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Potential of *Cosmos caudatus* Kunth. extract as a natural food sanitiser

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Abstract

Bacterial growth in food sources after harvesting and during food manufacturing has emerged as a major agricultural concern. Currently, several chemical preservatives have been added to foods to prevent or inhibit bacterial growth. These phenomena, however, become a concern when consumers begin to demand the safety of the food they consume, thus resulting in a search for natural plant extracts as an alternative. The antimicrobial activity of *Cosmos caudatus* leaf extract against six food pathogens was thus investigated in the present work. The disc-diffusion test, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) tests were used to determine the antibacterial potential. Time-kill kinetics were used to determine their killing endpoints at various incubation times. Thereafter, *Bacillus cereus* was selected as a representative bacterium to be viewed under the light sheet electron microscope (LSEM) to observe the morphological changes. For the application as a sanitiser, different concentrations of *C. caudatus* extract were applied to chicken meat and oyster mushroom to determine bacterial reduction and sensory acceptability. The toxicity test using brine shrimp lethality assay was also performed to determine the toxicity level of *C. caudatus* extract. Results showed that *C. caudatus* extract contain five major peaks of flavonoids. In the antibacterial test, all tested pathogens were inhibited, with *B. cereus* being the most susceptible, with a significant reduction time of more than 3 $\log_{10}$ at 3.125 mg/mL. Under LSEM, the population of *B. cereus* was reduced, and the shape was wrinkled. In the application, *C. caudatus* extract at 0.05% for 5 min treatment time was chosen as the best formulation, whereas the brine shrimp tests showed that *C. caudatus* extract was safe for human consumption (LC_{50}: 3.54 mg/mL). In conclusion, *C. caudatus* extract could have the potential to be further explored as a natural food sanitiser.

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Introduction

The emergence of infectious diseases caused by pathogenic food microorganisms has recently become a global concern. According to the World Health Organization (WHO), an estimated 600 million people in the global population suffer from foodborne illnesses every year (Lee and Yoon, 2021). In Malaysia, the Ministry of Health (MOH) reported that the trend continued to rise from 2005 to 2013, with 17.76 - 47.79 cases per 100,000 people (MOH, 2006; 2014). However, the actual number of cases is always higher than the reported number because infected people only experience mild illness, and simply allow it to heal at home. These circumstances are demonstrated by the current case, which occurred in one of Malaysia's states, where 59 people were hospitalised, and three of them died after eating a chicken dish contaminated with *Salmonella* (Rahman, 2019). *Pseudomonas* species, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* species are the most common food-associated pathogens.

There are several food preservative techniques practiced in the food industry (*e.g.*, physical, chemical, and natural preservation) to control microbial contamination, and extend the food shelf life (Kim *et al.*, 2024). However, this practice has become a concern for consumers seeking natural foods with only natural ingredients. Furthermore,

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chemical preservatives added to food products only slow down the growth of pathogens instead of eliminating them (Negi, 2012). In this regard, plant extracts have emerged as an alternative method to be investigated due to their potential health benefits, and there are various studies reporting the potential of plant extracts/and essential oils as food preservatives (Kim *et al.*, 2024).

Cosmos caudatus Kunth., locally known as *ulam raja*, is found throughout tropical regions, and always consumed as *ulam* or salad by most Malaysians and Indonesians. This plant belongs to family Asteraceae, and is best grown at temperatures ranging from 50 to 55°C (Latiff *et al.*, 2021). It grows to a height of 1 - 2 m, and produces pink, yellow, or white flowers and fruits (Uzbek and Shahidan, 2019). Its leaves and leaflets are variably lobed, and of different sizes, and when the shoots are harvested after planting, the plant will grow in height and branches (Moshawih *et al.*, 2017). Previously, this plant has been reported to have antibacterial, antifungal, antidiabetic, and anticancer properties (Salehan *et al.*, 2013; Perumal *et al.*, 2014; Seyedreihani *et al.*, 2017; Ramadhan *et al.*, 2018; Yusoff *et al.*, 2021). However, as far as we are concerned, their studies focused solely on the detection of antibacterial properties, with no output on the bacterial morphological changes that occurred after treatment. Moreover, there is no report to date on the development of *C. caudatus* extract as a food sanitiser. Therefore, the present work was conducted to determine the antibacterial activity of the extract of *C. caudatus* against selected foodborne pathogens, followed by the bacterial morphological changes after the treatment, and its potential to reduce the natural microflora in selected food materials, along with their sensory analysis.

Materials and methods

Plant sampling and extraction

Cosmos caudatus plant was purchased at Pasar Borong Selangor in Seri Kembangan, Selangor, and sent to the Institute of Bioscience, Universiti Putra Malaysia for taxonomic identification with the voucher number SK2668/15. The leaves were separated, cleaned under running tap water, dried at room temperature (27°C), and ground using a mechanical grinder until a fine powder formed. The powdered *C. caudatus* was then immersed in pure methanol for 7 d with occasional shaking. The mixture was then filtered using Whatman filter paper No. 1, concentrated in a rotary evaporator (50°C) to produce a crude extract, and stored at -20°C for further use.

Identification and quantification of flavonoid compounds in C. caudatus extract using HPLC and LC-MS/MS analyses

The compound identification was carried out using the Dionex Ultimate 3000 Rapid Separation LC system (ThermoFisher Scientific™, San Jose, CA), simultaneously linked to the Q Exactive Plus HRAM LC-MS/MS (ThermoFisher ScientificTM, San Jose, CA), and equipped with both positive and negative ionisation modes (ESI). Analyte separation was performed using Hypersil GOLD aQ column (100 × 2.1 mm ID). The temperature was set to 30°C, and the gradient elution was performed as follows: 0 min, 90% solvent A: 10% solvent B; 5.5 min, 80% solvent A: 20% solvent B; 10.50 min, 50% solvent A: 50% solvent B; 15.5 min, 30% solvent A: 70% solvent B; 18.0 min, 0% solvent A: 100% solvent B; 20.0 min, 0% solvent A: 100% solvent B; 21.0 min, 50% solvent A: 50% solvent B; 22.0 min, 90% solvent A: 10% solvent B; and 25.0 min, 90% solvent A: 10% solvent B. The flow rate was kept at 250 µL/min throughout the process, and the data were analysed using Xcalibur software (version 1.2).

For compound quantification, high performance liquid chromatography (HPLC, Agilent, 1100 Series) was used as adapted from Mustafa *et al.* (2010) with slight modifications. The ZORBAX Eclipse Plus C18 (4.6 mm × 250 mm × 5 µm) column (Agilent, USA) was employed, utilising deionised water with 2% acetic acid. The following solvent gradient elution was adopted: 0 min, 90% solvent A: 10% solvent B; 5 min, 90% solvent A: 10% solvent B; 10 min, 40% solvent A: 60% solvent B; 15 min, 10% solvent A: 90% solvent B; and 20 min, 90% solvent A: 10% solvent B. Briefly, *C. caudatus* extract was prepared at 1 mg/mL by dissolving in methanol, and filtered using a 0.2 um nylon membrane into a 2 mL vial. Thereafter, 20 µL of the sample was injected into the HPLC system using a 1 mL injection loop, and the detection peak was observed at 360 nm. Similar procedures were used for the standards, with the exception of the concentration, which was varied. For each standard, five different concentrations were prepared and injected into HPLC for the purpose of plotting the standard curves. The flow rate was maintained at 1.0 mL/min, and the UV

detection was at 360 nm. The standards used (*i.e.*, rutin, quercetin, and quercetin rhamnoside) were purchased from Sigma Chemicals (St. Louis, USA), and their selection was based on the major compounds in *C. caudatus* which had previously been reported by Mediani *et al.* (2012).

Antibacterial test: Disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC)

All antibacterial tests were conducted in accordance with Yusoff *et al.* (2014). For the disc diffusion test, 25 µL of pathogen inoculum (*i.e.*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus*) at 10^5 - 10^8 CFU/mL each, was streaked on Mueller Hinton Agar (MHA). A sterile commercial disc was placed onto the agar, and 10 µL of *C. caudatus* extract (10%) was pipetted onto each disc. Plates were incubated at 37°C for 24 h, and the inhibition zone (mm) was measured and recorded. A 0.1% chlorhexidine and 10% dimethyl sulfoxide (DMSO) served as positive and negative controls, respectively. The minimum inhibition concentration (MIC) and minimal bactericidal concentration (MBC) tests were performed as described in CLSI guidelines; M27-A3 (CLSI, 2008) and M07-A09 (CLSI, 2012). Briefly, all wells in the first column were filled with 200 µL of MHA/SDB, while wells from columns 2 to 12 were filled with inoculum (10^5 - 10^8 CFU/mL). Then, starting from well 12, a 2-fold dilution of 10% *C. caudatus* extract was performed, yielding the highest concentration of extract at well 12 (50 mg/mL) and the lowest at well 3 (0.1 mg/mL). After that, the microtiter plates were placed in an incubator at 37°C for 24 h. The MIC value was determined by visually observing the well with complete growth inhibition (Rukayadi *et al.*, 2013). Following MIC test, MBC test was performed by pipetting 10 µL of culture from each 96-well plate, and sub-culturing onto MHA. Inoculated plates were incubated, and the MBC value was determined by the lowest concentration that showed no visible growth.

Time-kill analysis

With slight modifications, time-kill analysis was performed according to Lorian (2005) and Pankey and Ashcraft (2009). Briefly, standardised inoculums (10^5 - 10^8 CFU/mL) were mixed with various concentrations of *C. caudatus* extract (*i.e.*, 0, 0.5, 1, 2, 4, and/or 8 MIC), at different exposure times (*i.e.*, 0, 0.25, 0.5, 1, 2, and 4 h). Next, 10 µL of each mixture was then serially diluted with 0.85% sterile phosphate buffer saline into several dilutions before pipetting them onto MHA, and incubating for 24 h at 37°C. The number of growing colonies was counted and recorded for graph plotting.

Light-sheet electron microscope (LSEM)

A standardised *B. cereus* inoculum (10^5 - 10^8 CFU/mL) was treated with 6.25 mg/mL of *C. caudatus* extract and left at 37°C for 24 h. The solution was then centrifuged at 4,500 rpm for 15 min, and the supernatant was discarded. The precipitate was then stained with crystal violet dye (Gram-positive bacteria) and left to settle for 15 min before being washed with sterile tap water. After that, agarose gel (2%) was added to the *B. cereus* precipitate, and kept in a water bath (45 - 50°C) to prevent the agarose gel from becoming solid. Finally, bacteria were drawn into a microscope needle, and observed under a Light Sheet Z.1 Microscope (Zeiss, Jena, Germany) at various magnifications.

Toxicity evaluation of C. caudatus extract using brine shrimp lethality assay

The toxicity of *C. caudatus* extract was determined according to LewisOscar *et al.* (2018). Briefly, a two-fold dilution method of *C. caudatus* methanolic extract was prepared to get a concentration range of 0.1 - 100 mg/mL, where artificial seawater was used as the diluent. Then, a total of 30 nauplii (*Artemia salina*) were added to each concentration and incubated for 24 h at room temperature. Previously, nauplii were hatched and separated from their eggs using a light microscope. Treatment using potassium dichromate served as a positive control, whereas *Artemia* alone in artificial saltwater served as a negative control. Results were recorded by counting the survived nauplii in each treatment, and the LC_{50} was calculated based on the plotted graph of mean percentage mortality (%) against the logarithm of *C. caudatus* extract concentrations.

Effect of C. caudatus extract at different concentrations and exposure times on raw food materials and sensory acceptability

Two types of fresh food materials, namely chicken meat and oyster mushroom, were used. Briefly, four concentrations of *C. caudatus* (*i.e.*, 0,

0.05, 0.50, and 5.00%) were prepared by diluting with deionised water (B. Braun Medical Industries, Penang, Malaysia). Next, 10 g of chicken meat that had been cut into cubes were immersed in the *C. caudatus* solution for varying immersion durations (*i.e.*, 0, 5, 10, and 15 min). Then, the sample was dried on the sterile filter paper to eliminate any excess solution prior to homogenising it in a stomacher machine (BagMixer 400-P Interscience, France) at 250 rpm for 2 min. After homogenisation, a serial dilution was performed and spread onto the selective agar for bacterial counting after the incubation period at 53 ± 2°C for 24 h. A similar procedure was applied for oyster mushroom, as adapted by Yusoff *et al.* (2022).

For sensory analysis, the method was performed according to Brasil *et al.* (2012), with slight modifications. All sensory acceptability was performed on each treatment of chicken meat and oyster mushroom, with tap water as a control. Briefly, the chicken meat samples with a weight of 10 g each were immersed in different concentrations of *C. caudatus* extract (*i.e.*, 0, 0.05, 0.5, and 5.00%) separately, dried on tissue paper to remove the excess solution, and given to the panellists for evaluation. A group of 25 panellists were provided with five-digit coded samples placed in a random order, and the acceptance was evaluated based on the 9-point hedonic scale provided, ranging from extremely disliked (on a scale of 1) to extremely liked (on a scale of 9). Similar procedures were also applied for the oyster mushroom. All these sensory tests were conducted on two different occasions, and scores ≥ 5 were considered "acceptable".

Statistical analysis

All experiments were performed in replicates, and the results were expressed as mean ± standard deviation. The statistical analysis was performed using MINITAB software (MINITAB version 16.0), and $p < 0.05$ was indicated as having a significant difference between treatments.

Results and discussion

In the present work, extraction of 100 g of dried *C. caudatus* yielded 16.49 g of crude extract (16.49% of total yield value). Previous studies have reported the extraction of *C. caudatus* with similar solvent extraction, although their yield percentages were different (Salehan *et al.*, 2013; Abdullah *et al.*, 2015) due to geographical factors, extraction methods, and preservation methods (Mediani *et al.*, 2012; Latiff *et al.*, 2021). A total of five major peaks were identified in *C. caudatus* extract, which comprised of flavonoids (*i.e.*, rutin, quercetin 3-*O*-glucoside, quercetin 3-*O*-arabinoside, quercetin 3-*O*-rhamnoside, and quercetin) (Table 1). All these identifications were performed based on their mass and fragmentation ions, and also compared with other studies (Mediani *et al.*, 2012). Three major representative compounds of the identified compounds were quantified using HPLC based on the regression line plotted in the calibration curve for each of the compounds. The highest compound was quercetin 3-*O*-rhamnoside (29.66 mg/g), followed by rutin (1.85 mg/g) and quercetin (0.95 mg/g); however, the other two detected compounds were not quantified due to a lack of standards in our collection. The quantification of these major compounds is crucial to determining the effectiveness of the extracts, where the amount of metabolites directly affects their biological activities. Details of the compound fragmentation ion and quantification of each compound in *C. caudatus* extract have been reported previously by Yusoff *et al.* (2021). Zongo *et al.* (2011) supported the findings that the biological activities of flavonoids are due to the presence of hydroxyl groups and phenolic rings, which make them hydrophobic, and easily accumulate in cell membranes, causing membrane disruption and death. Moreover, the hydrophobicity of phenolic compounds also plays a major role in cell inhibition and killing, as the key to their activity is to dissolve in the lipid phase of the cytoplasmic membrane, and cause alteration and disruption of the cells (Kumar and Pandey, 2013).

The antibacterial activity of *C. caudatus* extract was evaluated against six types of foodborne pathogens, as tabulated in Table 2. Results showed the potential of *C. caudatus* extract to suppress all tested foodborne pathogens in various diameters of clear zones. The disc-diffusion test showed that *B. cereus* resulted in the largest inhibition zone (9.80 mm) than others, whereas the MICs of *B. cereus* and *S. aureus* were found to be the most susceptible pathogens (6.25 mg/mL). The MBC had a wide range of values, ranging from 12.5 to 50.0 mg/mL. Both *B. cereus* and *S. aureus* are Gram-positive bacteria, and easily inhibited due to the thick peptidoglycan layer, which easily absorbs foreign materials like antibiotics

Table 1. Compounds identified in *C. caudatus* extract using LC-MS/MS analysis.

Peak	RT	$[M-H]^-$	MS/MS	Compound	Relative amount (mg/g)
1	7.18	609	463, 300, 179, 151	Rutin	1.85 ± 0.04
2	7.47	463	300, 179, 151	Quercetin 3-*O*-glucoside	-
3	8.26	433	300, 179, 151	Quercetin 3-*O*-arabinoside	-
4	8.47	447	300, 179, 151	Quercetin 3-*O*-rhamnoside	29.66 ± 0.19
5	10.03	301	151	Quercetin	0.95 ± 0.01

RT: retention time; $[M-H]^-$: molecular ion peak (deprotonated ion); (-): unidentified.

Table 2. Inhibition zone, MIC, and MBC of *C. caudatus* extract against foodborne pathogens.

Pathogen	Inhibition zone (mm)			MIC and MBC (mg/mL)	
	C. caudatus extract	0.1% CHX	10% DMSO	MIC	MBC
B. cereus	9.80 ± 0.29	9.00 ± 0.00	n.a.	6.25	50.00
E. coli O15:H7	8.70 ± 0.58	10.00 ± 0.71	n.a.	12.50	12.50
K. pneumoniae	8.70 ± 0.00	10.00 ± 0.00	n.a.	12.50	50.00
L. monocytogenes	8.70 ± 0.71	10.00 ± 0.71	n.a.	12.50	50.00
S. Typhimurium	8.00 ± 0.71	11.00 ± 0.71	n.a.	12.50	50.00
S. aureus	9.00 ± 0.00	16.00 ± 0.71	n.a.	6.25	12.50

Values are mean ± standard deviation. n.a.: no activity (6 mm); CHX: chlorhexidine (positive control); and DMSO: negative control. Diameter of inhibition zones in mm (including disc).

or detergents. Hany and Neelam (2020) reported the rapid inhibition of Gram-positive bacteria by plant extracts due to their semi-permeable outer membrane, which allows small hydrophilic molecules to pass into the cell. However, in the MBC result, the result was contradictory, demonstrating that *B. cereus* species were difficult to kill. This could have been due the ability of *B. cereus* to form spores, and become more resistant when experiencing extreme conditions (Soni *et al.,* 2016; Rodrigo *et al.,* 2021). In the food industry, the formation of spores on food surfaces is inevitable, and can cause food spoilage and contamination, and the presence of spores ranging from 10^5 - 10^8 CFU/g in food usually triggers infection in the people who consume it (Rodrigo *et al.,* 2021).

The killing kinetics of each of the tested food pathogens are illustrated in Figures 1a to 1f. Among them, the strongest bacteriostatic activity of *C. caudatus* extract was observed against *B. cereus,* at 3.125 mg/mL with more than 3 $\log_{10}$ CFU/mL of reduction after 2 h of treatment. The killing endpoint was reached at 50 mg/mL of *C. caudatus* extract after 2 h of treatment with *B. cereus*. A different study reported by Jobim *et al.* (2014) showed that no killing endpoint was achieved at 66.4 mg/mL of *Astrocaryum aculeatum* extract after 10 h of treatment on *B. cereus*. Meanwhile, *Widelia chinensis* extract was reported to kill *B. cereus* at 6.25 mg/mL after 48 h of exposure (Darah *et al.,* 2013). These studies showed that the differences were highly influenced by the presence of bioactive compounds in the plant extracts. As in *C. caudatus* extract, flavonoids are the major constituents, and have been widely reported to possess higher antimicrobial activity (Seyedreihani *et al.,* 2017; Alvarez-Martinez *et al*., 2021) due to their ability to complex with extracellular cells and proteins in microbial cell walls, thus initiating the disruption of cell walls (Dhiman *et al*., 2016). *Salmonella enterica* serovar Typhimurium was revealed to be the most resistant strain as compared to others, with a time-kill endpoint of 50 mg/mL after 4 h of treatment.

To observe the cell changes, *B. cereus* was selected as a representative bacterium to be observed under LSEM after being treated with 6.25 mg/mL of *C. caudatus* extract. Findings revealed that the untreated *B. cereus* showed a uniform shape, and was

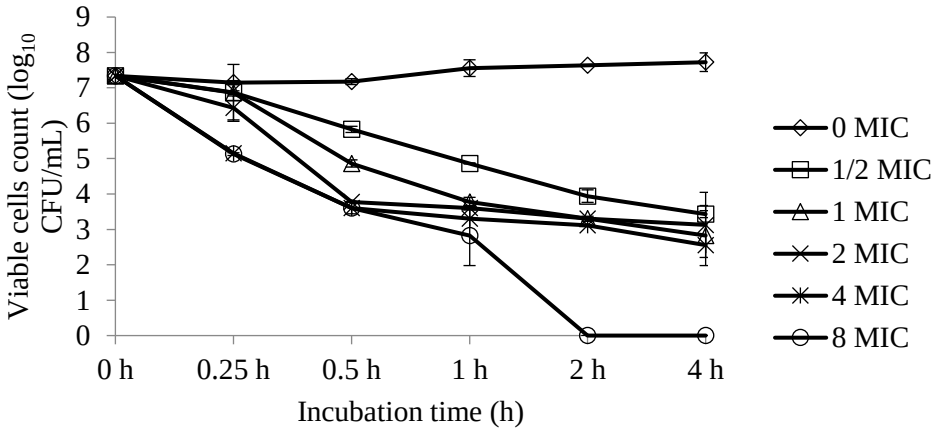

Figure 1(a). Rate of killing actions for *B. cereus* at different MIC levels.

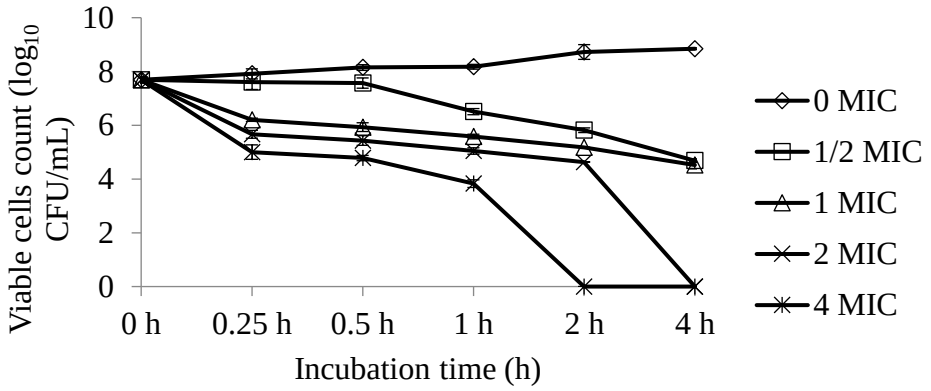

Figure 1(b). Rate of killing actions for *E. coli* O157:H7 at different MIC levels.

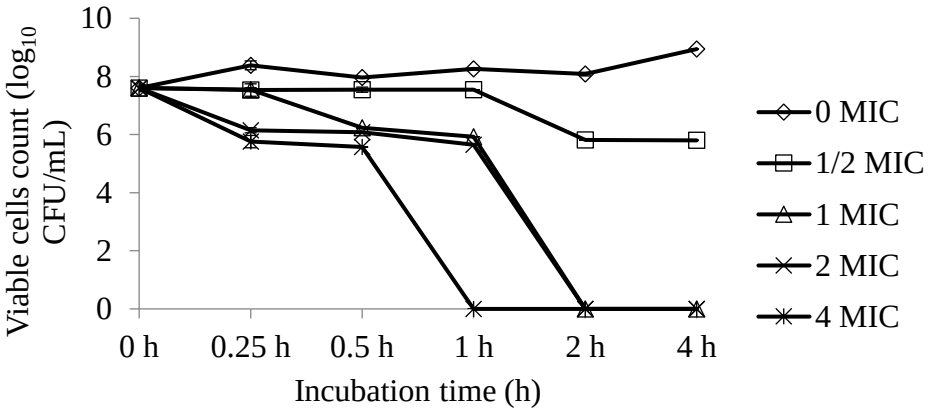

Figure 1(c). Rate of killing actions for *K. pneumoniae* at different MIC levels.

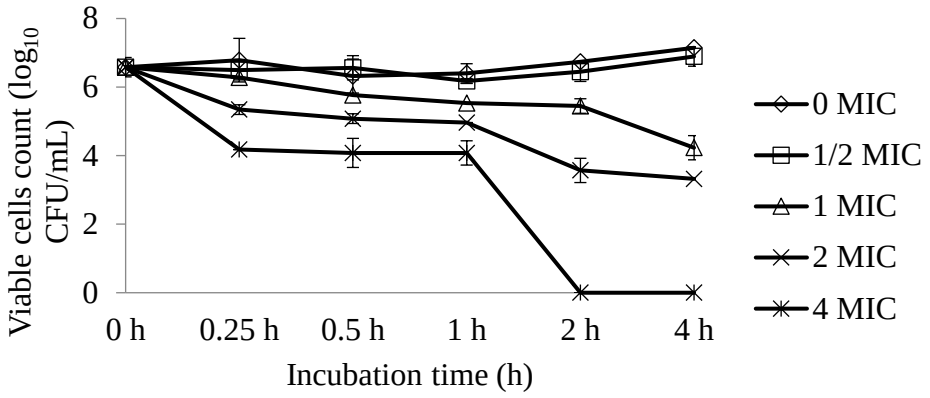

Figure 1(d). Rate of killing actions for *L. monocytogenes* at different MIC levels.

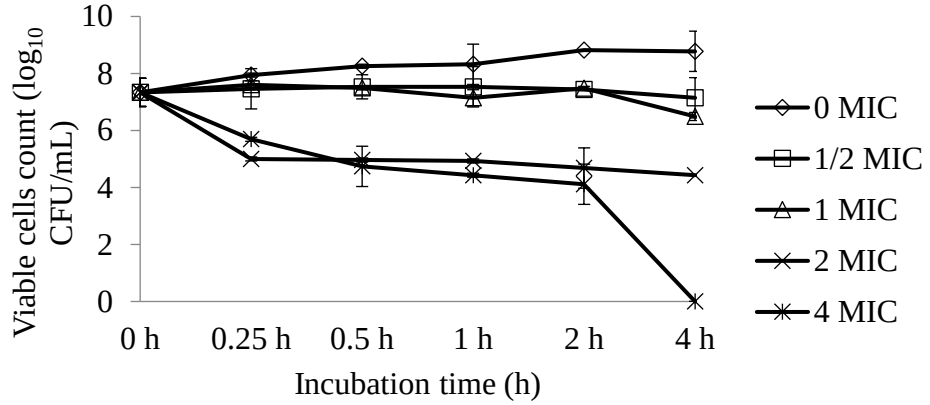

Figure 1(e). Rate of killing actions for *S. enterica* serovar Typhimurium at different MIC levels.

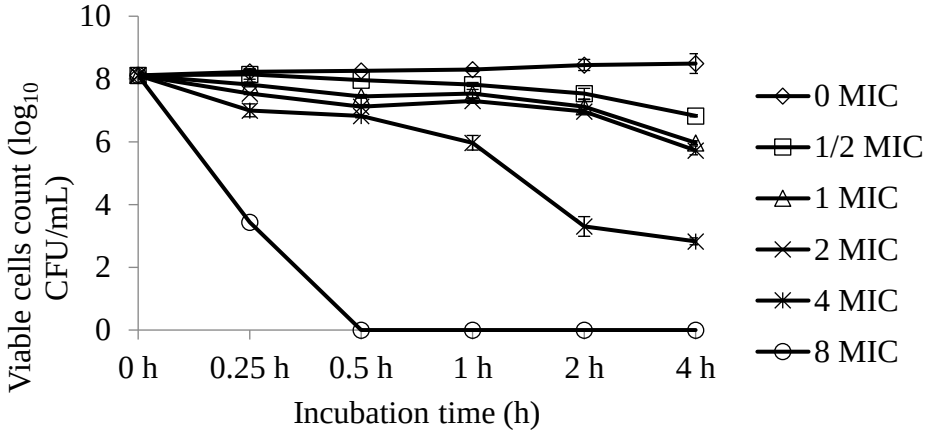

Figure 1(f). Rate of killing actions for *S. aureus* at different MIC levels.

in an active dividing stage (Figure 2a). However, after treatment, the populations decreased in number, and formed wrinkled bacilli shapes, and some of them were ruptured (Figure 2b). These consequences might have been due to the disruption of bacterial cell walls by the flavonoid compounds in *C. caudatus* extract (*i.e.*, rutin, quercetin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-arabinoside, and quercetin 3-*O*-glucoside), where their lipophilic character causes penetration into the cell membrane and coagulates their cell contents, which consequently interrupts cell energy metabolism and DNA synthesis in microorganism cells, thus leading to cell death (Gonelimali *et al.*, 2018; Khameneh *et al.*, 2019; Yu *et al.*, 2021).

As *C. caudatus* extract is intended to be applied as a food sanitiser, the toxicity level must be determined to evaluate the safety of this plant extract, which is why the brine shrimp lethality assay was performed. This test was performed because it is a quick and economical practice, and the results are also reliable to test the plant toxicity (Rajabi *et al.*, 2015). Results showed that the LC_{50} value of *C. caudatus* extract after exposure to *A. salina* for 24 h was 3.54 mg/mL. The value of LC_{50} was obtained from the best-fit line plotted of the percentage of nauplii killed against the concentration of *C. caudatus* extract (Figure 3). According to Meyer *et al.* (1982), plant crude extracts with LC_{50} values > 1 mg/mL are considered safe for human use, thus concluding that the *C. caudatus* extract would be safe for human use. Rameli *et al.* (2018) also reported the toxicity study of *C. caudatus* using brine shrimp lethality assay, and suggested using the extract below 100 µg/mL for

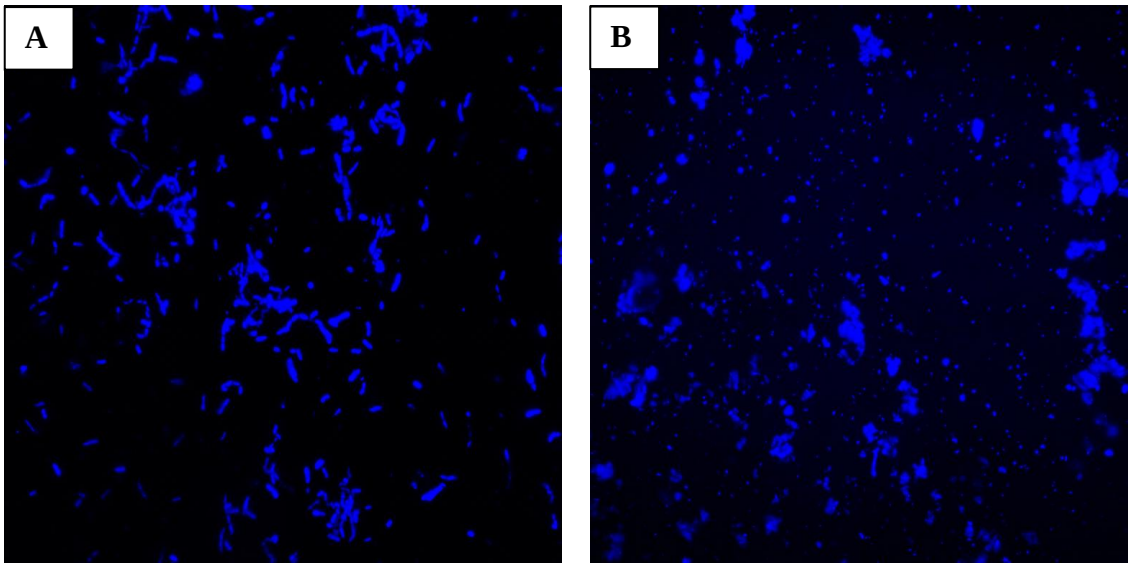

Figure 2. Stained *B. cereus* viewed under light-sheet electron microscope**. (A)** control; and **(B)** after treated with *C. caudatus* extract for 24 h.

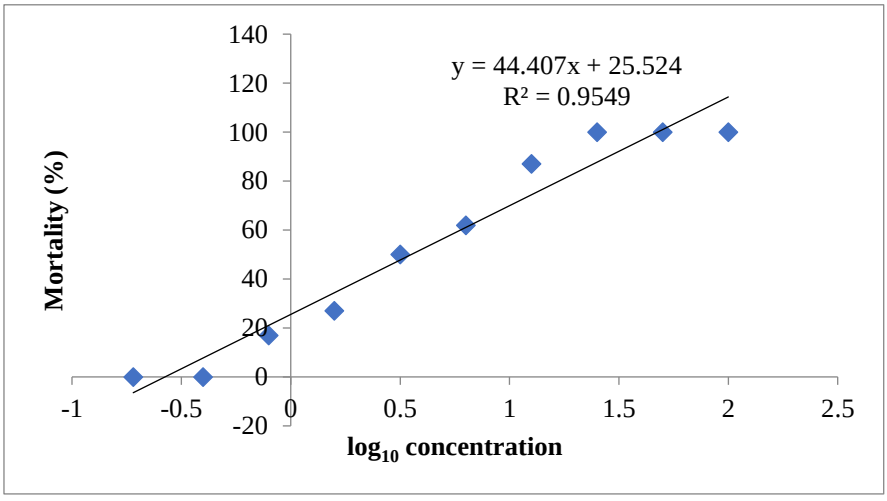

Figure 3. Standard curve of brine shrimp lethality assay after treated with *C. caudatus* extract for 24 h.

efficiency and safety. Another study on *C. caudatus* toxicity was also reported by Amna *et al.* (2013) where no toxicity signs or deaths were observed after the inclusion of 5,000 mg/kg of *C. caudatus* extract in the rat diet for 28 d of treatment. Herlina *et al.* (2021) reported that the administration of 333 mg/kg BW *C. caudatus* extract to rats for 14 d caused no severe pancreatic damage, and was considered safe. Ahda *et al.* (2023) recently reported that *C. caudatus* has the potential to improve insulin resistance in type-2 diabetic patients, and that the leaves are not toxic and pose no risk if taken.

For the application of *C. caudatus* extract in food materials, generally, results showed a reduction in natural microflora in both chicken meat and oyster mushroom (Tables 3 and 4). The bacterial populations were reduced in proportion to the increase in *C. caudatus* concentration and immersion time. Meanwhile, in the sensory evaluation, the minimum acceptance for most of the attributes in chicken meat and oyster mushroom was at 0.5 % (Table 5). No significant difference ($p > 0.05$) in texture even at 5.0%, which indicated that most panellists observed no changes between the *C. caudatus* treatment and the control. Meanwhile, in oyster mushroom, the minimum acceptance for most of the attributes was 0.05%, but in overall acceptability, the panellists still considered until they reached 0.50% treatment. These low acceptances for *C. caudatus* treatment might have been due to the rupture of some plant tissues when immersing at high concentrations, as these situations should also be avoided to reduce bacteria proliferation after washing. Abadias *et al.* (2011) reported a decrease in the number of bacteria that survived with an increase in sanitiser concentration and exposure time, but with no sensory evaluation data. A recent study reported the capability of *Moringa oleifera* extract to act as a hand sanitiser, where it could reduce the number of *S. aureus* (Arifan *et al.*, 2021). Ramli *et al.* (2017) also revealed the potential of *Syzgium polyanthum* leaf extract to cause a significant decrease in the natural microbial populations in grapefruits at 0.05% for a 5 min exposure time, which was accepted by the panellists during the sensory acceptability. Nascimento and Sao Jose (2022) reported a significant decrease in *Salmonella* Enteritidis, *E. coli*, *S. aureus* and *Enterococcus* sp. in fresh tomato surfaces after treatment with 30% green tea extract for 5 min contact. Özvural *et al.* (2016) also revealed a lower mesophilic aerobic count in hamburgers coated with edible green tea.

Conclusion

In the present work, results indicated that *C. caudatus* extract had broad range of antibacterial activities against all tested foodborne pathogens. All bacteria varied in their killing endpoints, depending on the concentration of *C. caudatus* extract and time of exposure. The major bioactive compounds, which mainly were from the phenolic and flavonoid groups (*i.e.*, rutin, quercetin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-arabinoside, and quercetin 3-*O*-glucoside), were identified by the HPLC and LC-MS/MS analyses. Generally, the increase in MIC value directly shortened the time for killing endpoints to occur. Through LSEM, *B. cereus* was observed to be inhibited due to the changes in membrane permeability, which consequently disrupted the membrane cells and the cytoplasm. The application of *C. caudatus* extract to chicken meat and oyster mushrooms as food sanitiser revealed the potential of this extract to reduce the microbial population in these food materials proportionally with the increase in *C. caudatus* extract concentration and immersion time. Based on these findings, it was suggested that *C. caudatus* extract at 0.5% for 5 min of immersion time could be promising to be further developed as a food sanitiser. However, more studies on the other food materials are crucial to determine their effectiveness and consumer acceptance. Data demonstrated that the killing activity varied depending on the concentration of *C. caudatus* extract and the type of pathogen used. Future studies including compound isolation and the antibacterial properties demonstrated by each single compound could be undertaken.

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Table 3. Effects of different concentrations and exposure times of *C. caudatus* extract on natural bacterial contaminants in chicken meat (log_{10} CFU/g).

Bacterial species	**TPC**			***B. cereus***			***E. coli***			***S. aureus***		
Initial bacterial load	6.17 ± 0.02^{Aa}			6.16 ± 0.02^{Aa}			5.90 ± 0.05^{Aa}			6.46 ± 0.09^{Aa}		
Exposure time (min) / Treatment (%)	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
Tap water	6.15 ± 0.04^{Aa}	6.09 ± 0.08^{Ab}	6.06 ± 0.04^{Ab}	5.96 ± 0.09^{Ab}	6.08 ± 0.15^{Ac}	5.96 ± 0.09^{Ac}	5.96 ± 0.01^{Ab}	5.82 ± 0.02^{Ac}	5.60 ± 0.04^{Ad}	6.42 ± 0.06^{Ab}	6.41 ± 0.04^{Ac}	6.28 ± 0.11^{Ad}
0.00	6.04 ± 0.01^{Ab}	6.00 ± 0.05^{Abc}	5.97 ± 0.07^{Ac}	4.85 ± 1.66^{Ab}	5.75 ± 0.02^{Ac}	5.59 ± 0.18^{Ad}	4.59 ± 0.18^{Ab}	5.46 ± 0.11^{Ac}	5.42 ± 0.16^{Ad}	6.17 ± 0.04^{Ab}	6.16 ± 0.16^{Ab}	6.09 ± 0.00^{Ac}
0.05	5.94 ± 0.12^{Ab}	5.81 ± 0.06^{ABc}	3.55 ± 0.04^{Bd}	3.77 ± 0.16^{Bb}	2.51 ± 1.25^{Bc}	1.67 ± 0.04^{Bd}	4.23 ± 1.30^{Bb}	3.28 ± 0.02^{Bc}	3.13 ± 0.03^{Bd}	6.11 ± 0.11^{ABb}	6.11 ± 0.01^{ABb}	5.42 ± 0.10^{Bc}
0.50	5.74 ± 1.22^{Bb}	3.47 ± 0.00^{Bc}	2.56 ± 1.31^{Cd}	2.66 ± 1.34^{BCb}	1.68 ± 0.00^{Cc}	1.63 ± 0.05^{Bc}	3.16 ± 0.04^{Cb}	3.07 ± 0.21^{BC}	1.59 ± 0.01^{BCd}	5.96 ± 0.01^{Bb}	5.63 ± 0.08^{BCc}	4.87 ± 1.69^{BCd}
5.00	3.72 ± 0.01^{Cb}	1.73 ± 0.03^{Dc}	1.65 ± 0.05^{CDd}	1.79 ± 0.14^{Cb}	0.00 ± 0.00^{Dc}	0.00 ± 0.00^{Cc}	1.64 ± 0.01^{Db}	0.00 ± 0.00^{Cc}	0.00 ± 0.00^{Cc}	5.01 ± 1.63^{BCb}	3.81 ± 0.04^{Dc}	2.74 ± 1.46^{Cd}

Values are mean ± standard deviation. Means with different uppercase superscripts within similar column and with different lowercase superscripts within similar row are significantly different ($p < 0.05$).

Table 4. Effects of different concentrations and exposure times of *C. caudatus* on natural bacterial contaminants in oyster mushroom (log_{10} CFU/g).

Bacterial species	**TPC**			***B. cereus***			***E. coli***			***S. aureus***		
Initial bacterial load	6.13 ± 0.04^{Aa}			6.15 ± 0.09^{Aa}			5.97 ± 0.04^{Aa}			6.46 ± 0.00^{Aa}		
Exposure time (min) / Treatment (%)	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
Tap water	6.12 ± 0.01^{Ab}	6.10 ± 0.03^{Ac}	5.45 ± 0.03^{Ad}	5.89 ± 0.01^{Ab}	5.71 ± 0.08^{Ac}	5.91 ± 0.05^{Ad}	5.59 ± 0.17^{Ab}	5.58 ± 0.00^{Ac}	5.43 ± 0.07^{Ad}	6.16 ± 0.50^{Aa}	5.99 ± 0.18^{Ac}	5.59 ± 0.02^{Ad}
0.00%	5.92 ± 0.11^{Aab}	6.00 ± 0.07^{Ab}	5.42 ± 0.03^{Ac}	5.46 ± 0.11^{Aa}	5.64 ± 0.07^{Aa}	5.87 ± 0.04^{Ab}	5.42 ± 0.16^{Ab}	5.31 ± 0.04^{Ab}	5.38 ± 0.04^{Ac}	5.94 ± 0.09^{Aa}	5.91 ± 0.02^{Aa}	5.33 ± 0.04^{Ab}
0.05%	5.44 ± 0.02^{Bb}	4.85 ± 0.05^{Bc}	2.62 ± 0.07^{Cd}	3.77 ± 0.15^{Bb}	1.77 ± 0.04^{Cc}	1.61 ± 0.02^{Cd}	4.79 ± 0.03^{Bb}	3.21 ± 0.13^{Cc}	2.90 ± 0.03^{Cd}	5.91 ± 0.01^{Ab}	5.56 ± 0.02^{Bc}	4.83 ± 0.07^{Bd}
0.50%	5.23 ± 0.01^{Bb}	3.31 ± 0.06^{Cc}	2.16 ± 0.09^{Dd}	2.64 ± 1.40^{Bb}	1.41 ± 0.04^{Cc}	0.00 ± 0.00^{Dd}	2.41 ± 1.13^{Cb}	1.87 ± 0.47^{Cc}	1.45 ± 0.02^{Dd}	5.30 ± 0.03^{Bb}	5.27 ± 0.03^{Bb}	3.85 ± 0.03^{Cc}
5.00%	3.10 ± 0.07^{Cb}	2.86 ± 0.05^{Dc}	1.49 ± 0.13^{Ed}	1.65 ± 0.04^{Cb}	0.00 ± 0.00^{Dc}	0.00 ± 0.00^{Dc}	1.61 ± 0.01^{Db}	1.34 ± 0.02^{Dc}	0.00 ± 0.00^{Ed}	3.44 ± 0.05^{Cb}	3.30 ± 0.06^{Cc}	2.78 ± 0.03^{Dd}

Values are mean ± standard deviation. Means with different uppercase superscripts within similar column and with different lowercase superscripts within similar row are significantly different ($p < 0.05$).

Table 5. Sensory acceptability of treated chicken meat and oyster mushroom at different concentrations of *C. caudatus* extract.

Attribute / Treatment (%)	Tap water (control)	0.05	0.50	5.00
		Chicken meat		
Colour	6.00 ± 1.65^{a}	6.40 ± 1.23^{a}	5.45 ± 1.32^{a}	4.40 ± 1.82^{b}
Odour	5.35 ± 1.57^{a}	4.95 ± 1.96^{ab}	4.95 ± 2.26^{ab}	3.75 ± 1.94^{b}
Texture	6.00 ± 1.81^{a}	5.15 ± 1.53^{a}	4.55 ± 2.14^{a}	5.15 ± 1.98^{a}
Overall acceptability	5.80 ± 1.47^{a}	5.35 ± 1.39^{ab}	5.00 ± 1.41^{ab}	4.70 ± 1.81^{b}
		Oyster mushroom		
Colour	6.60 ± 2.01^{a}	7.20 ± 1.06^{a}	5.55 ± 1.79^{b}	2.50 ± 1.19^{c}
Odour	6.50 ± 1.76^{a}	5.75 ± 1.41^{ab}	5.15 ± 1.57^{b}	3.35 ± 1.46^{c}
Texture	6.60 ± 1.46^{a}	6.65 ± 1.39^{a}	5.45 ± 1.43^{b}	4.20 ± 1.76^{b}
Overall acceptability	5.80 ± 1.47^{a}	5.35 ± 1.39^{ab}	5.00 ± 1.41^{ab}	4.70 ± 1.81^{b}

Values are mean ± standard deviation. Means with different lowercase superscripts within similar row are significantly different ($p < 0.05$).

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