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RESEARCH ARTICLE

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Chemical composition and antimicrobial activity of the essential oil of *Syzygium aromaticum* flower bud (Clove) against fish systemic bacteria isolated from aquaculture sites

Abstract This paper describes the chemical composition and antimicrobial activity of the essential oil of *Syzygium aromaticum* against *Vibrio* spp. ($n = 6$), *Edwardsiella* spp. ($n = 21$), *Aeromonas* spp. ($n = 2$), *Escherichia coli* ($n = 2$), *Flavobacterium* spp. ($n = 1$), *Salmonella* spp. ($n = 2$), *Streptococcus* spp. ($n = 1$) and *Pseudomonas* spp. ($n = 1$) isolated from aquaculture sites as well as seven reference strains of bacteria, namely, *Escherichia coli* (ATCC 25922), *Citrobacter freundii* (ATCC 8090), *Aeromonas hydrophila* (ATCC 49140), *Pseudomonas aeruginosa* (ATCC 35032), *Streptococcus agalactiae* (ATCC13813), *Edwardsiella tarda* (ATCC 15947) and *Yersinia enterocolitica* (ATCC 23715). Nowadays, most antibiotics are no longer effective in controlling diseases in aquaculture, especially fish systemic bacterial diseases, due to increasing incidences of antibiotic resistance among pathogenic bacteria. Furthermore, many countries have banned antibiotics in aquaculture use due to public health concerns and environmental hazards. Therefore, this study was carried out to evaluate the potential of the essential oil of *S. aromaticum* as an alternate commercial antibiotic to antimicrobial agents against fish systemic bacteria in aquaculture. The essential oil of *S. aromaticum* was prepared using a steam distillation method, and the chemical composition was analysed using Gas chromatography–mass spectroscopy (GC–MS). Minimum inhibitory concentration (MIC) values of the essential oils against the tested bacteria were determined using the broth two fold micro dilution method, with kanamycin and eugenol as positive controls. The MIC values of the essential oil of *S. aromaticum* ranged from 0.015 $\mu g \cdot mL^{-1}$ to 0.062 $\mu g \cdot mL^{-1}$ against the tested bacterial isolates. A total of nine chemical compounds were detected in the essential oil, with eugenol (49.0%) and caryophyllene (7.5%) being the major compounds. The results of the present study indicate that the essential oil of *S. aromaticum* shows a huge potential to substitute commercial antibiotics as antimicrobial agents for aquaculture use.

Keywords essential oil, *Syzygium aromaticum*, fish systemic bacteria, aquaculture sites

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1 Introduction

Syzygium aromaticum flower bud, commonly known as clove, is well known in food preparation, as an anticarcinogenic agent (Zheng et al., 1992), and as a traditional remedy for asthma (Kim et al., 1998), disorder of digestive system (Baytop, 1999), dental disorders, respiratory disorders, headaches and sore throat in Asian countries (Domaracky et al., 2007). Besides the reported antimicrobial, antifungal and antiviral properties, the essential oil of *S. aromaticum* shows anti-inflammatory, cytotoxic and anesthetic activities (Chaieb et al., 2007). Cai and Wu (1996) reported that *S. aromaticum* possessed antimicrobial activity against oral bacteria commonly associated with dental caries and periodontal disease. Therefore, *S. aromaticum* is used as an ingredient in toothpaste and mouth fresheners in India (Banerjee et al. 2006).

Nowadays, antibiotics residues are reported to be ineffective in controlling diseases in aquaculture due to the misuse or overuse of antibiotics by fish farmers. Furthermore, antibiotics residues are found as a threat to human health (Alderman and Hastings, 1998) and the environment (Cabello, 2006). Therefore, scientists have come out with an idea of applying natural antimicrobial

agents for aquaculture use, especially for fish bacterial disease treatment. Many studies have reported on the antimicrobial property of *S. aromaticum* against various types of bacteria. For instance, Fu et al. (2007) reported that the essential oil of *S. aromaticum* showed inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. Another study by Betoni et al. (2006) showed that the methanol extract of *S. aromaticum* possessed antimicrobial activity against clinical strains of *Staphylococcus aureus*. Furthermore, Lopez et al. (2005) claimed that the essential oil of *S. aromaticum* possessed inhibitory activity against four Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis*, and *Listeria monocytogenes*) and four Gram-negative bacteria (*Escherichia coli, Yersinia enterocolitica, Salmonella choleraesuis* and *Pseudomonas aeruginosa*). Another study of Yano et al. (2006) claimed that the aqueous extract of *S. aromaticum*, at a concentration of 0.04 $mg \cdot mL^{-1}$, was able to control *Vibrio parahaemolyticus*, a foodborne pathogen. However, until the present, no study has been conducted to reveal the antimicrobial property of the essential oil of the *S. aromati-cum* flower bud against fish bacterial diseases in aquaculture. Therefore, our study was carried out to reveal the potential of *S. aromaticum* essential oil as an antimicrobial agent as an alternative to commercial antibiotics in aquaculture use.

2 Materials and methods

2.1 Essential oil preparation

Syzygium aromaticum flower bud (Clove) was purchased from a market in Terengganu, Malaysia. The sample was subjected to steam distillation for 6 h using a piece of self-designed steam apparatus to produce the essential oil in a yield of 3.0% (v/w) based on the dry weight of the sample. The essential oil was stored in the dark at 4°C until use.

2.2 Bacterial isolates

A total of 36 bacterial strains isolated from 10 types of aquatic animals (*Penaeus vannamei, Penaeus monodon, Scylla* sp., *Rana catesbeiana, Macrobrachium rosenbergii, Trachinotus blochii, Clarias gariepinus, Tilapia* sp., *Monopterus albus* and *Trichogaster pectoralis*) were applied in the present study. There were *Vibrio* spp. (n = 6), *Edwardsiella* spp. (n = 21), *Aeromonas* spp. (n = 2), *Escherichia coli* (n = 2), *Flavobacterium* spp. (n = 1), *Salmonella* spp. (n = 2), *Streptococcus* spp. (n = 1) and *Pseudomonas* spp. (n = 1). *Escherichia coli* (ATCC 25922), *Citrobacter freundii* (ATCC 8090), *Aeromonas hydrophila* (ATCC 49140), *Pseudomonas aeruginosa* (ATCC 35032), *Streptococcus agalactiae* (ATCC13813), *Edwardsiella tarda* (ATCC 15947) and *Yersinia enterocolitica* (ATCC 23715) used as bacterial reference strains.

2.3 Minimum inhibitory concentration (MIC) test

The values of the minimum inhibitory concentration (MIC) of the essential oil of *S. aromaticum* as well as the positive controls, namely kanamycin and eugenol (Analar, UK), against bacterial isolates were determined through a two-fold broth micro dilution method (Daud et al., 2005). The essential oil was diluted using 0.01% methanol. The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature and the concentration of these cultures was adjusted to 10^9 $CFU \cdot mL^{-1}$ by using saline (0.85% of NaCl), and monitored with Biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate that contained a serial dilution of the isolated compound and the microplate was incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the isolated compound in the wells of the microtiter plate that showed no visible turbidity after the 24 h incubation.

2.4 Gas chromatography mass spectrometry (GC–MS)

The chromatographic procedure was carried out using a Shimadzu QP2010-GC-MS with autosampler. The sample was diluted 25 times with acetone, with 1 μL injected into the column. A fused silica capillary column HP5-MS (30 m×0.32 mm, film thickness 0.25 μm) was used. Helium was used as the carrier gas, and a split ratio of 1/100 was applied. The oven temperature used was maintained at 60ºC for 8 min. The temperature was then gradually raised at a rate of 3°C per min to 180°C per min and maintained at 180°C for 5 min. The temperature at the injection port was 250°C. The components of the test solution were identified by comparing the spectra with those of known compounds stored in the internal library.

3 Results and discussion

In the present study, the essential oil of *Syzygium aromaticum* was able to inhibit the growth of 36 bacterial isolates from ten types of freshwater and marine aquatic animals as well as seven ATCC reference bacterial strains. The minimum inhibitory concentration (MIC) values of the essential oil of *S. aromaticum* against the tested bacterial strains ranged from 0.015 $\mu g \cdot mL^{-1}$ to 0.062 $\mu g \cdot mL^{-1}$ (Table 1). Meanwhile, the MIC values of kanamycin and eugenol against the tested bacterial isolates ranged from 15 $\mu g \cdot mL^{-1}$ to 125 $\mu g \cdot mL^{-1}$ and 15625 $\mu g \cdot mL^{-1}$ to 250000 $\mu g \cdot mL^{-1}$, respectively. A total of nine chemical compounds were detected in the essential oil, with eugenol (49.0%) and caryophyllene (7.5%) being the major compounds.

Our study is the first report on the potential use of the essential oil of *S. aromaticum* as an alternative antimicrobial agent to commercial antibiotic for aquaculture use.

Table 1 Minimum inhibitory concentration (MIC) values of Kanamycin, Eugenol and *Syzygium aromaticum* against fish systemic bacteria isolated from aquaculture sites

types of bacterial	source	Kanamycin/($\mu g \cdot mL^{-1}$)	Eugenol/($\mu g \cdot mL^{-1}$)	essential oil *Syzygium aromaticum*/($\mu g \cdot mL^{-1}$)
Vibrio spp.	*Penaeus vannamei*	31	15625	0.015
Vibrio spp.	*Penaeus monodon*	31	15625	0.015
Vibrio spp.	*Scylla sp.*	62	15625	0.015
Edwardsiella spp.	*Rana catesbeiana*	15	250000	0.031
Aeromonas spp.	*Rana catesbeiana*	15	125000	0.031
Flavobacterium spp.	*Rana catesbeiana*	62	125000	0.031
Vibrio spp.	*Rana catesbeiana*	31	31250	0.015
Aeromonas spp.	*Macrobrachium rosenbergii*	31	62500	0.031
Escherichia coli	*Macrobrachium rosenbergii*	31	62500	0.031
Edwardsiella spp.	*Macrobrachium rosenbergii*	31	62500	0.062
Salmonella spp.	*Macrobrachium rosenbergii*	31	62500	0.062
Vibrio spp.	*Macrobrachium rosenbergii*	62	31250	0.015
Streptococcus spp.	*Trachinotus blochii*	62	62500	0.062
Escherichia coli	*Trachinotus blochii*	62	62500	0.062
Edwardsiella spp.	*Trachinotus blochii*	31	62500	0.062
Salmonella spp.	*Trachinotus blochii*	31	15625	0.062
Pseudomonas spp.	*Trachinotus blochii*	31	32500	0.062
Vibrio damsela	*Trachinotus blochii*	31	32500	0.015
Edwardsiella tarda	*Clarias gariepinus*	15	62500	0.062
Edwardsiella tarda	*Clarias gariepinus*	31	125000	0.062
Edwardsiella tarda	*Clarias gariepinus*	31	62500	0.062
Edwardsiella tarda	*Clarias gariepinus*	31	62500	0.062
Edwardsiella tarda	*Clarias gariepinus*	31	62500	0.031
Edwardsiella tarda	*Clarias gariepinus*	62	62500	0.031
Edwardsiella tarda	*Clarias gariepinus*	31	62500	0.062
Edwardsiella tarda	*Tilapia sp.*	125	15625	0.015
Edwardsiella tarda	*Tilapia sp.*	31	32500	0.015
Edwardsiella tarda	*Monopterus albus*	31	32500	0.015
Edwardsiella tarda	*Monopterus albus*	125	32500	0.015
Edwardsiella tarda	*Monopterus albus*	62	15625	0.062
Edwardsiella tarda	*Monopterus albus*	31	15625	0.062
Edwardsiella tarda	*Monopterus albus*	31	62500	0.015
Edwardsiella tarda	*Trichogaster pectoralis*	31	62500	0.062
Edwardsiella tarda	*Trichogaster pectoralis*	31	62500	0.031
Edwardsiella tarda	*Trichogaster pectoralis*	31	32500	0.031
Edwardsiella tarda	*Trichogaster pectoralis*	31	15625	0.015
Escherichia coli	ATCC 25922	31	32500	0.015
Citrobacter freundii	ATCC 8090	31	32500	0.015
Aeromonas hydrophila	ATCC 49140	31	62500	0.015
Pseudomonas aeruginosa	ATCC 35032	31	32500	0.015
Streptococcus agalactiae	ATCC13813	31	32500	0.015
Edwardsiella tarda	ATCC 15947	31	32500	0.031
Yersinia enterocolitica	ATCC 23715	31	62500	0.031

The essential oil successfully inhibited the growth of all bacterial isolates from various types of aquatic animals including seven ATCC reference bacterial strains, with an MIC value as low as 0.015 $\mu g \cdot mL^{-1}$. On the other hand, Fu et al. (2007) reported on the MIC value of essential oil of *S. aromaticum* against human pathogens; *Staphylococcus epidermidis*, *Escherichia coli* and *Candida albicans* ranged from 0.62 $mg \cdot mL^{-1}$ to 5 $mg \cdot mL^{-1}$. Other studies showed that the MIC value of the essential oil of *S. aromaticum* against human pathogens, four Gram positive bacteria (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis*, and *Listeria monocytogenes*) and three Gram-negative bacteria (*Escherichia coli, Yersinia enterocolitica* and *Salmonella choleraesuis*) ranged from 17.5 $mg \cdot mL^{-1}$ to 131 $mg \cdot mL^{-1}$, however, the essential oil failed to show inhibitory activity against *Pseudomonas aeruginosa* (Lopez et al., 2005). In the study of Moreira et al. (2005), the food grade essential oil of *S. aromaticum* (Nelson and Russell, England) purchased commercially was able to inhibit the growth of *E. coli* (ATCC 25158) at a concentration of 2.5 $mg \cdot mL^{-1}$. Here, we may conclude that bacterial isolates from aquatic animals were more susceptible to the essential oil of *S. aromaticum* compared with bacterial isolates from other hosts.

Many reports have claimed that eugenol is the major compound in the essential oil of *S. aromaticum*. For instance, Bauer et al. (2001) reported that the essential oil of *S. aromaticum* consisted of 75% to 85% eugenol. Farag et al. (1989) also mentioned that approximately 85% eugenol was found in the essential oil of *S. aromaticum*. Another study by Kong et al. (2004) claimed that the essential oil of *S. aromaticum* possessed 68% eugenol. However, the essential oil of *S. aromaticum* obtained in the present study only constituted 49.0% eugenol (Table 2). This may due to differences in methods for essential oil extraction, which resulted in a discrepancy of the percentage of eugenol in the essential oil. In the present study, a piece of self-designed steam apparatus was applied in obtaining the essential oil, whereas other studies used the Clevenger apparatus commonly used for essential oil extraction. Eugenol is reported to play an important role in inhibiting the growth of bacteria. Thoroski et al. (1989) claimed that eugenol could inhibit the production of amylase and proteases in the cell of *Bacillus cereus*. Another study of Wendakoon and Sakaguchi (1993) reported that eugenol could inhibit the growth of *Enterobacter aerogenes* by preventing enzyme action in the bacterial cells. Although the essential oil obtained in the present study constituted of a low percentage of eugenol compared with that of other studies, the MIC values of the essential oil in the present study against bacterial isolates from aquatic animals were lower than those in other studies. This may due to the combination of other chemicals with eugenol that are present in the essential oil, which synergically increased the potency of the antimicrobial property of the essential oil. This is supported by the finding of the present study that the MIC values of eugenol alone against the present bacterial isolates ranged from 15625 $\mu g \cdot mL^{-1}$ to 250000 $\mu g \cdot mL^{-1}$ and Kim et al. (1995) reported that the MIC value of eugenol against foodborne pathogens, *E. coli* and *Salmonella typhimurium*, was 1000 $\mu g \cdot mL^{-1}$ and 500 $\mu g \cdot mL^{-1}$, respectively, which were much higher than the MIC values of the essential oil obtained in the present study. However, further studies should be carried out before we come to a final conclusion.

Table 2 Composition compound in the essential oil of *Syzygium aromaticum*

compound	percentage/%
furan, tetrahydro-3-methyl	2.5
2-propanone, methylhydrazone	5.6
cyclopentane, methyl	4.0
pyrrolidine, 2-buthyl-1-methyl	0.1
2H-Pyran-2-one, tetrahydro-6,6-dimethyl	0.4
eugenol	49.0
copaene	0.5
caryophyllene	7.5
α-caryophyllene	1.4

In short, the result of our present study showed that the potential of the essential oil of *S. aromaticum* as an antimicrobial agent for aquaculture use is promising. However, further studies should be carried out to evaluate the efficacy of this essential oil in controlling bacterial disease infections in fish as well as production costs before the essential oil is introduced to fish farmers.

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