ORIGINAL ARTICLE

Inhibition of bacillus spores germination by cinnamon bark, fingerroot, and moringa leaves extract

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ABSTRACT

This study was intended to discover the natural food preservatives by comparing the antibacterial effect of the ethanolic extract of cinnamon bark, finger root, and moringa leaves toward *Bacillus cereus* both the vegetative cells and spores. The antibacterial activities of the investigated extracts were assessed against cells using the agar diffusion method. Whereas the sporicidal test was performed by observing the colony growth, after various times of incubation (1, 3, and 5 h). The investigated extracts produced inhibition in a diameter ranging from 10.6 to 35.3 mm, and it can be classified that the extract of cinnamon bark was the most potent extract to inhibit the vegetative cells form, followed by fingerroot and the moringa leaves extract. Consistently, the ethanolic extract of cinnamon bark and fingerroot significantly yielded sporicidal activities higher than the moringa leaves extract. Both extracts exerted sporicidal activity within 1 h of contact time at the lowest test concentration of 5% w/v, whereas moringa leaves extract required a longer contact time (5 h) at higher concentration of 20% w/v. It can be concluded that cinnamon bark and fingerroot extract have great potential as effective food preservative candidates to inhibit the *B. cereus* growth than moringa leaves extract.

Key words: *Bacillus cereus*, cinnamon bark, fingerroot, moringa leaves, spores, vegetative cells

INTRODUCTION

Bacillus cereus is ever present distributed in the milieu and known as a high-potential bacteria that can poison food manufacturing processes, improper food storage, refrigeration of improperly cooked food and ready to eat products, especially dairy products with its prevalence ranging at 2%–52%.[1-6] Therefore, everyone has a great potential to be infected by these bacteria. The Center for Disease Control website states that monitoring outbreaks of Bacillus-related food poisoning (not just *B. cereus*) from 1998 to 2015, there were 619 outbreaks, 7385 diseases, 14,681 hospitalization rate and 337 deaths.[7-10] *B. cereus* food poisoning is increasingly being reported as a serious and potentially fatal on the gastrointestinal or nonintestinal tract by secreting several toxins which can damage the target tissue.[11] These bacteria produce diarrheal toxins when they growth in the small intestine.[12] The food contamination may show diarrhea symptom if cells or spores in high numbers are ingested or may cause emetic symptom if the preformed toxins are ingested.[6] Furthermore, this bacterium may infect the respiratory tract, eye, and cause lesion.[13,14] From a clinical and a food industry standpoint,

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endospores are dangerous because of their resistance to environmental stress and sterilization methods due to their potential to be dormant and possess rigid structure.[15] Therefore, contamination of endospores into food is the main transmission route for *B. cereus* because endospores can exist anywhere.[16-19] Sterilization in the high temperature is reported to be effective at killing spores in foodstuffs; however, it should also be considered that overheating can damage organoleptic qualities and nutritional contents.[20] Therefore, this study explored the alternative ways to kill *B. cereus* spores by utilizing edible herbs, such as: Cinnamon Bark, fingerroot, and moringa leaves based on their antibacterial chemical content.

MATERIALS AND METHODS

Materials

All herbs collected from Manoko plantations in Lembang, Indonesia, in the periode of November–December 2020 and authenticated by Faculty of Biology, Padjadjaran University, Indonesia. The tested bacteria used in this study were *B. cereus* 11,778 and the bacteria was grown in Mueller Hinton Agar media (MHA-Oxoid CM0337).

Extraction

The plant materials were dried, weighed, and powdered using a grinder. A 500 g of each powdered materials was placed into macerator and soaked in ethanol (70% V/V) for 72 h at room temperature. The extract then analyzed to detect the secondary metabolites contents.[21]

Antibacterial activity

The antibacterial activities of the extracts were assessed against *B. cereus* using the standard agar diffusion method.

Sporicidal activity test

A total of one Ose of *B. cereus* colonies was suspended in a 5 mL sterile MHB and incubated for 96 h at 37°C to induce the spore forming. The suspension of vegetative cell was lysed for 5 min using sonication then centrifuged (12,000 × g for 2 min, 4°C) to isolate spores.[22,23] The collected spores were resuspended and rinsed using sterile distilled water by repeated centrifugation. The pellets containing spore were resuspended in sterile physiological saline solution and the condition of spore was observed by spore stain technique using carbol fuchsin ast the primer stain and methylene blue as the counter dye. The sporicidal activity of the extract was determined by observing the presence or absence of colony growth on agar media. Spore suspension (0.5 McFarland) was taken for 10 µL and challenged with 1 mL of each extract with various concentrations then incubated at 37°C for various times. The spores were isolated by centrifugation, following by pellets washing and resuspension in 1 mL of sterile saline solution. A 10 µL of spore suspension was spread on MHA and the viable spores were observed.

RESULTS AND DISCUSSION

A total of three crude ethanolic extracts was screened for the antibacterial potential against the vegetative cells form of *B. cereus*, presented in Figure 1 and Table 1. In this present study, the investigated extracts possessed different secondary metabolite content, Cinnamomum bark contains of flavonoids, tannins, polyphenolics, monoterpenoids and sesquiterpenoids, and quinones; fingerroot contains of flavonoids, polyphenolics, quinones, monoterpenoids and sesquiterpenoids; moringa contains of flavonoids, tannins, polyphenolics, and quinones. Another study reported that flavonoids substance containing in Roselle extract was suggested to play a role as the antibacterial agent against *B. cereus* cells.[24] Flavonoids can form complexes with dissolved proteins that are located outside the bacterial cell and disturb the DNA synthesis.[25-27] The polyphenolics substances were also predicted to have an important role in the inhibition of investigating extracts against *B. cereus*. Several studies have depicted that the polyphenolic compounds can modify the cell membrane permeability.[26,28-33] Meanwhile, Quinones work by inactivate the bacterial protein by irreversibly binding to the bacterial amino acids.[34] The antibacterial activity demonstrated that the Cinnamon bark extract generated the highest antibacterial potential against the vegetative cells, followed by fingerroot extract. Meanwhile, the effectivity of moringa leaf extract was less effective. These results were in accordance with the secondary metabolite content of each extract. The differences in the types and concentrations of secondary metabolites are thought to yield a great effect on the inhibition potency of the extract against *B. cereus*

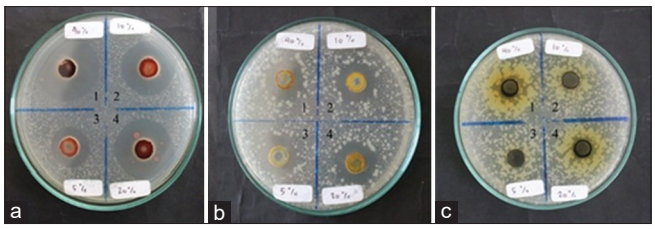

Figure 1: Antibacterial activity of cinnamon bark (a), Fingerroot (b) and Moringa leaves (c) ethanolic extract against vegetative cells of *Bacillus cereus*

Table 1: Antibacterial activity of all tested ethanolic extract against *Bacillus cereus* cells vegetative

Extract concentration (% w/v)	Diameter of inhibition (mm)		
	Cinnamon bark	Fingerroot	Moringa leaves
5	22.6±0.0100	15.0±0.0625	-
10	26.3±0.0025	15.9±0.0225	-
20	31.0±0.0225	17.2±0.0025	10.6±0.0225
40	35.3±0.0025	18.0±0.0000	12.4±0.0000

Perforator diameter=9 mm

cells.[35] The moringa leaves extract required a minimum concentration of 20%w/v to produce antibacterial activity against *B. cereus* cells. The sporicidal activities of the extracts are presented in Table 2. We revealed that the ethanolic extract of cinnamon bark and fingerroot significantly yielded sporicidal activities higher than the moringa leaves extract. Both extracts exerted sporicidal activity spores within 1 h at the lowest test concentration of 5% w/v, meanwhile moringa leaves extract required a longer contact time (5 h) at higher concentration of 20% w/v. The result showed that cinnamon bark and fingerroot extract have great potential as effective food preservative candidates to inhibit both forms of *B. cereus*. This is interesting because up to now, resistance cases of *B. cereus* spores against chemical substances and sporicidal enzymes have been identified, especially due to their integrity structure with triple layering of the spore (coat, protoplast membrane, and nucleus) and chemical composition to face the stressful environmental.[36-38] Meanwhile, for the spore integrity, the outer membrane is not an essential protective layer because its release was found not provide a significant effect on spore resistance.[36,37] In contrast to the outer, the inner membrane is a vigorous permeability constraint, importantly maintains the inside structure of the spore, especially the DNA, and have resistance mechanisms towards the anti-sporicidal chemical substances.[36-40] We suggested that the investigated extracts can penetrate and made the inner membrane of the spore to be lysed, thus, the spore unable to germinate and grow on the media. These findings provide strong evidence that the content of secondary metabolites in the studied extracts was responsible for the antibacterial activity against *B. cereus*. By comparing the secondary metabolite content in others plants extracts, we suggested that our investigated extracts had the same opportunity as the sporicidal agent candidate of *B. cereus*'s spore germination and outgrowth. As reported in another study, edible burdock root and olives extract that consist of flavonoid-types, terpenoids, several polyphenols and tannins, provided the sporicidal activity on *B. cereus* spores.[41,42] Thus, this finding is interesting because spores have a complex structure and are not easily to be penetrated. Moreover, considering that plant extracts that are effective against *B. cereus* spores have been reported less than those of *B. subtilis*. In addition, as Lau and Rukayadi said, reports of studies of sporicidal activity derived from plants are still rare.[43] Therefore, scientific evidence of the ability of ethanol extract of cinnamon bark and fingerroot provides a great opportunity in overcoming the problem of resistance to *B. cereus* spores that have existed so far.

Table 2: Sporicidal activities of the extracts

Extract	Extract concentration (% w/v)	*Bacillus cereus* colonies growth after contact, time (h)		
		1	3	5
Cinnamon bark	5	−	−	−
	10	−	−	−
	20	−	−	−
	40	−	−	−
Fingerroot	5	−	−	−
	10	−	−	−
	20	−	−	−
	40	−	−	−
Moringa leaves	5	+	+	+
	10	+	+	+
	20	+	+	−
	40	+	+	−
Control (+)		+	+	+
Control (−)		−	−	−

+: Colony presence, −: Colony absence

CONCLUSION

The present findings indicated that the ethanolic extract of cinnamon bark and fingerroot provided strong and significant antibacterial and sporicidal agents, compared to that of moringa leaves extract. Thus, cinnamon bark and fingerroot extracts might be potential to be further studied as a food preservative.

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Conflicts of interest

There are no conflicts of interest.

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