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Antibacterial Activity of Malaysian Edible Herbs Extracts on Fish Pathogenic Bacteria

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ABSTRACT

The study was conducted to investigate the antibacterial properties of five edible herbs against fish pathogenic bacteria. Herbs extracts including black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), curry leaf (*Murraya koenigii*), onion (*Allium cepa*) and Vietnamese coriander (*Persicaria odorata*) were screened against nine common fish pathogens namely *Escherichia coli, Edwardsiella tarda, Aeromonas hydrophila, Citrobacter freundii, Vibrio parahaemolyticus, Vibrio vulnificus, Staphylococcus aureus, Streptococcus agalactiae* and *Streptococcus anginosus*. Methanol extracts of *S. aromaticum* showed antibacterial properties against *V. parahaemolyticus, V. vulnificus, S. aureus* and *S. anginosus* with Minimum Inhibitory Concentration (MIC) values of 1.56, 3.13, 3.13 and 0.78 mg mL^{-1}, respectively. Water extract of *S. aromaticum* exhibited activity against *A. hydrophila* and *S. aureus* with MIC values of 12.5 and 50.0 mg mL^{-1}, respectively. Methanol extract of *M. koenigii* was active against *S. agalactiae* and its MIC was 0.39 mg mL^{-1} while methanol extract of *P. odorata* was active against *S. aureus* and its MIC was 3.13 mg mL^{-1}. For the heat stability test, methanol extract of *S. aromaticum* showed activity at temperatures of 37, 47, 57, 97 and 105°C with 7 to 8 mm in diameter of inhibition zone while another herbs were only active at 37°C. In general, methanol extract of *S. aromaticum* showed the greatest antibacterial activity among all herbs extracts tested.

Key words: Malaysian edible herbs, antibacterial activity, fish pathogenic bacteria, minimum inhibitory concentration, heat stability test, diameter of inhibition zone

INTRODUCTION

Bacterial diseases pose one of the major threats to aquaculture industry worldwide. Many bacteria have been reported to cause disease outbreaks in farmed fish culture resulting in serious economic loses to the industry. In Malaysia, the estimated loss of fin fish cultured in floating cages due to one type of pathogenic bacteria in Peninsular Malaysia alone in 1990 was about RM 20 million (Yusuf *et al*., 2007). Currently, fish treatment against bacterial diseases was via administration of antibiotics and chemotherapeutants. However, this has resulted in development of bacterial resistance against used antibiotics in fish, aquatic environment and sediments (Thakare, 2004). The occurrence of antibiotic resistant bacteria associated with fish disease is a worldwide problem in aquaculture and continues to increase due to the absence of a more effective and safer use of antibiotics (Banasamir *et al*., 2006).

Treatments of bacterial diseases with herbs alleviate many side effects that are associated with synthetic antibiotics (Punitha *et al*., 2008). For the past two decades, there has been an increasing interest in the investigation of various extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi, 2004). It has also been widely observed that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Duraipandiyan *et al*., 2006). The most important of these bioactive phytocomponent of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga *et al*., 2005). In addition, antimicrobial activity could be due to the presence of alkaloids (Osadebe *et al*., 2008).

The present study investigated on herbal potentials as an alternative to the commonly used antibiotics in aquaculture, particularly against bacterial diseases. Edible herbs were selected and known to have human medicinal properties in order to avoid the risk of poisonous side effect to fish culture as well as to the consumers. Black pepper (*P. nigrum*), clove (*S. aromaticum)*, curry leaf (*M. koenigii*), onion (*A. cepa*) and Vietnamese coriander (*P. odorata*) were screened for antimicrobial potential against commonly found gram-positive and gram-negative bacteria in fish culture. Minimum Inhibitory Concentration (MIC) and heat stability test were established for herbs extracts that exhibited positive activity.

MATERIALS AND METHODS

Test microorganisms: Study was carried out in 2010. Test organisms for this study were obtained from bacterial stock collection of Fish Disease Laboratory of Universiti Malaysia Terengganu. Six gram-negative bacteria tested were *Escherichia coli, Edwardsiella tarda, Aeromonas hydrophila, Citrobacter freundii, Vibrio parahaemolyticus* and *Vibrio vulnificus* while three gram-positive bacteria were *Staphylococcus aureus, Streptococcus agalactiae* and *Streptococcus aginosus*. For the purpose of antimicrobial evaluation, the microorganisms were cultured in Tryptone Soya Broth (TSB) (Oxoid, England) at room temperature for 24 h and were adjusted to 10^7 cfu mL^{-1} with sterile saline. The Optical Density (OD) at 540 nm of each culture was measured by using ELISA microplate reader (Biorad, Japan).

Herbs materials: Five herbs including black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), curry leaf (*Murraya koenigii*), onion (*Allium cepa*) and Vietnamese coriander (*Persicaria odorata*) were utilized in this study.

Extracts preparation: Approximately, about 0.20 kg of fresh plant material were dried in oven at 37°C for three days. They were blended into powder form and macerated in both methanol and distilled water for three days. The extracts were filtered through Whatman® filter paper (No. 1, 125 mm) and evaporated to dryness at 37°C. The culture extracts were scored at 4°C for further analysis.

Antibacterial activity test: The antibacterial activity of the herbs was determined by the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). A swab was dipped into TSB culture of selected bacteria and streaked onto Mueller-Hinton agar (Oxoid, England) surface for a lawn of growth. Sterile paper discs (Whatman® paper disc No. 1) with 6 mm in diameter were impregnated in 20 μL of each herb stock solution, dried at 37°C and sterilized under ultraviolet light for about 15 min. By using sterile forceps, the discs were gently placed on the previously inoculated agar surface in triplicate. The plates were incubated for 24 h at 37°C. A standard antibiotic disc of

Oxytetracycline (OTC) was included as positive control and another disc containing methanol served as negative control. Antimicrobial activity was determined by the diameter of clear inhibition zone around the discs as described by Daud *et al.* (2005).

Minimum inhibitory concentration determination: Two hundred milligram dry-crude extract was exposed to ultraviolet light before dissolving it in serial particular solvents. The herbs extracts were then diluted with TSB in two-fold dilutions in sterile 96 well micro plate. The serial dilution was made in range of 200 to 0.10 mg mL^{-1}; then the residue was discarded. Five μL of bacteria was placed into each tube containing 0.25 mL diluted extracts. The mixture was incubated at room temperature for 48 h. The lowest concentration which completely inhibited the microbial growth was recorded as MIC (mg mL^{-1}). The turbidity was measured by ELISA microplate reader at 540 nm and OD value that increased twice its initial value was considered to have produced positive growth.

Effect of temperature on the antibacterial activity: Dry-crude methanol extracts of *S. aromaticum, M. koenigii* and *P. odorata* and water extract of *S. aromaticum* were exposed to different temperatures from 37, 47, 57, 67, 77, 87, 97 and 105°C for 15 min. After heating, the extracts were reconstituted to 200 mg mL^{-1} and 20 μL was pipetted onto paper discs and subsequently tested for antibacterial activity using Kirby-Bauer disc diffusion. The effect of temperature was measured by the inhibition zone.

RESULTS

Preliminary study for antibacterial activity using paper disc diffusion assay showed that among five herbs extracts tested, both methanol and water extracts of *S. aromaticum* and methanol extract of *M. koenigii* and *P. odorata* were active against some of the bacteria tested. Methanol extract of *S. aromaticum* was active against *V. parahaemolyticus, V. vulnificus, S. aureus* and *S. aginosus* by forming clear inhibition zones between 7.0 to 15.3 mm in diameter while the water extract of *S. aromaticum* was active against *A. hydrophila* and *S. aureus* with 15.3 and 8.3 mm in diameter of inhibition zone, respectively. *M. koenigii* inhibited growth of *S. agalactiae* with diameter of 9.3 mm while *P. odorata* exhibited inhibition zone of 8.0 mm in diameter. Oxytetracycline OTC served as positive control exhibited antimicrobial activity of *E. coli, A. hydrophila, V. parahaemolyticus* and *S. aureus* in a range of 11 to 36.0 mm in diameter. Both methanol and water extracts of *P. nigrum* and *A. cepa* showed no positive results of antimicrobial activity (Table 1).

MIC values were expressed in mg mL^{-1} and were presented in Table 2. MIC values for methanol extract of *S. aromaticum* against *V. parahaemolyticus* was 1.56 mg mL^{-1}, *V. vulnificus* was 3.13 mg mL^{-1}, *S. aureus* was also 3.13 mg mL^{-1} and *S. aginosus* was 0.78 mg mL^{-1}. MIC values for water extract of *S. aromaticum* were considerably high which was 12.5 mg mL^{-1} against *A. hydrophila* and 50.0 mg mL^{-1} against *S. aureus*. *M. koenigii* was active against *S. agalactiae* with MIC value of 0.39 mg mL^{-1} while *P. odorata* against *S. aureus* was 3.13 mg mL^{-1}. For the heat stability test, methanol extract of *S. aromaticum* showed inhibition against gram-positive and gram-negative bacteria at 37, 47, 57, 97 and 105°C with 7 to 8 mm in diameter of inhibition zone while other herbs were only active at 37°C. Antimicrobial activity at different temperatures of the extracts against pathogenic bacteria was presented in Fig. 1.

Table 1: Antibacterial activity test of five herbs of both water and methanol extracts against nine fish pathogenic bacteria

	Mean of inhibition zone diameter (mm)										
	P. nigrum		*S. aromaticum*		*M. koenigii*		*A. cepa*		*P. odorata*		
Bacteria	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	OTC
E. coli	-	-	-	-	-	-	-	-	-	-	18.2
E. tarda	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	-	-	15.3	-	-	-	-	-	-	-	11.4
C. freundii	-	-	-	-	-	-	-	-	-	-	-
V. parahaemolyticus	-	-	-	15.3	-	-	-	-	-	-	35.7
V. vulnificus	-	-	-	7.0	-	-	-	-	-	-	-
S. aureus	-	-	8.3	10.0	-	-	-	-	-	8.0	27.0
S. agalactiae	-	-	-	-	-	9.3	-	-	-	-	-
S. aginosus	-	-	-	8.0	-	-	-	-	-	-	-

OTC: Oxytetracycline, -: No zone of inhibition

Table 2: Minimum Inhibitory Concentration (MIC) of selected herb extracts against fish pathogenic bacteria

Herbs	Extracts	Bacteria tested	MIC (mg mL^{-1})
S. aromaticum	Water	*A. hydrophila*	12.50
		S. aureus	50.00
	Methanol	*S. aureus*	1.56
		V. parahaemolyticus	1.56
		V. vulnificus	3.13
		S. aginosus	0.78
M. koenigii	Methanol	*S. agalactiae*	0.39
P. odorata	Methanol	*S. aureus*	3.13

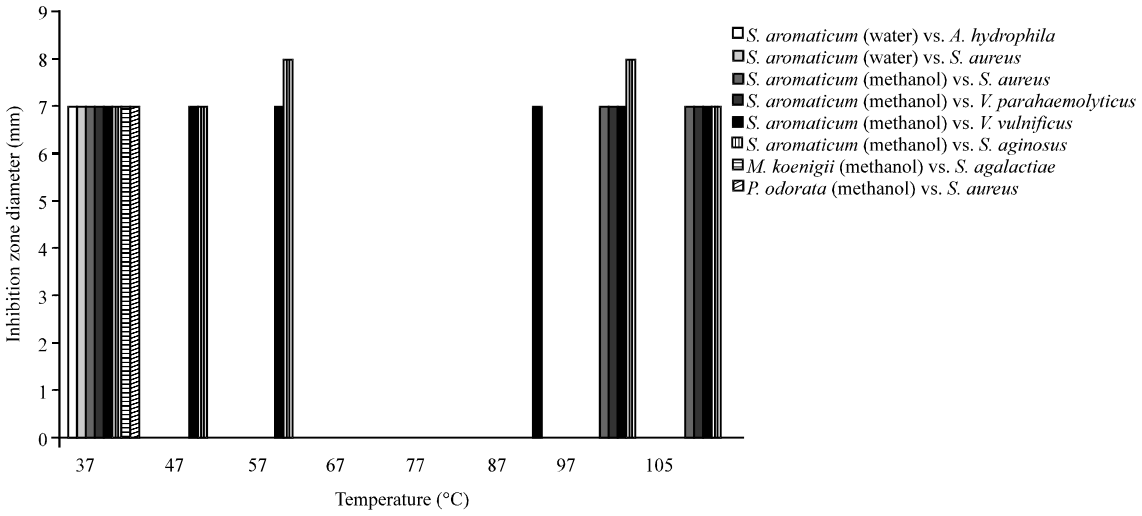

Fig. 1: Heat stability test of herbs extracts against fish pathogenic bacteria

DISCUSSION

The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant bacteria

(Kone *et al*., 2004). It was clearly established that many plants contain microbial inhibitors (Delaquis *et al*., 2002). All herbs tested were famously used in many dishes. This finding further supported that a number of edible herbs are obviously a potential source of inhibitory substances for some important pathogenic bacteria (Voravuthikunchai *et al*., 2006; Saadabi *et al*., 2006). However, there was no antibacterial activity in extracts of *P. nigrum* and *A. cepa* against tested pathogens at the specific dose. The results are contradictory to some researchers who reported antibacterial activity of those herbs (Kalemba and Kunicka, 2003). This variation may be due to amount of dose used, the method of extraction of the herbs, the method of antibacterial study, the generic variation of the herbs and age of the plant or the environment (Thakare, 2004). It would be advantageous to standardize methods of extraction and *in vitro* testing so that future research could be more systematic and interpretation of results would be facilitated. Besides, alternative mechanisms of infection, prevention and treatment should be included in initial activity screenings (Cowan, 1999).

Among five herbs tested, *S. aromaticum* showed greatest antibacterial activity against fish bacterial pathogens. *S. aromaticum* contained 14 to 20% volatile oil which consisted of 70 to 90% eugenol tannins (Thomson, 1978). It was a well-characterized, representative found in oil of *S. aromaticum* and considered bacteriostatic against both fungi and bacteria. Active compounds of *M. koenigii* such as the essential oils, terpenoids and bioactive alkaloids most probably contributed for antibacterial activity of the herb against *S. agalactiae* as many studies suggested these constituents had the antimicrobial activities (Ramsewak *et al*., 1999; Rahman *et al*., 2011). On the other hand, *P. odorata* contained aldehydes and essential oil that constituted to antimicrobial activity (Hunter, 1996). Earlier, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1977). The results of this study supported the point of view that different bacterial species exhibited different sensitivities towards these compounds (Chamberlin, 1991).

MIC values for water extract of *S. aromaticum* against *A. hydrophila* and *S. aureus* were high which were 12.5 and 50.0 mg mL^{-1}. This indicated the low degree of inhibition of the herb extract. Contrast to this, methanol extracts of *S. aromaticum, M. koenigii* and *P.odorata* showed low MIC values indicating the better degree of inhibition. However, the degree of inhibition of each herb against different bacteria was different and this may be related to the different active compounds of the herbs that act in different degree of inhibition (Chamberlin, 1991; Khanahmadi *et al*., 2010).

Some of the herbs were effective than traditional antibiotics to combat the pathogenic bacteria studied. The chance to find antimicrobial activity was more apparent in methanol than water extracts of the same herbs (Voravuthikunchai *et al*., 2006). Though MIC values obtained that range from 0.39 to 12.5 mg mL^{-1} for the three herbs selected might not good enough to present antimicrobial potential of the herbs tested commercially, extraction methods of both and susceptibility test methods could be manipulated to provide more scientific validation for the traditional medicinal use of these herbs as antimicrobial agents (Geissman, 1963).

For heat stability test, all herbs tested were affected by different temperatures. Only methanol extract of *S. aromaticum* showed a slight activity at high temperature. Similarly, Muskhazli *et al*. (2008) found that antibacterial activity of *Quercus infectoria* (Olivier) showed the largest inhibition zone at 30°C when compared to higher temperatures tested. This effect was probably explained that heat could denature the active compound of the herbs (Chandarana *et al*., 2005). There were also many factors that could influence the antimicrobial activity under the heat such as air exposure, lights and activities of plant enzymes. However, a study by Chandarana *et al*. (2005)

found that heated extracts have higher activity against bacteria than unheated extracts due to the fact that, biologically active compounds are easily extracted and reacted at higher temperature (Chandarana *et al*., 2005).

Now a days, most pathogenic organisms are becoming resistant to antibiotics. The present study suggested the potential of some herbs as an alternative to commercial and synthetic antibiotics which could be used in aquaculture industries.

CONCLUSION

In conclusion this present study showed that *S. aromaticum* showed strong antibacterial property when compared to other herbs. It is anticipated that this herb can be potentially used in combating fish bacterial diseases. However, further study on safety and toxicity are warranted.

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