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RESEARCH ARTICLE

Effect of Different pHs and Temperatures on Stability and Mode of Action of Ethanolic *Kayu Manis Hutan (Cinnamomum iners)* Extract Against Foodborne Pathogens

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Abstract Pathogenic bacteria found in raw foods have the potential to contaminate processed goods and cause them to deteriorate. Processed foods are divided into groups based on the necessary degree of heat treatment and pH levels in order to provide quality and safety. This study aims to evaluated the effect of different pHs and temperatures on the stability and mode of action of ethanolic *Cinnamomum iners* leaves extract against foodborne pathogens. Two different stages of leaf maturity were selected, namely young and old. The susceptibility activities of extracts after treated with different pHs (5, 7 and 9) and temperature (30°C, 50°C and 80°C) against foodborne pathogens ranged between 8.33 ± 0.76 to 11.33 ± 0.58 mm and 8.67 ± 0.58 to 12.67 ± 0.58 mm, respectively. Young leaves showed better susceptibility toward foodborne pathogens than old leaves against *P. mirabilis* (10.33 ± 0.58 and 11.33 ± 0.58 mm) at pH 5 and pH 9, while *E. coli* (10.33 ± 0.58 mm) at pH 7. In terms of temperature for foodborne pathogens, *B. cereus* showed the highest inhibition zone (12.67 ± 0.58 and 10.00 ± 0.00 mm) at 30°C and 50°C, while *B. megaterium* (11.00 ± 1.00 mm) at 80°C. The MIC and MBC from both extracts tested showed at the ranged between 0.31 to 5.00 after being treated at different pHs and temperatures. The SEM analysis showed morphological features in selected treated microorganisms namely, *B. cereus* and *K. pneumoniae* changed in the cell wall shape, ruptured, and the cytoplasm leaked. Meanwhile, untreated cells assume normal rod with a smooth surface. In conclusion, *C. iners* leaf extract exhibited antibacterial activity particularly young leaf, which showed stability after being subjected to different pHs and temperatures and can be developed as natural food preservatives.

Keywords: Antibacterial activity, *Cinnamomum iners*, foodborne pathogens, extract stability, SEM.

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Introduction

Raw foods like vegetables and fruits may carry microorganisms from soil or air, and even washing may not remove all of them. Meat and milk produced by animals can be contaminated with bacteria from the animal's skin and intestines throughout slaughtering and milking [31]. The pathogenic bacteria found in raw foods have the potential to contaminate processed goods and cause them to deteriorate without proper cleaning [10]. Food products contaminated by microorganisms will deteriorate because of the presence of a wide variety of metabolic by-products during metabolites process [35] which causes

changes in food color, smell, taste, texture and other sensory properties, such as a putrid sour smell, off-odors and flavors [33] and it is undesirable or unacceptable for human consumption [37]. Thus, processed foods are divided into groups based on the necessary degree of heat treatment and pH levels in order to provide quality and safety [26].

A few preservation procedures, including salting, acidification, drying, heat treatment, and sanitizing have been used in the food processing industry to preserve food from spoiling and from growing harmful microbes [2]. Food preservatives are important to enhance food shelf life during storage and to maintain the physical characteristics of food. Preservatives also inhibit the growth of bacteria, yeasts, and molds in foods, but synthetic preservatives are widely used such as benzoates in foods as antimicrobial preservatives [25]. However, the usage of chemical preservatives is controversial due to their harmful effects and have been suspected to cause allergies and asthma [30]. As a result, new eco-friendly approaches are needed to minimize harmful bacteria development and extend the shelf-life of food goods without the use of chemical preservatives [16].

Cinnamomum iners, commonly known as *Kayu manis hutan*, has been studied for its antimicrobial properties, with several reports highlighting its efficacy against various microbial strain. The leaves of *C. iners* extract demonstrated moderate antibacterial activity (13 mm) against periimplantitis-triggering microbes such as *E. coli* and *S. aureus*. The study found that cinnamic acid in the extract was responsible for its antibacterial properties [24]. Similarly, the methanolic extract of *C. iners* leaves was effective against clinical isolate of methicillin-resistant *S. aureus* (MRSA) and *E. coli*, with xanthorrhizol identified as the active compound [28]. Previous reports mentioned the uses of clinical strain in their studies while few using foodborne pathogen. The *C. iners* leaf and bark extract has shown antimicrobial activity against bacterial strains such *E. coli*, *S. aureus*, *Serratia marcescens*, *K. pneumoniae* and *Pseudomonas aeruginosa* and fungal strains such as *Trichophyton rubrum*, *Aspergillus fumigatus* and *Candida albicans* [40]. It also has been reported to possess analgesic, antioxidant activity and toxicity [41] due to robustness of bioactive compounds [39].

The plants possess ethnobotanical attributes due to the presence of secondary metabolites such as cinnamaldehyde, kaempferol-3-glucopyranoside, quercetin-3-rutinoside and eugenol [29]. These secondary metabolites may have direct impacts on enzymes and other cell activities, altering the shape of microorganisms, aggregating in the cell membrane, causing instability and damage, varying membrane permeability, and causing cytoplasmic membrane rupture [23]. Almost every part of the cinnamon tree including the bark, leaves, flowers, fruits and roots, has some medicinal or culinary use [36] but the ethnobotanical reports of its leaves remain lacking.

Scanning Electron Microscopy (SEM) is a powerful imaging technique that offers the ability to visualize features at nanometer to micrometer scales, making it invaluable for characterizing a diverse range of materials, including biological samples [11]. It facilitates the observation of surface properties, such as roughness, texture, and fine structures, which are essential for understanding the functionality and performance of various materials and biological structures [34]. But since it doesn't quantify the extent of differences from the control, it can only offer a qualitative assessment. Instead, it shows that the cell's physiological state has been physically disrupted [43].

Although there are reports on the antibacterial properties of *C. iners*, previous study focused on specific parts of the plant, such as the bark and essential oils, leaving the leaves underexplored despite their ethnobotanical significance and accessibility. Additionally, there lacking information on how external factors like pH and temperature affect the antibacterial efficacy of *C. iners* leaves extracts. This study fills that gap by evaluating the antibacterial activity of young and old *C. iners* leaves, while assessing the stability of these extracts under varying pH conditions and elucidating its mode of action. Understanding these factors is essential for optimizing the practical use of *C. iners* extracts, particularly in food preservation.

Thus, this study aimed to evaluate the antibacterial activity of young and old *C. iners* leaves extracts against foodborne pathogens namely, *B. cereus*, *B. megaterium, K. pneumoniae*, *E. coli* and *P. mirabilis*, to determine the effect of pHs and temperature on extract stability and to observed the mode of action of the extracts.

Materials and Methods

Plant Collection and Extraction

The young leaves (YL) and old leaves (OL) of *C. iners* were purchased from Putra Agriculture Centre, UPM. The color of the leaves-OL, dark green, and YL, bright/pale green-was used to visually identify their maturity. They were cleaned, dried in an oven at 60^{o}C for 48 h, and then ground into fine powders

using a separate Panasonic grinder (model MX-EX1001WSK, Malaysia). Over the course of 48 h, 100 g of the ground leaf were immersed in 400 mL of denatured ethanol (R and M Chemicals, Essex, UK, 95%). The solutions were concentrated using a rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at 40°C for 3 h after being filtered through a Whatman No. 2 filter (Whatman International Ltd., Middlesex, England). The crude extracts were subjected to freeze drying for 48 h and stored in the fridge (-20°C) for use in further studies. The crude extracts were diluted to a concentration of 10 mg/mL in 10 % DMSO (R and M Marketing, Essex, UK, 99.9%) prior experiment.

Inoculums Preparation

Selected foodborne pathogens strains namely *Bacillus cereus* ATCC33019, *B. megaterium* ATCC14581, *Escherichia coli* ATCC10536, *Klebsiella pneumoniae* ATCC13773 and *Proteus mirabilis* ATCC14153 are obtained from American Type Culture Collection (ATCC) (Rockville, Maryland, United States). Each strain was incubated onto Mueller-Hinton broth (MHB) (Oxoid Ltd., United Kingdom) for 24 h at 37ºC for antibacterial activity tests. For the susceptibility test, bacteria turbidity was adjusted between 10^6 to 10^8 CFU/mL by using standard broth microdilution method [8] or by referencing 0.5 McFarland (Chemiz, Malaysia) turbidity. The 10% DMSO served as the negative control, while 1 mg/mL of chlorhexidine (CHX) (Sigma-Aldrich, USA) served as the positive control.

Effect of Different pHs and Temperatures on Antibacterial Activity of Extract

The method was followed according to Yusoff et al. [44] to examine the effect of extracts on various pHs and temperatures against foodborne pathogens using antibacterial methods namely disc diffusion assay (DDA), minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). Using 0.1 M of sodium hydroxide (NaOH, Sigma Aldrich, United States) or 0.1 M of hydrochloric acid (HCl, Merck, Darmstadt, Germany), respectively, the extract was adjusted to pHs of 5, 7, and 9. While for the stability of temperature, the extracts were exposed to various temperatures starting from 30°C, 50°C and 80°C using a water bath for 15 min each. The extracts were left to cool at ambient temperature before analysis was carried out. Each of the treated extracts was evaluated for the DDA, MIC, and MBC after treatment. The control was an untreated extract at pH 6.6 and room temperature (25 ± 2ºC).

Scanning Electron Microscopy (SEM)

Observation of the treated strains under SEM was referring to Wong et al. [42]. Fresh *B. cereus* and *K. pneumoniae* were selected to represent Gram-positive bacteria and Gram-negative bacteria, and were treated with YL and OL of *C. iners* extracts at the tested MIC value. The extracts were applied to both cultures, and they were then incubated at 37°C in MHB for 24 h. Centrifugation at ×5000 g for 10 min was used to produce the pellets, which were subsequently fixed in 2.5% glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 4-6 h at 4°C. Next, the pellets were immersed in 0.1 M sodium cacodylate buffer (Sigma-Aldrich, St. Louis, MO, USA) for 10 minutes each of the three-rinse cycle. Following a 2 h post fixation at 4ºC with 1% osmium tetroxide (Sigma-Aldrich, St. Louis, MO, USA), the pellets underwent a third washing with 0.1 M sodium cacodylate buffer for 10 minutes. After that, the pellets were dehydrated in 35, 50, 75, and 95% acetone for 15 minutes each. Next, for 15 minutes, the pellets were fully dehydrated with 99% acetone (Merck Millipore, Darmstadt, Germany). The cell suspension was added to a specimen basket made of albumin-coated aluminium foil which was then dried for 0.5 h. The sputter was coated with gold and the specimen was set atop a stub. Using the SEM instrument (JSM 6400, JEOL Ltd., Tokyo, Japan), the morphology was observed and images were recorded.

Statistical Analysis

The data was analyzed using Minitab 19 (version 19, LLC, Pennsylvania, United States), and two-way analysis of variance (ANOVA) was applied to the outcomes of several parameters. Tukey's multiple range test was used to establish the significance of the mean difference, and different letters denote significant differences ($p<0.05$).

Results and Discussion

The sample of YL and OL *C. iners* extracts have been extracted via the maceration method. 100 g of dried *C. iners* leaves has been immersed into 400 mL ethanol solvent and has yielded in semi-viscous crude that appears dark greenish with total yields of 17.46% (YL) and 11.72% (OL). Extraction is the first crucial step to separate the desired natural product from the raw materials. Ethanol is the most widely used solvent, as it efficiently extracts polar and non-polar compounds, and extraction at low temperatures minimizes loss and prevents degradation of bioactive compounds [45]. Result indicates young leaves higher yield due higher herbivore attack rates compared to mature leaves, attributed to their rapid growth, elevated metabolic activity, and increased concentrations of specific phytochemicals typically present at

the plant canopy's top [4]. The amounts of active substances, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, will differ between the two leaf age groups in response to growth and environmental factors such as temperature and salinity [21].

Effect of Different pHs on Stability of *C. iners* Extracts

The YL and OL *C. iners* extracts were tested at various pHs levels against foodborne pathogens to evaluate their inhibitory efficacy in terms of DDA, MIC and MBC (Table 1). Based on DDA, the *C. iners* extracts both displayed inhibitory zones with diameters between 8.67 ± 0.58 to 11.33 ± 0.58 mm and 8.33 ± 0.76 to 10.33 ± 0.29 mm, respectively. It has been demonstrated that YL *C. iners* extracts exhibit higher sensitivity to the studied foodborne pathogens than OL, but at pH 5, OL *C. iners* extracts exhibit stronger inhibitory values than YL extracts. The inhibitory zone, however, did not appear to differ much amongst the several pHs examined. According to the MIC and MBC, the findings demonstrated that both YL and OL *C. iners* extracts were within the same range, which was 0.31 to 2.50 mg/mL for MIC and 0.63 to 5.00 mg/mL for MBC, respectively, at different pH values. Based on the findings, the *C. iners* extracts were discovered to have bacteriostatic effects at pH 7 and 9, although their MBC was found to be 4 times higher at 5.00 mg/mL than their MIC value of 0.31 mg/mL for young leaf for *B. cereus* and *K. pneumoniae*. In general, as compared to the original pH of the untreated extract, the antibacterial activity of the YL and OL *C. iners* extracts after being treated with various pHs demonstrated stable levels. The pH level of a solution greatly affects the ionization state, solubility, and chemical reactivity of the compounds present in a plant extract. For example, polyphenols are more stable as the pH value of the solution is lower [7]. In consensus, Bouarab Chibane et al. [6] showed that several naturally occurring plant bioactive components, such as polyphenolic, which are consumed by humans, are harmed by high pH levels. For instance, extracts from *Cinnamomum zeylanicum* have shown similar stability of polyphenolic compounds such as gallic acid and p-coumaric acid at acidic and neutral pH levels, with high antibacterial efficacy [32]. Similarly, *Laurus nobilis* (bay leaf), another member of Lauraceae, exhibits consistent antibacterial properties across a range of pH levels, largely due to the stability of its essential oils and phenolic acids such as caffeic acid [1]. Hoque et al. [14] stated the highest antibacterial activity was found at pH 5.0 for essential oil of cinnamon against foodborne bacterial, *L. monocytogenes, E. coli, S.* Enteritidis, and *B. cereus.* The range of DDA, MIC and MBC values suggest that the *C. iners* extracts are stable under different pH conditions and could serve as potential antibacterial agents for food applications.

Table 1. The effects of pHs on the antibacterial of *C. iners* against selected foodborne pathogens

Strains/ Extract	Zone of Inhibition (mm)				MIC				MBC			
	pH 5	pH 6.6	pH 7	pH 9	pH 5	pH 6.6	pH 7	pH 9	pH 5	pH 6.6	pH 7	pH 9
Bc												
YL	9.00±0.00^{Ba}	9.50±0.71^{ABa}	9.33±0.58^{Aba}	10.33±0.58^{Aa}	1.25	0.63	0.31	0.63	5.00	5.00	5.00	5.00
OL	9.33±0.58^{Aba}	9.50±0.71^{ABa}	9.00±0.00^{Ba}	9.00±0.00^{Bb}	0.63	1.25	1.25	0.63	5.00	2.50	5.00	5.00
Bm												
YL	9.00±1.00^{Ba}	9.50±0.71^{ABa}	9.00±0.00^{Ba}	10.00±0.00^{Aa}	0.63	0.63	0.63	0.63	1.25	1.25	5.00	0.63
OL	9.33±0.58^{Aba}	10.00±0.00^{Aa}	10.33±0.58^{Aa}	9.33±0.58^{ABb}	0.31	0.63	2.50	0.63	1.25	1.25	5.00	1.25
Ec												
YL	9.33±0.58^{Aba}	9.50±0.71^{ABa}	10.33±0.58^{Aa}	11.00±1.00^{Aa}	0.31	0.63	2.50	1.25	0.63	1.25	5.00	2.50
OL	9.00±1.00^{Ba}	9.25±0.35^{ABa}	9.33±0.58^{Aba}	9.00±0.00^{Bb}	0.31	1.25	2.50	1.25	0.63	2.50	5.00	5.00
Kp												
YL	8.67±0.58^{Ba}	9.00±0.00^{ABa}	8.61±0.58^{Bb}	10.00±0.00^{Aab}	1.25	1.25	0.63	0.31	5.00	5.00	5.00	5.00
OL	9.67±0.27^{Aba}	9.50±0.71^{ABa}	8.33±0.76^{Bb}	10.33±0.29^{Aa}	0.63	0.63	1.25	1.25	5.00	5.00	5.00	5.00
Pm												
YL	10.33±0.58^{Aa}	9.00±0.00^{Ba}	10.00±0.00^{Aa}	11.33±0.58^{Aa}	0.63	0.63	0.63	0.63	1.25	2.50	5.00	1.25
OL	9.67±0.58^{ABa}	10.00±0.00^{Aa}	10.00±0.00^{Aa}	9.33±0.29^{ABb}	0.63	0.63	1.25	2.50	2.50	2.50	5.00	5.00

Bc: *Bacillus cereus*; *Bm*: *Bacillus megaterium*; *Ec*: *Escherichia coli*; *Kp*: *Klebsiella pneumoniae*; *Pm*: *Proteus mirabilis*; YL: young leaf, OL: old leaf, ATCC: American Type Culture Collection, pH 6.6 indicated control pH of extracts. Values are mean ± SD of replications (n = 3). Values with different superscript capital letters within the same rowand small letters within same column are significantly different (p<0.05)

Effect of Different Temperatures on Stability of *C. iners* Extracts

The YL and OL *C. iners* extracts were tested at various temperatures levels against foodborne pathogens to evaluate their inhibitory efficacy in terms of DDA, MIC and MBC (Table 2). The diameter inhibition zones (DDA) for the YL and OL *C. iners* extracts were 9.00 ± 0.00 to 12.67 ± 0.58 mm and 9.25 ± 0.35 to 10.00 ± 0.00 mm, respectively. It was discovered that YL has the best antibacterial action compared to OL towards all tested foodborne pathogens at 30ºC exposed to *C. iners* extracts. Both YL and OL *C. iners* extracts were shown to be most harmful to *B. cereus*, whereas OL were harmful to *E. coli, B. cereus* and *P. mirabilis*. After being exposed to higher temperatures of 50°C and 80°C, the inhibitory activities of YL *C. iners* extracts exhibited small reductions in the zone of inhibition compared to 30°C but remained greater than those of control (25°C). This reduction may result from the depolymerization and heat degradation of polyphenols. Depolymerization degrades polymeric polyphenols, such as proanthocyanidins, into smaller unit, whereas heat degradation changes chemical structure, reducing the antibacterial efficacies [22]. The MIC for extracts from YL and OL *C. iners* extract were 0.16 to 1.25 mg/mL and 0.31 to 2.50 mg/mL, respectively. For MBC, the ranges for extracted YL and OL *C. iners* extract were at 0.31 to 5.00 mg/mL and 0.63 to 5.00 mg/mL, respectively. While, *B. cereus, B. megaterium, E. coli* and *P. mirabilis* were discovered to be stable at temperatures of 30°C and 50°C, their MIC and MBC values exhibited a slight decrease at 80°C. Any product development process must take into account the stability of functional food items throughout processing and storage [13]. Enzymatic browning, pH, temperature and microbial population may impact how stable the bioactive components of a food product are both before and after processing [15]. In addition to current study, the strongest antibacterial activity was identified for *Cinnamomum verum* EO at high temperature against the majority of the bacterial combinations, indicating that high temperatures do not impact the activity of the EO [9]. As reported by Muhammad et al. [27], exposure to temperatures of 60°C and 80°C for 120 h reduced polyphenol retention in *Cinnamomum burmanii* extract-loaded nanoparticles to approximately 90% and 70%, respectively. High temperatures also cause non-enzymatic condensation, a chemical reaction to form complex molecule further decreasing phenolic content. This reduction in polyphenols also correlates with a decrease in antibacterial efficacy, as polyphenols are key contributors to the antimicrobial properties of the extracts. Gong et al. [12], reported that the volatilization and/or physical and chemical changes during heating cause natural compounds to lose their antibacterial effectiveness. This serves as valuable indicator that *C. iners* extract remain stable after treatment with high temperature, making it suitable for food applications. Specifically, it refers to its antibacterial effectiveness against *B. megaterium* and *E. coli* at 80ºC.

Table 2. The effects of temperatures on the antibacterial of *C. iners* against selected foodborne pathogens

Strains/ Extract	Zone of Inhibition (mm)				MIC (mg/mL)				MBC (mg/mL)			
	25ºC	30ºC	50ºC	80ºC	25ºC	30ºC	50ºC	80ºC	25ºC	30ºC	50ºC	80ºC
Bc												
YL	9.50±0.71^{Bb}	12.67±0.58^{Aa}	12.00±0.00^{Aa}	9.67±0.58^{Bb}	1.25	0.31	0.31	0.31	2.50	2.50	0.63	0.63
OL	9.50±0.71^{Bb}	9.67±0.58^{Bb}	9.67±0.58^{Bb}	10.00±0.00^{Aba}	1.25	0.63	0.63	1.25	5.00	1.25	0.63	2.50
Bm												
YL	9.50±0.71^{Ab}	11.33±1.15^{Aab}	10.33±0.58^{Aab}	11.00±1.00^{Aa}	0.63	0.31	0.63	1.25	1.25	0.63	1.25	2.50
OL	10.00±0.00^{Ab}	9.67±0.58^{Ab}	9.33±0.58^{Ab}	9.67±0.58^{Ab}	0.63	1.25	0.63	1.25	5.00	2.50	1.25	2.50
Ec												
YL	9.50±0.71^{Bb}	11.33±0.58^{Aab}	10.33±0.58^{ABab}	10.67±1.53^{Aa}	0.63	0.31	0.31	1.25	2.50	0.63	0.63	5.00
OL	9.25±0.35^{Bb}	9.00±0.00^{Bb}	9.67±0.58^{Abb}	10.33±0.58^{Aba}	2.50	0.63	0.31	2.50	5.00	1.25	0.63	5.00
Kp												
YL	9.00±0.00^{Bb}	12.00±1.00^{Aa}	10.00±1.00^{ABab}	9.67±0.58^{Bb}	1.25	0.31	0.16	0.31	2.50	0.63	0.31	0.63
OL	9.50±0.71^{Bb}	8.67±0.58^{Bb}	9.33±1.15^{Bb}	10.00±1.00^{Aba}	1.25	0.63	0.63	0.63	5.00	1.25	1.25	1.25
Pm												
YL	9.00±0.00^{Cb}	12.00±1.00^{Aa}	11.00±0.00^{Aba}	10.00±0.00^{BCa}	0.31	0.16	0.16	0.15	1.25	0.31	0.31	0.31
OL	10.00±0.00^{BCa}	9.67±0.58^{BCb}	9.67±0.58^{BCb}	9.33±0.58^{Cb}	0.63	0.63	0.31	0.63	2.50	1.25	0.63	1.25

Bc: *Bacillus cereus*; *Bm*: *Bacillus megaterium*; *Ec*: *Escherichia coli*; *Kp*: *Klebsiella pneumoniae*; *Pm*: *Proteus mirabilis*; YL: young leaf, OL: old leaf, ATCC: American Type Culture Collection, 25ºC indicated control temperature of extracts. Values are mean ± SD of replications (n = 3). Values with different superscript capital letters within the same rowand small letters within same column are significantly different (p<0.05)

Mode of Action of *C. iners* Extracts Against Foodborne Pathogens Using SEM

Fresh *B. cereus* and *K. pneumoniae* were selected to represent Gram-positive bacteria and Gram-negative bacteria. The SEM pictures (5000 × magnification) in Figure 1 presented the images of untreated and treated/injured cells of *B. cereus* and *K. pneumoniae* with YL *C. iners* extract at 2.50 and 1.25 mg/mL, respectively. In control group, the morphology appears as rod-shaped cells, turgid, whole with individual cells varying in length and width, and separated from one another for *B. cereus* (Figure 1a) and *K. pneumoniae* (Figure 1c). On the other hand, strains treated with the YL *C. iners* extract at the MIC value had clear negative effects on the shape of cell membranes of *B. cereus* (Figure 1b) and *K. pneumoniae* (Figure 1d). Treatment-induced *B. cereus* and *K. pneumoniae* were found to have an incomplete and distorted shape, no cell walls, and a significant increase in the release of cell components, which resulted in cytoplasm secretion and cell lysis. This result of this study was consistent with those of Behbahani et al. [5]. The SEM images also capture surface appendages, such as flagella or pili, which play essential roles in bacterial motility and adhesion to surfaces. The capsule (outermost layer), which serves as a virulence factor, can be observed as a protective layer surrounding the bacterial cell, contributing to its ability to evade the host immune system [18]. YL was chosen based on current study antibacterial efficacy and was reported by their relatively early stage of development, during which they actively synthesize and accumulate various bioactive compounds [19], such as xanthorrhizol, eugenol, cinnamic aldehyde [28]. These bioactive compounds are known to play essential roles in plant defense mechanisms against environmental stressors, pathogens, and herbivores, as well as in regulating various physiological processes [3]. Shu et al. [38] stated that the vegetative cell of *B. cereus* was exhibited apparent morphological and ultrastructural changes, further affirming the disruption of cell membrane and the unbalance of cellular environments when treated with mannosylerythritol lipids, a type of natural glycolipid. Kim et al. [20] mentioned that the onion peel extract prepared using subcritical water extraction was observed to damage the cell of *B. cereus*. According to Jamal et al. [17], *K. pneumoniae* forms biofilms, where bacterial cells adhere to surfaces and produce a matrix of extracellular substances, providing protection and enhancing bacterial resilience. Further study using SEM can aid in elucidating the mechanisms behind bacterial virulency, antimicrobial resistance, and adaptation to different niches.

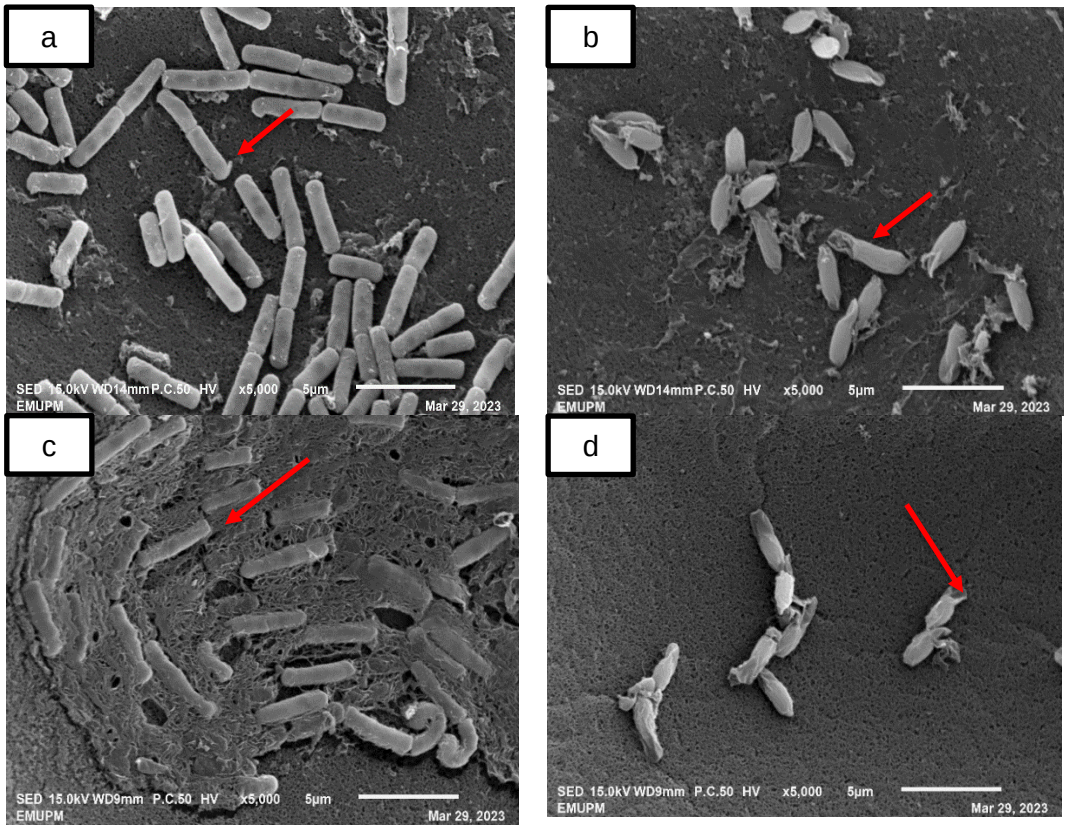

Figure 1. Bacterial morphology observation treated with YL *C. iners* extract by using SEM. *B. cereus* (a) control (b) treatment and *K. pneumoniae* (c) control (d) treatment, at 5000 x magnification

Conclusions

In this study, it was shown that extracts of *C. iners* leaves have antibacterial properties. The results show that the extracts have potential antibacterial action because they include antibacterial chemicals that actively attack both Gram-positive and Gram-negative bacteria's cell membranes. When evaluated in vitro, the stability of the extracts efficiently controlled all foodborne pathogens in a pH- and temperature-dependent manner. The high-resolution images obtained from SEM provide valuable data for studying the morphological characteristics of *B. cereus* and *K. pneumoniae*, which were essential for understanding its pathogenicity, interactions and mode of action with the host and the *C. iners* leaf extracts. The findings of this study will also help to innovate the use of *C. iners* extract for natural food preservative purposes.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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