mg/ml, the values of the MBC were much higher for all the plant crude extracts of *J. gendarussa*, *S. crispus* and *M. odorata* extracts. Meanwhile for *C. odorata*, *T. crispa* and *A. indica* extract, the bactericidal effect can only be observed at 10mg/ml. This means that at the concentrations between the MIC and MBC values, the methanolic crude extracts could only act as bacteriostatic agents rather than as bactericidal for *B. cereus*, because at this concentration, the bioactive compound was unable to eliminate *B. cereus* or sustain the activity for a long period, and thus allowing the

bacteria to grow. Two possible explanations for this bacteriostatic effect are: (i) the bioactive compound in the extract was not adequate to cause a significant mortality to the bacteria (Basri and Fan, 2005); and (ii) the sensitivity of the bioactive compound towards a certain type of solvent might cause or enhance the rate of deactivation or degradation (Mutu and Staden, 2003).

In general, the plant extracts are much more active against Gram-positive bacteria than against Gram-negative bacteria (Lin *et al.*, 1999; Cimanga *et al.*, 2002), and this was demonstrated by the positive effects of several plants extracts on the other Gram-positive bacteria such as *S. epidermidis, Bacillus subtilis* (Fatima *et al.*, 2001) and *Pseudomonas aeruginosa* (Nimri *et al.*, 1999). The fact that *B. cereus* was categorized as a Gram-positive bacteria has some contribution towards the effectiveness of the plant methanolic crude extract as a bacteriostatic or bactericidal agent. The density of the lipopolysaccharide layer in the outer surface of bacterial cell wall is much lower in the Gram-positive bacteria as compared to the Gram-negative bacteria (Burn, 1988). Without this layer, certain antibacterial compounds can easily reach the peptidoglycan layer of the bacterial cell wall and penetrate into the cytoplasm to cause the loose of cell's turgor pressure, with a subsequent disorganization of the internal organelle (Clements *et al*., 2002). This might explain the sensitivity of *B. cereus* towards the plant extracts.

In conclusion, this study demonstrated the ability of all the methanolic crude extracts to act as a bactericidal agent. Among the six methanolic crude extracts tested, *Strobilanthes crispus* was found to be the most active, i.e. by showing the largest mean of diameter inhibition zones at the concentration of 20mg/ml. However, this is much dependent on the concentration applied, the types of plant extract and the extraction process. The potential of these plants, particularly the extracts of *S. crispus, J. gendarussa* and *M. odorata*, in pharmacology as an antibacterial agent is enormous. However, further biochemical analysis is still required to prove this.

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