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Shorea macrophylla (Engkabang) phytochemical fruit extract exhibits antimicrobial susceptibility against *Salmonella enterica* and *Citrobacter freundii*

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ABSTRACT

Bacterial infections are a major concern in aquaculture, and although antibiotics are commonly used for treatment, they may lead to toxicity and environmental risks. In search of safer alternatives, this study explores the antibacterial potential of natural compounds from the fruit of *Shorea macrophylla* (Engkabang), a native species known for its bioactive phytochemicals. The objective was to evaluate the antibacterial activity of engkabang fruit extracts against five aquaculture-relevant bacteria: *Pseudomonas koreensis, Citrobacter freundii, Enterobacter hormaechei, Salmonella enterica*, and *Bacillus subtilis*. Using the disc diffusion method, extracts were tested at concentrations of 20 mg/disc, 10 mg/disc, and 2 mg/disc with hexane, isopropanol, and acetone as solvents. Notably, *C. freundii* showed inhibition zones at all concentrations with isopropanol and hexane extracts, while *S. enterica* exhibited inhibition zones at all concentrations with isopropanol and acetone extracts. These results indicate significant antibacterial activity, highlighting engkabang fruit extract as a promising natural alternative to antibiotics in aquaculture.

Introduction

Aquaculture has a significant impact on the global economy and research [1–4]. Approximately half of the world's fish food supply comes from aquaculture, which has grown dramatically over the last 50 years to reach about 52.5 million tonnes [5,6]. Numerous challenges like pathogens and diseases are present in agriculture [7,8] and aquaculture, like the infectious abdominal dropsy (IAD), which is caused by *Aeromonas* bacterium. Invasive pathogens tend to thrive and proliferate when the inflammation of the fish gut microbiota population is disrupted [9]. Furthermore, *Pseudomonas* sp. and *Flavobacterium* sp. (enteritis), *Vibrio* sp., *Aeromonas* sp., and *Shewanella* sp. (Red-operculum disease), and *Aeromonas salmocida* (furunculosis) are other common bacteria that cause diseases in aquaculture [10]. Thus, several control strategies have been employed to prevent further economic loss of the aquaculture industry.

The combat of bacterial infections in the aquaculture industry is a decades-long predicament. Disease outbreaks in aquaculture have been controlled with the use of antibiotics [11]. Although these antibiotics are an effective treatment for aquatic diseases, it poses high toxicity, carcinogenic effects, and environmental issues when utilizing them. Furthermore, the bacterial strains might be able to develop resistance strains towards antibiotics, leading to difficulty in treating aquaculture diseases. Therefore, the development of plant oils or extracts for treating bacterial infections is driven by the cost-effectiveness of plant-based natural products, their minimal side effects, and the low risk of developing drug resistance [12]. In this respect, *Shorea macrophylla* is a potential candidate, where initial research has reported that its saturated fatty acids possibly possess antibacterial properties [13].

S. macrophylla, commonly known as engkabang, is a native species in Borneo,where it typically can be found in lowland and hill forests with high rainfall [14,15]. The engkabang is a fruit of a plethora of uses as it contains >50 % triglycerides, including stearic acid, oleic acid, and palmitic acid [16]. High-quality fats are essential for fish feed production, and the engkabang fruit is commonly incorporated into the diets of empurau and tilapia [17–21]. It has been recently discovered that the engkabang leaf and bark extracts exhibited antioxidant and protective effects against oxidative stress in brine shrimps [22], but the potential of

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the fruit extract has not been explored to date. Previous studies have demonstrated that plant-based extracts exhibit notable antibacterial activity against aquaculture-relevant pathogens. For instance, neem (*Azadirachta indica*) and garlic (*Allium sativum*) extracts have shown inhibitory effects against *Salmonella enterica* and *Citrobacter freundii*, reducing bacterial load in aquatic systems [23,24]. Similarly, extracts from *Ocimum sanctum* (holy basil) and *Curcuma longa* (turmeric) have been effective against *Pseudomonas* spp. and *Enterobacter* spp., pathogens commonly implicated in opportunistic infections among stressed fish [25,26]. Although *Bacillus subtilis* is typically considered a probiotic, certain strains may act as contaminants or interact negatively with other bacterial communities in aquaculture environments. Therefore, evaluating the antibacterial potential of *S. macrophylla* fruit extracts against these five bacteria provides a well-rounded assessment of its application in aquaculture health management. In this study, the antimicrobial properties of *S. macrophylla* (engkabang) fruit extracts were examined against five commonly found pathogenic bacteria in aquaculture.

Materials and methods

Fruit powder preparation and extraction

Sundried engkabang fruits were collected from LTT Aquaculture Sdn Bhd, Kuching, Sarawak. The engkabang fruits were ground into powder in liquid nitrogen. The powder was weighed into 20 g, placed in an amber bottle, and stored at −20 °C until further use. The ground engkabang fruit was weighed for each extraction procedure, using 20 g of weight. The Soxhlet extraction was conducted emulating that of Muleta et al. [27]. The extraction parameters, such as extraction temperature, drying temperature, and incubation period, were optimized to ensure the maximum yield obtained. The optimized parameters are as follows. The 200 mL solvents were separated from the extract at 70 °C for 5 h, and the extracts were stored in amber bottles and left in a fume hood at room temperature (25 °C) overnight (at least 8 h) for further solvent evaporation. The experiment was conducted in triplicate (three biological and technical replicates each). The extraction process was repeated using ethanol, methanol, isopropanol, acetone, ethyl acetate, dichloromethane, and diethyl ether. The extraction efficiency was calculated using the following formula:

Extraction Efficiency (%) = (Weight of dried extract / Weight of raw sample) × 100

This formula quantifies the percentage yield of the extract obtained from the initial plant material, allowing for comparison between different solvents and extraction conditions.

Gas chromatography-mass spectrometry (GC–MS)

Stored engkabang extracts were melted in a water bath before performing the esterification process to convert them into fatty acid methyl esters (FAMEs). A total of 200 µL engkabang extracts were dissolved in 1 mL hexane in a sonicator conical flask and sonicated at 40 kHz for 1 min at 50 °C. The following procedures were emulated from those of Younis et al. [28]. A total of 1 mg engkabang extract was redissolved in 1 mL of hexane. The GC–MS (Shimadzu single quadrupole GC–GC-MS-OP2020 NX, USA) analyzed the solution using a fused, non-polar silica column composed of 5 % phenyl polysilphenylene-siloxane with a size of 30 m x 0.25 mm x 0.25 µm. The detector used 99.9 % helium gas at a flow rate of 1 mL/min and an ionization energy of 70 eV. The equipment was injected with 1 µL of engkabang extract at 280 °C. The temperature started at 50 °C and was held there for 1 min. After that, it rose to 260 °C, and finally, it was held at 280 °C for 10 min. Then, the National Institute of Standards and Technology (NIST) library's databases were used to interpret the mass spectrum of the extracts. The list of secondary metabolites detected with >0.5 % composition was tabulated and documented. All compounds are listed for ethyl acetate and ethanol extracts.

Bacterial culture and growth optimization

Pseudomonas koreensis CM-01 was obtained from Lau et al. [29], whereas *C. freundii* (ATCC 13,316), *Enterobacter hormaechei* (ATCC 49, 162), *S. enterica* (ATCC 14,028), and *B. subtilis* (ATCC 6051) were purchased from ATCC. Their vital growth parameters, such as growth media, incubation temperature, oxygen level, culture period, and pH were first optimized. The optimal culture conditions determined for all five bacterial strains are Luria Bertani (LB) culture media, atmospheric oxygen level, 24 h of culture period, as well as pH 7.0. The optimal incubation temperature for *P. koreensis, C. freundii,* and *E. hormaechei* was determined at 25 °C, whereas the optimal incubation temperature for *S. enterica* and *B. subtilis* is 37 °C. The growth curve of these bacterial strains also exhibited high similarity to that documented by the manufacturer, with growth phases such as lag phase, log phase, stationary phase, and death phase. The bacterial strains are harvested for downstream analysis and application at the log (exponential) phase.

Antibacterial susceptibility testing via disc diffusion

In an amber microcentrifuge tube, the extracts were weighed into 20 mg, 10 mg, and 2 mg. Each amber microcentrifuge tube was added with 200 µL of hexane after the extracts melted in the 70 °C water bath and allowed to dissolve the extracts. Subsequently, the extracts were placed onto 0.6 cm x 0.6 cm paper disc, allowed to dry, and the process was repeated until 200 µL of the extracts were concentrated on the disc. In our study, the extract was dissolved in hexane due to its nonpolar nature, which is more suitable for extracting specific phytochemicals present in *S. macrophylla*. Prior to disc application, the discs were left in a sterile environment under a laminar flow hood to allow complete evaporation of hexane. Only after full evaporation were the discs placed on the agar plates. This procedure ensures that no residual solvent remains to influence bacterial growth, thereby preserving the accuracy of the disc diffusion results. The well-prepared disc containing engkabang crude extracts was stored in the dark until use. *P. koreensis* CM-01, *C. freundii* (ATCC 13,316), *E. hormaechei* (ATCC 49,162), *S. enterica* (ATCC 14,028), and *B. subtilis* (ATCC 6051) were cultured according to their optimized culture conditions. Three concentrations of the engkabang extracts, 20 mg/disc, 10 mg/disc, and 2 mg/disc, were prepared in disc form. The culture was streaked across the surface of the LB agar plate using an inoculation loop. Then, using forceps, a blank disc was picked and saturated in 0.2 % DMSO as a negative control; the disc was placed on the streaked agar plate. The antibiotics disc was used as a positive control, and three concentrations of engkabang extracts were placed on each section respectively. For each bacterial sample, the procedure was repeated. The plates were incubated at 25 °C for *P. koreensis, C. freundii* and *E. hormaechei*, as well as 37 °C for *S. enterica* and *B. subtilis,* for 24 h. The process was replicated three times for each bacterium. The zone of inhibition (average ± standard deviation) was determined once inhibition was formed.

Results and discussion

Phytochemical extraction

In this study, eight solvents with varying polarities, namely ethanol, methanol, isopropanol, acetone, ethyl acetate, dichloromethane, diethyl ether, and hexane were used to extract engkabang fruit using the Soxhlet extraction method. Among the eight solvents, acetone had the highest extraction efficiency, which is 38.3 %, followed by diethyl ether (37.0 %), dichloromethane (34.1 %), isopropanol (32.9 %), ethyl acetate (30.9 %), hexane (30.8 %), methanol (10.6 %), and ethanol had the lowest extraction efficiency among them, which is 8.8 %. The extraction efficiency of each solvent was illustrated in Fig. 1.

The extraction efficiency of organic solvents is influenced by a number of variables such as dielectric constant, dipole interactions, and

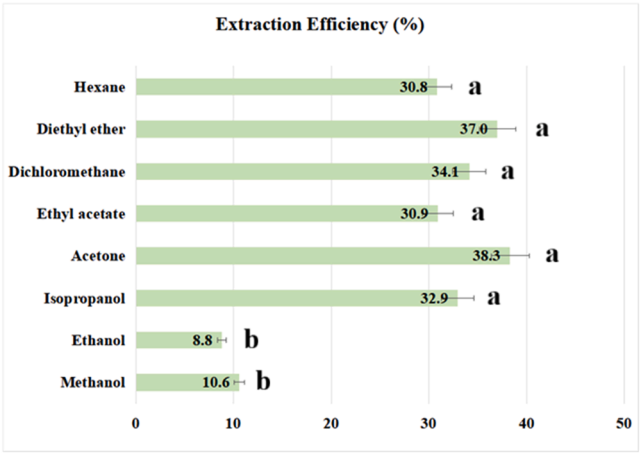


Fig. 1. Extraction efficiency of eight different solvents. Significant groups were labelled with different alphabets after ANOVA and Dunn's post hoc tests.

the solvent's ability to dissolve polar or non-polar particles [30]. As no single solvent will be able to successfully extract every phytochemical compound that exists in the plant material, different phytochemicals are extracted in solvents with varying polarities based on their chemical composition [31]. The high extraction efficiency of the engkabang crude extracts in this study, obtained using non-polar solvents, suggested that most of the compounds available in the engkabang fruit is non-volatile. This could be explained by the fact that it has a higher concentration of polyunsaturated fatty acids. This enables it to give itself a higher diffusivity and lower viscosity when using non-polar solvents for extraction, which contributes to an increase in the extraction yield [32].

Muleta et al. [27] had attempted to optimize the Soxhlet extraction protocol to be applied to avocado peel oil. The optimization parameters considered are extraction time and extraction temperature to obtain the highest yield. Comparing the extraction parameters optimized by Muleta et al. [27] on avocado peel oil, the highest oil yield was obtained at 3 h extraction time period as compared to 2, 4 and 5 h extraction time, whereas in this study, the highest engkabang oil yield was obtained at 5 h extraction period from all extracts. The maximum oil yield for avocado peel oil was achieved at 70 °C extraction temperature as compared to other temperatures such as 60 °C and 65 °C [27]. Similarly, the maximum oil yield of engkabang was obtained at 70 °C extraction temperature for all extracts.

GC-MS secondary metabolites detection

Phytochemical compounds, also known as bioactive compounds, are naturally occurring chemical compounds found in plants. These compounds can interact with biological systems in various ways, including within cells, to influence biochemical pathways [33]. A high match score (usually above a certain threshold, like 80 out of 100) indicates a good match. According to the analysis from GC-MS, engkabang fruit mostly consists of methyl stearate, methyl elaidate, methyl palmitate, and methyl arachidate, which are non-polar fatty acids [34]. This is correlated to the high extraction efficiency obtained using non-polar solvents, which are diethyl ether, dichloromethane, isopropanol, ethyl acetate, hexane, with the range between 30–40 %, compared to polar solvents, ethanol and methanol.

The list of secondary metabolites detected from the engkabang phytochemical extracts were tabulated in Table 1. Generally, the top three secondary metabolites of methanol, ethanol, isopropanol, ethyl acetate, dichloromethane, and diethyl ether are the same, namely methyl stearate, 9-Octadecenoic acid methyl ester, as well as hexadecanoate, with varying compositions. Interestingly, 9-octadecenoic acid methyl ester appears in the top three of all engkabang extracts. The glycidyl palmitate was only found exclusively abundant in the acetone extract (within the top three positions), and it topped the list with 23.78 % abundance. The Bis(2-ethylhexyl) adipate was found exclusively (within the top three positions) in the hexane extract with 10.45 % abundance. These secondary metabolites detected from engkabang extracts are all essential fatty acids that are believed to have contributed towards the fatty acid buildup of the wild empurau fish that eventually made the fish scrumptiously palatable with a unique fragrance and texture [35,36]. Similar compounds were also detected from avocado seed extract via GC–MS, namely 9-Octadecenoic acid methyl ester and hexadecanoate [37]. Interestingly, the methyl stearate was also unearthed as the most abundant fatty acid species in cucumber seed oil detected via GC–MS [38], which closely mirrored that of the engkabang in this study. Besides, Ibrahim et al. [39] have also performed GC-MS fatty acid content analysis on sesame (*Sesamum Indicum* L.), sunflower, and peanut. As a result, the major fatty acid species identified across all three plant extracts selected are methyl stearate, 9, 12-octadecadienoic acid (Z,Z)-methyl ester, 9-octadecenoic acid methyl ester, eicosanoic acid methyl ester, hexadecanoic acid as well as heptadecadienoic acid [39], in which most of them resembles the dominant fatty acid species found in the engkabang phytochemical fruit extract.

Disc diffusion analysis

The antibacterial activities of engkabang extracts were evaluated on *P. koreensis, C. fruendii, E. hormaechei, S. enterica*, and *B. subtilis*. These bacterial strains were selected because they represent a mix of potential pathogens and beneficial microbes in the aquaculture environment [40]. Table 2 showcases the summary of zones of inhibition detected across all five selected bacteria species and the positive controls used in this study. It was discovered that there were zones of inhibition observed against the growth of *S. enterica* and *C. fruendii* at concentrations of 20 mg/disc, 10 mg/disc, and 2 mg/disc. In contrast, the growth of *P. koreensis, E. hormaechei*, and *B. subtilis* was not inhibited by all eight engkabang crude fruit phytochemical extracts utilized in this study. The antibacterial activity of plant materials against tested bacteria can be influenced by the presence of bioactive compounds in adequate concentrations [41]. The low concentrations of antibacterial bioactive compounds in engkabang extracts in this study are probably insufficient to combat the selected bacteria.

In the disc diffusion test for *C. freundii*, imipenem was used as a positive control at a concentration of 10 mg/disc. At concentrations of 20 mg/disc, 10 mg/disc, and 2 mg/disc, two extracts, isopropanol and hexane showed an antibacterial effect against the growth of *C. freundii* (Table 2 & Fig. 2). The mean inhibition zones exhibited by isopropanol extract were 11.00 mm ± 0.00 mm at 20 mg/disc, 9.00 mm ± 0.00 mm at 10 mg/disc, and 7.00 mm ± 0.00 mm at 2 mg/disc. Besides that, the hexane extracts showed inhibition against *C. freundii* growth, with mean measured zones of 7.67 mm ± 1.15 mm at 20 mg/disc, 6.67 mm ± 0.58 mm at 10 mg/disc, and 6.00 mm ± 0.00 mm at 2 mg/disc. Tkachenko et al. [42] discovered that the growth of *C. fruendii* was inhibited by seven medicinal plant extracts with zones of inhibition ranging from 11 to 14 mm. An research conducted by Thanigaivel et al. [43] unearthed that *Azadirachta indica*, or neem (at a concentration of 150 mg/L), can inhibit the growth of *C. fruendii* in sick tilapia. Surprisingly, a recent research by Hasan et al. [44] showed that the ethanolic extract of clove is the most effective candidate against this bacterium among all other extracts examined. On the side note, methanolic extracts of *Calamus leptospadix* were recently observed to be effective against *C. freundii* [45]. Hence, the hexane and isopropanol extracts from this study may be presented as potential candidates for the aquaculture industry to combat against *C. freundii* manifestations.

Zones of inhibition were observed across isopropanol and acetone engkabang fruit extracts against the *S. enterica* in all tested concentrations (20 mg/disc, 10 mg/disc, and 2 mg/disc) (Table 2 & Fig. 2). The mean zones of inhibition for acetone extracts were 18.50 mm ± 4.50

Table 1
The list of all secondary metabolites (with >0.5 % composition) obtained from engkabang fruit phytochemical extracts. All compounds are listed for ethyl acetate and ethanol extracts.

Extracts	Methanol	Ethanol	Isopropanol	Acetone	Ethyl acetate	Dichloromethane	Diethyl ether	Hexane
Detected Compounds (% composition)	Methyl stearate (36.25 %)	Methyl stearate (44.97 %)	Methyl stearate (36.88 %)	Glycidyl palmitate (23.78 %)	Methyl stearate (45.16 %)	Methyl stearate (42.68 %)	Methyl stearate (40.3 %)	Methyl stearate (24.69 %)
	9-Octadecenoic acid, methyl ester (35.5 %)	9-Octadecenoic acid, methyl ester (32.96 %)	9-Octadecenoic acid, methyl ester (24.17 %)	Methyl stearate (20.58 %)	9-Octadecenoic acid, methyl ester (29.67 %)	9-Octadecenoic acid, methyl ester (28.09 %)	9-Octadecenoic acid, methyl ester (25.8 %)	9-Octadecenoic acid, methyl ester (16.92 %)
	Hexadecanoate (17.14 %)	Hexadecanoate (17.03 %)	Hexadecanoate (13.83 %)	9-Octadecenoic acid, methyl ester (13.74 %)	Hexadecanoate (21.81 %)	Hexadecanoate (15.6 %)	Hexadecanoate (14.58 %)	Bis(2-ethylhexyl) adipate (10.45 %)
	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.66 %)	Octadecanoic acid, ethyl ester (1.4 %)	Eicosanoate ⟨methyl-⟩ (1.73 %)	9-Octadecenoic acid (Z)-, oxiranylmethyl ester (13.5 %)	Eicosanoate ⟨methyl-⟩ (1.36 %)	Eicosanoate ⟨methyl-⟩ (1.94 %)	Butylated Hydroxytoluene (2.52 %)	Hexacosane ⟨*n*-⟩ (10.44 %)
	Eicosanoate ⟨methyl-⟩ (1.5 %)	Eicosanoate ⟨methyl-⟩ (1.08 %)	5-Phenyl-7-(trifluoromethyl)pyrazolo [1,5-a]pyrimidine-2-carboxylic acid (0.92 %)	Hexadecanoate ⟨methyl-⟩ (8.06 %)	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (0.63 %)	Myristic acid glycidyl ester (1.45 %)	Eicosanoate ⟨methyl-⟩ (2.09 %)	Hexadecanoate ⟨methyl-⟩ (8.88 %)
		Ethyl Oleate (1.05 %)	Stigmasta-3,5-diene (0.9 %)	Glycidyl palmitate (4.35 %)	tetrahydro-4,5-dihydroxy-, [3R-(3. alpha.,4.alpha.,5. alpha.,6.alpha.)]- (0.45 %)	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.11 %)	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (0.69 %)	Bis(2-ethylhexyl) adipate (8 %)
		Hexadecanoic acid, ethyl ester (0.66 %)	Tetrachloroethylene (0.85 %)	Glycidol stearate (2.98 %)	9-Octadecenoic acid (Z)-, 2,3-dihydroxy-propyl ester (0.27 %)	Bis(2-ethylhexyl) adipate (0.91 %)	3-Demethylthiocolchicine, N-decarbonyl(0.6 %)	Bis(2-ethylhexyl) phthalate (3.16 %)
		9,12-Octadecadienoic acid (Z,Z)-, methyl ester (0.66 %)	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (0.82 %)	Glycidyl (Z)-9-nonadecenoate (2.1 %)	Methyl 3-undecenoate (0.24 %)	9-Octadecenoic acid (Z)-, methyl ester (0.67 %)	2-Chloro-4-(diphenylamino)-6-morpholino-1,3,5-triazine (0.5 %)	3-Ethoxy-4-hydroxyphenylacetonitrile (1.14 %)
		Oxalic acid, isohexyl propyl ester (0.11 %)	1,3-Didodecylurea (0.8 %)	Glycidyl palmitate (1.44 %)	2-Butanone, 3-(2,5-dihydro-4,5,5-trimethyl-2-pyrimidinyl)-3-methyl-, (.±.)- (0.21 %)			Eicosanoate ⟨methyl-⟩ (1.11 %)
		Heptadecanoic acid, methyl ester (0.09 %)	Bis(2-ethylhexyl) adipate (0.79 %)	Eicosanoate ⟨methyl-⟩ (1.36 %)	Cholest-5-en-3-ol (3. beta.)-, propanoate (0.2 %)			Phthalic acid, butyl phenyl ester (0.87 %)
				Docosanoic acid, methyl ester (1.1 %)				Trichothec-9-ene-3,4,7,8,15-pentol, 12,13-epoxy-, 4,15-diacetate, (3.alpha.,4.beta.)- (0.73 %)
				Oleic Acid (0.91 %)				
				Octadecanoic acid (0.91 %)				
				Decyl sulfide (0.79 %)				
				n-Hexadecanoic acid (0.72 %)				

Table 2
The summary of zones of inhibition detected across all five selected bacteria species with three readings, mean value, standard deviation (value after ±) and ANOVA statistical analyses. Significantly different groups were labelled with asterisk (*) based on Dunn's post hoc tests.

Bacteria/Extracts	Zone of inhibition							
	Methanol	Ethanol	Isopropanol	Acetone	Ethyl acetate	Dichloromethane	Diethyl ether	Hexane
Pseudomonas koreensis **(Positive control: 5 mg/disc Ciprofloxacin)**	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–
Citrobacter fruendii **(Positive control: 10 mg/disc Imipenem)**	–	–	11.00 ± 0.00 mm; 11.00 ± 0.00 mm; 11.00 ± 0.00 mm; Mean: 11.00 ± 0.00 mm (20 mg/disc)*	–	–	–	–	7 ± 1.15 mm; 7 ± 1.15 mm; 9 ± 1.15 mm; Mean: 7.67 ± 1.15 mm (20 mg/disc)
	–	–	9.00 ± 0.00 mm; 9.00 ± 0.00 mm; 9.00 ± 0.00 mm; Mean: 9.00 ± 0.00 mm (10 mg/disc)	–	–	–	–	7 ± 0.58 mm; 6 ± 0.58 mm; 7 ± 0.58 mm; Mean: 6.67 ± 0.58 mm (10 mg/disc)
	–	–	7.00 ± 0.00 mm; 7.00 ± 0.00 mm; 7.00 ± 0.00 mm; Mean: 7.00 ± 0.00 mm (2 mg/disc)	–	–	–	–	6.00 ± 0.00 mm; 6.00 ± 0.00 mm; 6.00 ± 0.00 mm; Mean: 6.00 ± 0.00 mm (2 mg/disc)
Enterobacter hormaechei **(Positive control: 30 mg/disc Tetrcycline)**	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–
Salmonella enterica **(Positive control: 10 mg/disc Ampicillin)**	–	–	12 ± 1.53 mm; 11 ± 1.53 mm; 9 ± 1.53 mm; Mean:10.67 ± 1.53 mm (20 mg/disc)	18 ± 6.00 mm; 12 ± 6.00 mm; 24 ± 6.00 mm; Mean: 18.00 ± 6.00 mm (20 mg/disc)	–	–	–	–
	–	–	9 ± 1.15 mm; 11 ± 1.15 mm; 9 ± 1.15 mm; Mean: 9.67 ± 1.15 mm (10 mg/disc)	14.5 ± 4.50 mm; 10 ± 4.50 mm; 19 ± 4.50 mm; Mean: 14.50 ± 4.50 mm (10 mg/disc)	–	–	–	–
	–	–	9 ± 1.15 mm; 9 ± 1.15 mm; 7 ± 1.15 mm; Mean: 8.33 ± 1.15 mm (2 mg/disc)	11.5 ± 4.50 mm; 7 ± 4.50 mm; 16 ± 4.50 mm; Mean: 11.50 ± 4.50 mm (2 mg/disc)	–	–	–	–
Bacillus subtilis **(Positive control: 30 mg/disc Aztreonam)**	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–

mm at 20 mg/disc, 11.50 mm ± 4.50 mm at 10 mg/disc, and 18.00 mm ± 6.00 mm at 2 mg/disc. The mean zones of inhibition for isopropanol extracts were 8.33 mm ± 1.15 mm at 2 mg/disc, 10.67 mm ± 1.53 mm at 20 mg/disc, and 9.67 mm ± 1.15 mm at 10 mg/disc concentrations (Table 2). Interestingly, the isopropanol extract is capable of inhibiting both *C. freundii* and *S. enterica* bacteria species, resulting in zones of inhibition observed. This phenomenon can be explained through the different types of mycotoxin derivative compounds discovered in isopropanol extracts that are effective against inhibiting foodborne bacteria such as *Salmonella typhimurium, Escherichia coli, Vibrio anguillarum, Staphylococcus aureus,* and *B. subtilis* in previous research [46–48]. In the case of acetone, Patel et al. [49] conducted a study where acetone was used to extract phytochemical compounds from five medicinal plants to evaluate their antibacterial properties against eight bacterial strains. The phytochemical screening identified the presence of secondary metabolites such as triterpenoids, flavonoids, and alkaloids. Out of the eight bacterial strains tested, seven of them were inhibited by the acetone extracts from these medicinal plants [49]. In this study, triterpenes were discovered in the acetone extract via GC–MS and this compound may be responsible for this antibacterial activity against *S. enterica.* Acetone leaf extract of *Hippocratea indica* was recently found to be effective against *Salmonella typhi* [50]. The *Punica granatum* L. and Areca nut (P.A) combined extracts were found to be effective in microbial inhibition of *Staphylococcus aureus* (98.98 %), *Enterobacter aerogenes* (88.55 %), and *Staphylococcus aureus* (94.98 %) [51]. Ethanol-based extracts from *Punica granatum* L. and Areca nut demonstrated a combined synergistic antibacterial effect against *E. coli* [52]. All aqueous, ethanolic, and methanolic extracts of Areca Nut Fruit were found to be effective against *S. aureus, S. enterica, E. coli*, and *E. aerogenes* bacteria using agar disc diffusion technique [53]. In this study, disc diffusion investigations showed that engkabang fruit crude acetone and isopropanol extracts exhibited inhibition on *S. enterica*. Therefore, it is proposed that fruit crude extracts from engkabang might be an effective antibacterial shield against *Salmonella* sp. in various aquaculture settings.

Conclusion

Overall, the engkabang fruit was extracted, and phytochemical screening was carried out to investigate its antibacterial properties. Then, disc diffusion analysis was carried out on *P. koreensis, C. freundii, E. hormaechei, S. enterica*, and *B. subtilis* using the engkabang crude fruit extracts. In this study, higher extraction efficiency was achieved when non-polar solvents were employed, suggesting that the engkabang fruit

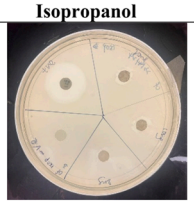
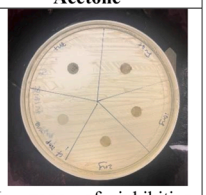
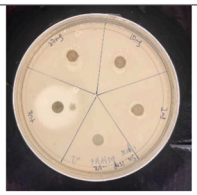
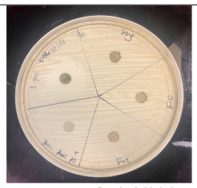
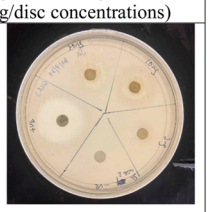
Bacteria/Extracts	Isopropanol	Hexane	Acetone
Citrobacter fruendii			 (No zone of inhibition observed across 20 mg/disc, 10 mg/disc, and 2 mg/disc concentrations)
Salmonella enterica		 (No zone of inhibition observed across 20 mg/disc, 10 mg/disc, and 2 mg/disc concentrations)	

Fig. 2. The zones of inhibition observed across *Citrobacter fruendii* and *Salmonella enterica.*

extract is non-volatile. In addition, the isopropanol and hexane extracts showed antibacterial properties against *C. fruendii*. The isopropanol and acetone extracts also showed similar effects against *S. enterica*. The research question of this study has been answered, and it was found that the engkabang fruit extract exhibited antimicrobial properties. The limitation of this study is that only preliminary antibacterial tests have been conducted in this study, future research should expand the work to other bacterial species.

Data availability statement

Data will be made available upon request.

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CRediT authorship contribution statement

Hung Hui Chung: Validation, Supervision, Funding acquisition, Writing – review & editing. **Pei Xuan Hew:** Writing – original draft, Investigation, Formal analysis, Data curation, Visualization. **Ivy Yee Yen Chew:** Methodology, Investigation, Formal analysis, Data curation. **Crystal Jia Jing Lim:** Methodology, Investigation, Formal analysis, Data curation. **Kristene Ling Yong:** Methodology, Investigation, Formal analysis, Data curation. **Siong Fong Sim:** Writing – review & editing, Resources. **Lesley Maurice Bilung:** Writing – review & editing, Resources. **Leonard Whye Kit Lim:** Writing – review & editing, Validation, Formal analysis, Writing – original draft.

Declaration of competing interest

The authors declared no conflict of interest.

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References

[1] A.W. Aminan, L.W.K. Lim, H.H. Chung, B. Sulaiman, Morphometric analysis and genetic relationship of *Rasbora* spp, Sarawak, Malaysia. Trop. Life Sci. Res. 31 (2) (2020) 33–49, https://doi.org/10.21315/tlsr2020.31.2.3.

[2] H.H. Chung, C.K.A. Kamar, L.W.K. Lim, J.S. Roja, Y. Liao, et al., Sequencing and characterization of complete mitogenome DNA of *Rasbora tornieri* (Cypriniformes: cyprinidae: rasbora) and its evolutionary significance, J. Genet. 99 (2020) 67, https://doi.org/10.1007/s12041-020-01221-x.

[3] L.W.K. Lim, H.H. Chung, S.D. Ishak, K. Waiho, Zebrafish (*Danio rerio*) ecotoxicological ABCB4, ABCC1 and ABCG2a gene promoters depict spatiotemporal xenobiotic multidrug resistance properties against environmental pollutants, Gene Rep. 23 (2021) 101110, https://doi.org/10.1016/j.genrep.2021.101110.

[4] L.W.K. Lim, H.H. Chung, Y.L. Chong, N.K. Lee, Isolation and characterization of putative liver-specific enhancers in proboscis monkey (*Nasalis larvatus*), Pertanika J. Trop. Agricu. Sci. 42 (2) (2019) 627–647.

[5] A. Bordoni, Insight into the sustainability of the Mediterranean diet: the water footprint of the recommended Italian diet, Nutrients. 15 (9) (2023) 2204, https://doi.org/10.3390/nu15092204.

[6] L.W.K. Lim, Implementation of artificial intelligence in aquaculture and fisheries: deep learning, machine vision, big data, internet of things, robots and beyond, J. Comput.Cogn. Eng. 3 (2) (2024) 112–118, https://doi.org/10.47852/bonviewJCCE3202803.

[7] M.S. Jee, L.W.K. Lim, M.A. Dirum, S.I.C. Hashim, M.S. Masri, et al., Isolation and characterization of avirulence genes in *Magnaporthe oryzae*, Borneo J. Resource Sci. Technol. 7 (1) (2017) 31–42, https://doi.org/10.33736/bjrst.389.2017.

[8] L.W.K. Lim, H.H. Chung, Salt tolerance research in sago palm (*Metroxylon sagu* Rottb.): past, present and future perspectives, Pertan. J. Trop. Agric. Sci. 43 (2) (2020) 91–105.

[9] H. Liu, X. Guo, R. Gooneratne, R. Lai, C. Zeng, F. Zhan, W. Wang, The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels, Sci. Rep. 6 (1) (2016), https://doi.org/10.1038/srep24340.

[10] T. Li, H. Li, F.J. Gatesoupe, R. She, Q. Lin, X. Yan, J. Li, X. Li, Bacterial signatures of "red-operculum" disease in the gut of crucian carp (*Carassius auratus*), Microb. Ecol. 74 (3) (2017) 510–521, https://doi.org/10.1007/s00248-017-0967-1.

[11] S. Thanigaivel, N. Chandrasekaran, A. Mukherjee, J. Thomas, Seaweeds as an alternative therapeutic source for aquatic disease management, Aquaculture 464 (2016) 529–536, https://doi.org/10.1016/j.aquaculture.2016.08.001.

[12] M.K. Swamy, M.S. Akhtar, U.R. Sinniah, Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review, Evid. Based Complem. Altern. Med. 2016 (2016) 1–21, https://doi.org/10.1155/2016/3012462.

[13] S.N. Rao, A.I. Redhwan, H.C. Ha, S.I. Nguang, A. Kari, W.S. Yong, F.H. Yong, C. F Komilus, A review of Borneo Buah engkabang (*Shorea macrophylla*) as potential omega-6 lipid source for fish feed, E3S Web. Conf 442 (2023) 02034, https://doi.org/10.1051/e3sconf/202344202034.

[14] G. Ismaili, K.K. Abdul Rahim, A. Duju, I. Openg, Z. Ismaili, Strength classification of Aras as fast-growing indigenous species timber in Sarawak, Appl. Mech. Mater. 695 (2014) 617–621, https://doi.org/10.4028/www.scientific.net/amm.695.617.

[15] H.H. Chung, A.A.L. Soh, M.M.L. Lau, H.M. Gan, S.F. Sim, L.W.K. Lim, The first engkabang jantong (*Rubroshorea macrophylla*) genome survey data, Data Brief. 58 (2025) 111248, https://doi.org/10.1016/j.dib.2024.111248.

[16] I.Y. Chew, H.H. Chung, L.W.K. Lim, M.M. Lau, H.M. Gan, B.S. Wee, S.F. Sim, Complete chloroplast genome data of *Shorea macrophylla* (Engkabang): structural

features, comparative and phylogenetic analysis, Data Brief. 47 (2023) 109029, https://doi.org/10.1016/j.dib.2023.109029.
[17] M.M.L. Lau, C.J.Y. Kho, L.W.K. Lim, S.C. Sia, H.H. Chung, et al., Microbiome analysis of gut bacterial communities of healthy and diseased Malaysian Mahseer (*Tor tambroides*), Malays. J. Microbiol 18 (2) (2022) 170–191, https://doi.org/10.1101/2021.12.08.471852.
[18] M.M.L. Lau, L.W.K. Lim, H.H. Chung, H.M. Gan, The first transcriptome sequencing and data analysis of the Javan mahseer (*Tor tambra*), Data Brief. 39 (2021) 107481, https://doi.org/10.1016/j.dib.2021.107481.
[19] M.M.L. Lau, L.W.K. Lim, S.D. Ishak, A. Abol-Munafi, H.H. Chung, A review on the emerging Asian aquaculture fish, the Malaysian Mahseer (*Tor tambroides*): current status and the way forward, Proc. Zool. Soc. 74 (2021) 227–237, https://doi.org/10.1007/s12595-021-00368-4.
[20] L.W.K. Lim, H.H. Chung, M.M.L. Lau, F. Aziz, H.M. Gan, Improving the phylogenetic resolution of Malaysian and Javan mahseer (Cyprinidae), *Tor tambroides* and *Tor tambra*: whole mitogenomes sequencing, phylogeny and potential mitogenome markers, Gene 791 (2021) 145708, https://doi.org/10.1016/j.gene.2021.145708.
[21] M.M.L. Lau, C.J.Y. Kho, F.S. Zaidi, J.K. Fong, et al., Gut and skin microbial profiling of healthy and dropsy diseased Malaysian Mahseer Empurau (*Tor tambroides*) following exposure to antimicrobial agents, Malays. J. Microbiol 18 (2) (2025) 170–191, https://doi.org/10.21161/mjm.230206.
[22] S. Ramli, N.N. Sukri, N.A. Zulkifli, D.M. Ariestanti, C.Y. Choo, R.J. James, Antioxidant activities and protective effects of *Shorea macrophylla* leaf and bark extracts, Pharm. Sci. Res. 11 (1) (2024). https://scholarhub.ui.ac.id/psr/vol11/iss1/4/.
[23] E. Circella, G. Casalino, F. D'Amico, N. Pugliese, G. Bozzo, In vitro antimicrobial effectiveness tests using garlic (*Allium sativum*) against *Salmonella enterica* subspecies enterica Serovar Enteritidis, Antibiotics. (Basel) 11 (11) (2022) 1481, https://doi.org/10.3390/antibiotics11111481.
[24] M.R. Wylie, D.S. Merrell, The antimicrobial potential of the neem tree *Azadirachta indica*, Front. Pharmacol. 13 (2022) 891535, https://doi.org/10.3389/fphar.2022.891535.
[25] E.O. Odo, J.A. Ikwuegbu, E.I. Obeagu, S.A. Chibueze, R.E. Ochiaka, Analysis of the antibacterial effects of tumeric on particular bacteria, Medicine (Baltimore) 102 (48) (2023) e36492, https://doi.org/10.1097/MD.0000000000036492.
[26] T. Tabassum, A. Islam, K.M.S. Andalib, B. Sarker, M. Mia, A. Habib, Antibacterial activity of *Ocimum teniflorum* against drug resistant bacteria isolated from raw beef, J. Microbiol. Biotechnol. 35 (2025) e2409028, https://doi.org/10.4014/jmb.2409.09028.
[27] Muleta, M.D., Daba, B.J., Dube, A.M., & Ramesh, R. (2022). Extraction of avocado peel oil using a soxhlet extractor and investigation of its physicochemical properties. Retrieved 10 January 2025, from https://doi.org/10.21203/rs.3.rs-1918209/v1.
[28] I.Y. Younis, A.R. Khattab, N.M. Selim, M. Sobeh, S.S. Elhawary, M. Bishbishy, Metabolomics-based profiling of 4 avocado varieties using HPLC–MS/MS and GC/MS and evaluation of their antidiabetic activity, Sci. Rep. 12 (2022) 4966, https://doi.org/10.1038/s41598-022-08479-4.
[29] M.M.L. Lau, C.J.Y. Kho, H.H. Chung, A. Zulkharnain, Isolation, identification and characterisation of Pseudomonas koreensis CM-01 isolated from diseased Malaysian mahseer (*Tor tambroides*), Fish Shellifish Immunol. 148 (2024) 109518, https://doi.org/10.1016/j.fsi.2024.109518.
[30] Z. Zhang, Polar and dispersive surface tension components of water-guanidinium chloride (Gdmcl) binary mixtures, Colloids Surf. A 676A (2023) 132223, https://doi.org/10.1016/j.colsurfa.2023.132223.
[31] H. Nawaz, M.A. Shad, N. Rehman, H. Andaleeb, N. Ullah, Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds, Braz. J. Pharm. Sci. 56 (2020), https://doi.org/10.1590/s2175-97902019000417129.
[32] Y. Li, K. Bundeesomchok, N. Rakotomanomana, A.S. Fabiano-Tixier, R. Bott, Y. Wang, F. Chemat, Towards a zero-waste biorefinery using edible oils as solvents for the green extraction of volatile and non-volatile bioactive compounds from rosemary, Antioxidants 8 (5) (2019) 140, https://doi.org/10.3390/antiox8050140.
[33] A. Guaadaoui, S. Benaicha, N. Elmajdoub, M. Bellaoui, A. Hamal, What is a bioactive compound? A combined definition for a preliminary consensus, Int. J. Food Sci. Nutr. 3 (3) (2014) 174, https://doi.org/10.11648/j.ijnfs.20140303.16.
[34] I.Y. Chew, In-silico and In-Vitro Analysis of Lipogenesis Effect of *Shorea macrophylla* Fruits' Crude Extract (Unpublished Master Dissertation), University of Malaysia Sarawak, 2023.
[35] L.W.K. Lim, Eco-economically indispensable borneo-endemic flora and fauna: proboscis monkey (*Nasalis larvatus*), Malaysian Mahseer (*Tor tambroides*), Engkabang (*Shorea macrophylla*), Sarawak Rasbora (*Rasbora sarawakensis*) and Sago Palm (*Metroxylon sagu*), Int. J. Zool. Animal Biol. 5 (3) (2022) 000381, https://doi.org/10.23880/izab-16000381.
[36] L.W.K. Lim, Cultivated meat in Singapore: the road to commercialization, Int. J. Zool. Animal Biol. 6 (4) (2023) 1–5, https://doi.org/10.23880/izab-16000488.
[37] P.P. San, T. Kyaw, Chemical characterization, antioxidant activity and GC-MS analysis of the extracted oil from seeds of *Persea americana* Mill, (Avocado). Yadanabon Univ. Res. J. 10 (1) (2019) 1–11.
[38] J. Yuenyong, P. Pokkanta, N. Phuangsaijai, S. Kittiwachana, S. Mahatheeranont, P. Sookwong, GC-MS and HPLC-DAD analysis of fatty acid profile and functional phytochemicals in fifty cold-pressed plant oils in Thailand, Heliyon. (2021), https://doi.org/10.1016/j.heliyon.2021.e06304.
[39] N. Ibrahim, M.O. Hussein, M. Ali, E. Ali, Physiochemical properties and fatty acids content of selected Sudanese edible vegetable oils, J. Nucl. Radiat. Sci. 1 (2) (2022) 31, https://doi.org/10.5455/jnrs.2022.02.003.
[40] M. Bentzon-Tilia, E.C. Sonnenschein, L. Gram, Monitoring and managing microbes in aquaculture – Towards a sustainable industry, Microbial Biotechnology 9 (5) (2016) 576–584.
[41] J.M. McRae, Q. Yang, R.J. Crawford, E.A. Palombo, Antibacterial compounds from *Planchonia careya* leaf extracts, J. Ethnopharmacol. 116 (3) (2008) 554–560, https://doi.org/10.1016/j.jep.2008.01.007.
[42] H. Tkachenko, L. Buyun, E. Terech-Majewska, Z. Osadowski, Antibacterial activity of ethanolic leaf extracts obtained from various Ficus species (Moraceae) against the fish pathogen, *Citrobacter freundii*, J. Ecol. Protec. Coast. 20 (2016) 117–136.
[43] S. Thanigaivel, S. Vijayakumar, S. Gopinath, A. Mukherjee, N. Chandrasekaran, J. Thomas, *In vivo* and *in vitro* antimicrobial activity of *Azadirachta indica* (Lin) against *Citrobacter freundii* isolated from naturally infected Tilapia (*Oreochromis mossambicus*), Aquaculture 437 (2015) 252–255, https://doi.org/10.1016/j.aquaculture.2014.12.008.
[44] H.J. Hasan, M.T. Abdulwahid, H.N. Ayyez, *In vitro* antimicrobial activity of clove extract against gram negative bacteria isolated from chickens, Int. J. Health Sci. 6 (S4) (2022) 2392–2408, https://doi.org/10.53730/ijhs.v6nS4.7372.
[45] U. Dutta, M.J. Goswami, T. Seema, T. Payum, T.N. Ullah, P.K. Hui, D. Kakati, Antibacterial, antidiabetic and antioxidant bioevaluation of *Calamus leptospadix* Griff. And isolation of a flavan type compound, Heliyon. 10 (14) (2024) e34638, https://doi.org/10.1016/j.heliyon.2024.e34638.
[46] S. Li, M.W. Shao, Y.H. Lu, L.C. Kong, D.H. Jiang, Y.L. Zhang, Phytotoxic and antibacterial metabolites from fusarium proliferatum zs07 isolated from the gut of long-horned grasshoppers, J. Agric. Food Chem. 62 (36) (2014) 8997–9001, https://doi.org/10.1021/jf502484n.
[47] J. Zhang, G.Y. Chen, X.Z. Li, M. Hu, B.Y. Wang, B.H. Ruan, H. Zhou, L.X. Zhao, J. Zhou, Z.T. Ding, Y.B. Yang, Phytotoxic, antibacterial, and antioxidant activities of mycotoxins and other metabolites from *Trichoderma* sp, Nat. Prod. Res. 31 (23) (2017) 2745–2752, https://doi.org/10.1080/14786419.2017.1295235.
[48] R. Li, Z. Su, C. Sun, S. Wu, Antibacterial insights into alternariol and its derivative alternariol monomethyl ether produced by a marine fungus, Appl. Environ. Microb. 90 (4) (2024), https://doi.org/10.1128/aem.00058-24.
[49] J.P. Patel, B. Gami, K. Patel, R. Solanki, Antibacterial activity of methanolic and acetone extract of some medicinal plants used in indian folklore, Int. J. Phytomed 3 (2) (2011) 261.
[50] A.O. Owolabi, O.B. Akpor, J.A. Ndako, S.O. Owa, A.P. Oluyori, R.M Asaleye, Antimicrobial potential of Hippocratea indica Willd. Acetone leaf fractions against *Salmonella typhi*: an in vitro and in silico study, Sci. Rep. 14 (2024) 25222, https://doi.org/10.1038/s41598-024-75796-1.
[51] N. Jam, R. Hajimohammadi, P. Gharbani, A. Mehrizad, Antibacterial activity of *Punica granatum* L. and Areca nut (P.A) combined extracts against some food born pathogenic bacteria, Saudi. J. Biol. Sci. 29 (3) (2022) 1730–1736, https://doi.org/10.1016/j.sjbs.2021.10.057.
[52] P. Gharbani, N. Jam, H. Doshmanfekan, A. Mehrizad, Optimization of synergic antibacterial activity of *Punica granatum* L. and Areca nut (P.G.L.A.N) extracts through response surface methodology, Sci. Rep. 13 (2023) 6098, https://doi.org/10.1038/s41598-023-32900-1.
[53] N. Jam, R. Hajimohammadi, P. Gharbani, A. Mehrizad, Evaluation of antibacterial activity of aqueous, ethanolic and methanolic extracts of areca nut fruit on selected bacteria, Biomed. Res. Int. 2021 (1) (2021) 6663399, https://doi.org/10.1155/2021/6663399.