Research Article

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ARECA CATECHU NUT EXTRACT: AN ANTIMICROBIAL AGENT FOR AQUACULTURE USES

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ABSTRACT

This paper deals with the antimicrobial activity of *Areca catechu* nut extract against various species of bacterial isolates obtained from aquaculture sites. A total of 49 bacterial isolates were applied in the present study, namely *Aeromonas hydrophila* (n = 6), *Escherichia coli* (n = 5), *Edwardsiella tarda* (n = 6), *Flavobacterium* spp. (n = 6), *Klebsiella* spp. (n = 5), *Salmonella* spp. (n = 6), *Vibrio alginolyticus* (n = 5), *V. parahaemolyticus* (n = 5), *V. cholerae* (n = 2) and *Pseudomonas* spp. (n = 3). The antimicrobial property of *A. catechu* was revealed using two-fold broth micro dilution method whereas chemical composition of the plant extract was characterized using Gas Chromatography - Mass Spectrometry (GC –MS). The present study revealed that *A. catechu* exhibited antimicrobial activity to all tested bacterial isolates with the minimum inhibitory concentration (MIC) values ranged from 1.95 to 250 mg/L. A total of 10 compounds was successfully identified. Hexadecanoic acid, methyl ester (29.85%) is the major compound in *A. catechu* nut extract followed by 9-Octadecenoic acid, methyl ester, (E)- (19.71%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (14.52%), Acetic acid (11.80%), 4-Hydroxy-2-methylacetophenone (5.22%), Pentadecanoic acid, 14 –methyl-, methyl ester (4.30%), Pantolactone (3.70%), Octadecanoic acid methyl ester (3.09%), 9-Octadecenamide (Z)- (3.06%), Acetamide, N-(2-phenylethyl)- (2.58%) and an unidentified compound (2.17%).The present study showed that antimicrobial property of *A. catechu* nut extract is promising.

Keywords: antimicrobial, chemical compound, *Areca catechu*, bacteria, aquaculture

INTRODUCTION

Traditionally, *Areca catechu* nut was used as an ingredient in chewing with betel leaves in many Asia countries, in which itwas believed to improve oral hygiene, increase appetite, anti-diabetic and reduce cholesterol[1]. *Areca catechu* nut was reported to possess several biological properties such as antihelmintic, antifungal, antibacterial, anti-inflammatory, antioxidant, insecticide and larvicidal[2] and therefore became famous in South East Asia as traditional herbal medicine. Besides application for human, *A. catechu* may be applied for animal uses as well. The increasing antibiotic resistance cases among pathogenic bacteria in aquaculture prompted fish farmer to seek for alternative natural resources in the treatment of fish diseases. From the literature survey, we found that the study of antimicrobial activity of *A. catechu* was started as early as 1965[3]. The study of antimicrobial activity of *A. catechu* was then characterized by Joseph and Ranjit Singh[4] and Anthikat and Michael[1]. Till present, no study has documented the antimicrobial activity of *A. catechu* to be used as an antimicrobial agent against bacterial isolates from aquaculture sites. Therefore, this study was conducted to reveal antimicrobial activity of *A. catechu* nut extract and the potential of this plant extract to be used as antimicrobial agent in aquaculture industry.

MATERIALS AND METHODS

Preparation of *Areca catechu* nut extract

Areca catechu extract was prepared immediately after being bought from herbal nursery located at Pasir Puteh, Kelantan, Malaysia. The plant samples were subjected to the procedures reported by Lee et al.[5] and Das et al.[15] Briefly, the fresh plant sample was oven dried at 37 °C for 4 days. Next, the plant sample was freeze-dried prior to extraction using 70% methanol and concentrated at 1 $g.mL^{-1}$. Finally, the plant extraction was kept in -20 °C until further use.

Bacterial isolates

A total of 49 bacterial isolates were used in the present study. All bacterial isolates were provided by Universiti Malaysia Kelantan namely *Aeromonas hydrophila* (n = 6), *Escherichia coli* (n = 5), *Edwardsiella tarda* (n = 6), *Flavobacterium* spp. (n = 6), *Klebsiella* spp. (n = 5), *Salmonella* spp. (n = 6), *Vibrio alginolyticus* (n = 5), *V. parahaemolyticus* (n = 5), *V. cholerae* (n = 2) and *Pseudomonas* spp. (n = 3). These bacteria were isolated from various aquatic animals including *Artemia* sp., *Corbicula fluminea*, *Clarias gariepinus*, *Tilapia* sp., *Pangasius sutchi*, *Penaeus vannamei*, and *Scylla* sp. (Table 1), identified with biochemical tests and further confirmed with bacterial identification kit (BBL, USA). These bacterial isolates were kept in tryptic soy agar (TSA) for further uses.

Determination of minimum inhibitory concentration (MIC) of *Areca catechu* crude extract

The values of minimum inhibitory concentration (MIC) of kanamycin as positive control and *A. catechu* (Voucher: UMK 01) crude extract against tested bacterial isolates were determined through two-fold broth micro dilution method[9]. The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature and the concentration of these cultures were adjusted to 10^9 CFU 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 plant extract (0.24 to 500 $mg.L^{-1}$) and kanamycin (0.24 to 500 $\mu g.L^{-1}$).The microplate was incubated at room temperature for 24 h (Figure 1.). The MIC values were defined as the lowest concentration of the plant extract in the wells of the microtiter plate that showed no visible turbidity after 24 h incubation.

Bioactive compound characterization

Bioactive compound characterization of the plant extract was carried out as described by Lee et al.[9]. The chromatographic procedure was carried out using Shimadzu QP2010 Gas Chromatography – Mass Spectrometry (GC-MS) with auto sampler. The sample was diluted 25 times with acetone and 1 µl of sample was injected into a column. A fused silica capillary column HP5-MS (30 mm x 0.32 mm, film thickness 0.25 µm) was used. Helium was the carrier gas, and a split ratio of 1/100 was used. The oven temperature was maintained at 60 ºC for 8 min. The temperature was then gradually raised at a rate of 3 ºC per min to 180 ºC and maintained at 180 ºC for 5 min. The temperature at the injection port was 250 ºC. The components of the test solution were identified by comparing the spectra with those of known compounds stored in internal library.

Table 1: Minimum inhibitory concentration (MIC) values of kanamycin and *Areca catechu* nut extract against bacteria isolated from various aquatic animals

Types of bacteria	Sources	Kanamycin $\mu g.L^{-1}$	*Areca catechu* nut extract $mg.L^{-1}$
Vibrio parahaemolyticus	*Artemia* sp.	62.5	1.95
Vibrio alginolyticus	*Artemia* sp.	250	125
Edwardsiella tarda	*Artemia* sp.	125	62.5
Aeromonas hydrophila	*Artemia* sp.	7.81	31.25
Flavobacterium spp.	*Artemia* sp.	3.90	15.63
Vibrio cholerae	*Artemia* sp.	1.95	62.5
Klebsiella spp.	*Artemia* sp.	62.5	125
Vibrio parahaemolyticus	*Corbicula fluminea*	1.95	1.95
Vibrio alginolyticus	*Corbicula fluminea*	125	3.90
Edwardsiella tarda	*Corbicula fluminea*	7.81	62.5
Aeromonas hydrophila	*Corbicula fluminea*	62.5	7.81
Flavobacterium spp.	*Corbicula fluminea*	3.90	125
Escherichia coli	*Corbicula fluminea*	7.81	3.90
Salmonella spp.	*Corbicula fluminea*	62.5	250
Klebsiella spp.	*Corbicula fluminea*	1.95	62.5
Edwardsiella tarda	*Corbicula fluminea*	250	7.81
Aeromonas hydrophila	*Corbicula fluminea*	250	1.95
Flavobacterium spp.	*Corbicula fluminea*	3.90	62.5
Escherichia coli	*Corbicula fluminea*	125	1.95
Salmonella spp.	*Corbicula fluminea*	7.81	125
Klebsiella spp.	*Corbicula fluminea*	1.95	250
Salmonella spp.	*Tilapia* sp.	125	250
Edwardsiella tarda	*Tilapia* sp.	62.5	3.90
Aeromonas hydrophila	*Tilapia* sp.	250	3.90
Flavobacterium spp.	*Tilapia* sp.	125	250
Escherichia coli	*Tilapia* sp.	3.90	125
Klebsiella spp.	*Tilapia* sp.	1.95	250
Salmonella spp.	*Pangasius sutchi*	250	7.81
Edwardsiella tarda	*Pangasius sutchi*	125	62.5
Aeromonas hydrophila	*Pangasius sutchi*	3.90	250
Flavobacterium spp.	*Pangasius sutchi*	250	125
Escherichia coli	*Pangasius sutchi*	62.5	3.90
Klebsiella spp.	*Pangasius sutchi*	250	1.95
Vibrio parahaemolyticus	*Penaeus vannamei*	7.81	1.95
Vibrio alginolyticus	*Penaeus vannamei*	125	62.5
Pseudomonas spp.	*Penaeus vannamei*	250	3.90
Vibrio parahaemolyticus	*Penaeus vannamei*	3.90	125
Vibrio alginolyticus	*Penaeus vannamei*	250	1.95
Pseudomonas spp.	*Penaeus vannamei*	125	7.81
Vibrio parahaemolyticus	*Scylla* sp.	1.95	3.90
Vibrio alginolyticus	*Scylla* sp.	62.5	250
Vibrio cholerae	*Scylla* sp.	250	1.95
Edwardsiella tarda	*Scylla* sp.	125	3.90
Aeromonas hydrophila	*Scylla* sp.	250	62.5
Flavobacterium spp.	*Scylla* sp.	1.95	7.81
Salmonella spp.	*Scylla* sp.	1.95	125
Pseudomonas spp.	*Scylla* sp.	125	250
Klebsiella spp.	*Scylla* sp.	7.81	3.90
Escherichia coli	*Scylla* sp.	3.90	62.5

Table 2: Compound composition of *Areca catechu* nut extract

Compound	Compound Composition (%)
Hexadecanoic acid, methyl ester	29.85
9-Octadecenoic acid, methyl ester, (E)-	19.71
9,12-Octadecadienoic acid, methyl ester, (E,E)-	14.52
Acetic acid	11.80
4-Hydroxy-2-methylacetophenone	5.22
Pentadecanoic acid, 14 –methyl-, methyl ester	4.30
Pantolactone	3.70
Octadecanoic acid, methyl ester	3.09
9-Octadecenamide, (Z)-	3.06
Acetamide, N-(2-phenylethyl)-	2.58
Unidentified compounds	2.17
Total	100

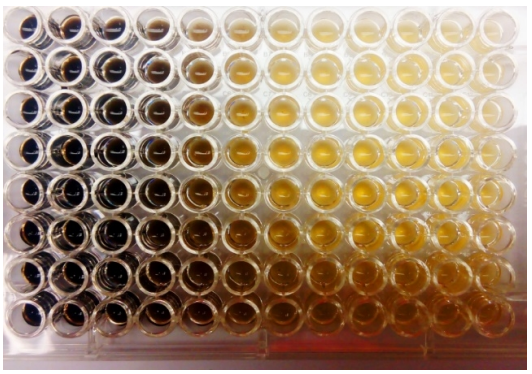

Figure 1: Minimum Inhibitory Concentration (MIC) assay using microtiter plate

RESULT & DISCUSSION

In the present study, the minimum inhibitory concentration (MIC) values of *A. catechu* nut extract against the tested bacterial isolates ranged from 1.95 to 250 $mg.L^{-1}$ whereas the MIC values of kanamycin ranged from 1.95 to 250 $\mu g.L^{-1}$ (Table 1). Antimicrobial agent can be any compound or substance or combination of several compounds in which can inhibit the growth of microorganism. Further study need to be carried out soon to reveal selective chemical composition of the plant extract that may contribute to the antimicrobial property of the plant extract as well as its mode of action.

A total of 10 compounds was successfully identified by GC-MS. Hexadecanoic acid, methyl ester (29.85 %) is the major compound in *A. catechu* nut extract followed by 9-Octadecenoic acid, methyl ester, (E)- (19.71 %), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (14.52 %), Acetic acid (11.80 %), 4-Hydroxy-2-methylacetophenone (5.22 %), Pentadecanoic acid, 14 –methyl-, methyl ester (4.30 %), Pantolactone (3.70 %), Octadecanoic acid methyl ester (3.09 %), 9-Octadecenamide (Z)- (3.06 %), Acetamide, N-(2-phenylethyl)- (2.58 %) and an unidentified compound (2.17 %) (Table 2).

The antimicrobial property of *A. catechu* against various species of pathogens was quite well documented. In the study by Anthikat and Michael reported that this plant extract can inhibit the growth of *Streptococcus mutans*, causative agent of dental caries[1]. The study also found that the plant extract showed inhibitory activity against *E. coli*, *K. pneumoniae*, *S. typhimurium* isolated from animals. Furthermore, they also claimed that *Pseudomonas* sp., *Klebsiella* sp., *Proteus* sp. and *E. coli* isolated from human were sensitive to *A. catechu* extract. Another study of Joseph and Ranjit Singh claimed that *A. catechu* possesses inhibitory activity against *E. coli*, *P. aeruginosa*, *S. paratyphi*, *V. cholerae* and *V. fischeri*[4]. Hexadecanoic acid and octadecenoic acid are the major compounds the found in the plant extract that may play an important role in the antimicrobial activity of *A. catechu* nut extract. Other compound that was found in large percentage in the plant extract was acetic acid. For instance, Ryssel *et al*. claimed that acid acetic at 3% of concentration can inhibit the growth of *Proteus vulgaris*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*[7]. Fraise et al. was also reported that acetic acid can be applied as antimicrobial agent against *P. aeruginosa* for burn injuries patient[8].

The antimicrobial property of plant extracts toward pathogenic bacteria from aquaculture were well documented. The plant extracts were found promising and can be alternative to the present antibiotics in controlling bacterial diseases in aquaculture. For example, essential oil of *Syzygium aromaticum* flower bud (clove)[5], *Allium sativum*[6], *Murdannia bracteata* leaf extract[9], *Sauropus androgynus* stem extract[10], *Ficus deltoidea* Jack. leaf extract[11], *Andrographis paniculata* leaf extract[12]; *Peperomia pellucida* leaf extract[13], *Michelia champaca* seed and flower extracts[14].

CONCLUSION

In conclusion, our investigation on *A. catechu* nut extract found that it is effective to control or inhibit the growth and propagation of all the tested bacterial isolates. However, further study by *in vivo* test should be carried out in the near future for better understanding of its application in aquaculture.

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