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Antimicrobial activities of blinding tree, *Excoecaria agallocha* against selected bacterial pathogens

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In aquaculture, the occurrence of bacterial resistance to synthetic antibiotics has become a serious problem. Therefore, research has been focused on finding new antimicrobial antibiotics from natural products to replace synthetic antibiotics. The aim of this study was to investigate the antimicrobial properties of mangrove plant blinding tree *Excoecaria agallocha* against selected fish pathogens namely *Flavobacterium indicum*, *Chryseobacterium indologenes*, *Chryseobacterium gleum* and *Elizabethkingia meningoseptica* previously named *Flavobacterium meningosepticum*. Mangrove leaves were obtained via extraction with 100 ml of methanol. The antimicrobial susceptibility test showed that the bacteria were resistant to Nitrofurantion, Gentamycin and Neomycin, and were sensitive to Flumequine. The minimum inhibitory concentration (MIC) of *E. agallocha* was 3.12 mg/ml, and minimum bactericidal concentration (MBC) was 6.25 mg/ml. Inhibition zones were significantly different ($p< 0.05$) depending on concentrations (100, 300 and 500 mg/ml) of the crude extraction of *E. agallocha*. The highest activity with LC_{50} of *E. agallocha* was 94.19 (mg/ml). Methanolic extract of *E. agallocha* exhibited strong antimicrobial activity against these bacteria.

Key words: Antimicrobial activities, *Excoecaria agallocha*, inhibition zone, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), LC_{50}.

INTRODUCTION

Antimicrobial agents have been widely used in aquaculture worldwide to treat infections caused by a variety of fish bacterial pathogens. Excessive use of antimicrobial agents in aquaculture has led to antimicrobial resistance among bacteria including those that are not fish pathogens. Many studies have been carried out on the plant antimicrobial properties against aquatic bacterial pathogens (Lee et al., 2008; Najiah et al., 2011a; Najiah et al., 2011b). Laith (2012) reported the presence of antimicrobial activities of mangrove plants such as *Sonneratia caseolaris* and *Rhizophora apiculata* on fish pathogenic bacteria. *Excoecaria agallocha* latex is well known to cause skin irritants, rapid blistering, and temporary blindness in human. This latex has been used as a poison for fish by adding it to water as well as to poison arrowheads. The plant is also used to treat flatulence (Karalai et al., 1994). Mangrove contains biologically active antibacterial, anti-fungal and antiviral compounds (Vadlapudi et al., 2009). Mangrove is a natural resource of tannin and is of great value; however, the antioxidant and antifungal potentials against fish pathogen is limited (Dhayanithi et al., 2012). Nowadays, interest in the plant extract of *E. agallocha* bark herbal preparation due to its antimicrobial activities is increasing. Plant extract has been suggested as beneficial in the healing of severe infections, in addition to anti-tumour, anti-microbial, anti-wound killing and anti-oxidant properties (Thirunavukkarasu et al., 2009). The antibacterial activity of methanolic extract of 10 plants

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species was carried out *in vitro* by disc diffusion method against 10 both Gram-positive and Gram-negative bacteria. The antibacterial results revealed three levels of activities; maximum, moderate and minimum activity on bacteria, this might be as a result of the presence of flavanoids, alkaloids, phenolic and glycosides compounds in the plant extracts (Gothandam et al., 2010). The value of these secondary metabolites is increasing due to the constant discoveries of their potential role in health care and drug development (Remani et al., 2012).

Flavobacteria are found in soil and water, several species are identified in causing diseases in fish such as *Flavobacterium psychrophilum* in salmond and rainbow trout fry- a disease known as bacterial cold water disease. *Flavobacterium columnare* was reported to cause cotton-wool disease in freshwater fishes (Bernardet et al., 1994).

The *Chryseopbacterium* genus was a member of the Flavobacteriaceae such as *Chryseopbacterium meningosepticum*, *Chryseopbacterium indologenes*, *Chryseopbacterium indoltheticum*, *Chryseopbacterium scophthalmum* and *Chryseopbacterium gleum* (Vandamme et al., 1994). *C. meningosepticum* and *C. miricola* have been reclassified into the new genus *Elizabethkingia* (Kim et al., 2005). In the study of Montasser (2005), isolation of *C. meningosepticum* from chicken and tick, *Argas persicus* was performed. *E. meningoseptica* is a significant bacterial group in human clinical infections. Recently, *E. meningoseptica* was isolated from tiger frogs, *Rana tigerina rugulosa* with cataract disease in China (Xie et al., 2009). *Chryseobacterium* species, including *C. indologenes* and *C. meningosepticum*, have been documented as human pathogens in hospitalized patients implanted with indwelling devices (Hsueh et al., 1996; Hsueh et al., 1997). *C. indologenes* is responsible mostly for nosocomial infections linked to the use of intravascular devices (Hsueh et al., 1996). *Chryseobacterium* spp. is known to exhibit resistance to aminoglycosides, tetracyclines, chloramphenicol and erythromycin (Kim et al., 2005). *C. indologenes* appears to be an emerging problem in Taiwan because of its multi-resistance to antibiotics (Hsueh et al., 1997).

Therefore, the aim of the present study was to determine the antimicrobial activity of methanolic extracts from leaves of the mangrove plant blinding tree, *E. agallocha* against selected bacterial pathogens.

MATERIALS AND METHODS

Bacterial stock

Fish bacterial stock was obtained from Fish Disease Laboratory, University Malaysia Terengganu (UMT) Malaysia. They were *Flavobacterium indicum*, *Chryseobacterium indologenes, Chryseobacterium gleum and Elizabethkingia meningoseptica* (*Chryseobacterium* spp, *Elizabethkingia* spp, were previously put under Flavobacterium family).

Plant material

E. agallocha leaves were collected from the mangrove rural area in Terengganu, Malaysia. The plant was identified at the Plant Taxonomy Laboratory, University Malaysia Terengganu (UMT), Malaysia. The weight of *E. agallocha* leaves after extraction was determined and the percent yield of the leaves was calculated using the formula (Sule et al., 2011):

Percentage yield = $X_1 - (X_2 / X_1) \times 100.0\%$

Where, X_1 = Weight before extraction; X_2 = Weight after extraction.

Extract preparation

The plant leaves were washed with tap water and then with distilled water to remove epiphytes and other debris. The leaves were then air dried in a shaded area for 3 weeks before being grounded to powder (15 g dry weight) and then extracted with 100 ml of methanol for 48 h according to the methods of Bele et al. (2009). The extract was filtered using a filter paper. This procedure was repeated three times and the samples were pooled. The solvent was evaporated from the crude extract by a rotator evaporator (Buchi, Switzerland) and the dried extracts were stored at 4°C until further use. The percentage yield was calculated from the dry extract powder. Then, 3,250 mg/ml of the extracts were dissolved in methanol for the antibacterial assays and in dimethyl sulfoxide (DMSO) for the cytotoxicity assays. The samples for the cytotoxicity screening were further diluted to 5 mg/ml with growth medium (DMEM or RPMI 1640) to reduce the concentration of DMSO.

Antibiotics susceptibilities tests (disk diffusion method)

Antibiotic susceptibility was determined following the methods as described by Bauer et al. (1966). Isolates were tested *in vitro* for their sensitivity to 18 different antibiotics namely; Oxolinic Acid (2 µg), Flumequine (30 µg) Deoxycycline (30 µg), Ampicillin (10 µg), Oleandomycin (15 µg), Fosfomycin (50 µg), Nitrofurantion (50 µg), Spiramycin (100 µg), Lincomycin(15 µg), Neomycin (10 µg), Tetracycline (10 µg), Florfenicol (30 µg), Amoxicillin (25 µg), Erythromycin (15 µg), Colistin Sulphate (25 µg), Novobiomycin (30 µg), Gentamycin (10 µg) and Polymyxin B (30 µg) (Oxoid, England).

The isolates in the current study were cultured in Anacker and Ordal's broth (EAOB) at 28°C for 48 h. Minispin tube (Eppendorf, Germany) was used to centrifuge the bacterial cells at 14,500 rpm for 5 min. Bacterial cell concentrations were adjusted to McFarland 0.5 ($1.5 \times 10^8 CFUmL^{-1}$). 100 µl of aliquots were spread over Anacker and Ordal's agar (EAOA) surface using sterile cotton buds. After a time lapse of 10 min, antibiotic disks were placed on the surface of the inoculated agar plates using sterile forceps. The plates were incubated at 28°C for 48 h. After incubation, the diameter of inhibition zones around the discs were measured in millimetre (mm) and characterized as sensitive (S), intermediate (I) and resistant (R) according to Clinical and Laboratory Standard Institute (CLSI, 2006).

Antibacterial assay

Disk diffusion assay

Disk diffusion assay was carried out on Anacker and Ordal's agar

(EAOA) following the method described by Barker et al. (1995). Briefly, the disc (6 mm in diameter) was impregnated with 10 mg/ml extract (20 µg/disc) and placed on inoculated agar. Antibiotic disc was used as positive control (Flumequine 30 µg/disc) and discs impregnated with (20 µl) of methanol was used as negative controls. Discs were then air dried and placed equidistantly onto the EAOA agar surface layered with bacterial pathogens and incubated at 28°C for 48 h. The growth inhibition was assessed as the diameter (mm) of the inhibition zone around the discs. The experiment was carried out in triplicate.

Agar-well diffusion method

Anacker and Ordal's broth (EAOB) was used to grow bacterial isolates for 48 h prior to use. The concentration of the suspensions was adjusted to achieve a turbidity of 0.5 McFarland 1.5×10^8 $CFUmL^{-1}$. Sterilized cotton swabs were used to seed isolates on Anacker and Ordal's agar (EAOA). A hole in the wells of the agar medium was made by means of a sterilized 6 mm cork borer. Wells were later filled with 100 µl solution of various concentrations of extracts (100, 300 and 500 mg/well) and 100 µl of methanol (negative control) was dispensed into separate wells. The standard antibiotic disc flumequine (30 µg) was left to set on the agar surface as positive control. The plates were laid inside an incubator at 28° C for 48 h (Perez et al., 1990). Following that, plates were inspected for zone of inhibition. The experiment was carried out in triplicate.

Determination of minimum inhibitory concentration (MIC)

The MIC of plants crude extracts was determined for active component that showed antimicrobial activity against test organisms. The micro titre broth dilution technique was performed, according to standards methods, using sterile 96 well-micro titre plates (CLSI, 2006). Bacteria were cultured overnight in Anacker and Ordal's broth (EAOB) at 28°C and adjusted to achieve a turbidity of 0.5 McFarland $1.5 \times 10^8 CFUmL^{-1}$. A 100 (µl) of Anacker and Ordal's broth (EAOB) were allocated to each wells. For each assay, the first wells were inoculate with 100 (µl) of 100 (mg/ml) crude extracts, followed by the two fold dilution until 0.098 mg/ml. Microbial suspension of 10µl were used as inoculants. Positive growth control included broth and inoculums (without extracts suspension). Micro titre plates were sealed with parafilm to ensure the bacteria does not become dehydrated. Plates were further incubated at 28°C for 48 h. After incubation, turbidity of each wells was observed visually and the optical density (OD) were measured at 540 nm by a micro-titre reader model 680 (Bio-rad, US). Data dilution values lower than the yielding value ≥ 2, the doubling concentrations were interpreted as MIC results. The results were confirmed with micro dilution assays with the addition of 10 µl of 0.1% 2,3,5-triphenyltetrazolium chloride (TTC) (w/v) (Merck, Germany) into each well and incubated for 1 h for reaction. Colour changes from purple to pink were observed visually where the bacteria were able to reduce the TTC into formazon. The lowest concentrations that inhibit the growth of bacteria with the absence of visual colour changes were recorded as MIC. Tests were run in triplicate.

Determination of Minimum bactericidal concentrations (MBC)

MBC extract was determined according to Ilavenil et al. (2010). Plates that displayed negative results from the MIC assay were taken as samples and sub-cultured onto a new Anacker and Ordal's agar (EAOA); they were incubated at 28°C for 48 h. The MBC inhibited bacterial growth on agar plate surface and therefore was the lowest concentration of extract. Experiments were performed in triplicate of three and the mean readings were recorded. The ratio of MBC /MIC determined the results. If ratio of MBC/MIC was ≤ 2, the active crude extract was considered as bactericidal; if not, it was considered as bacteriostatic. If ratio was ≥16, then the active crude extract is considered to be ineffective (Shanmughapriya et al., 2008).

Brine shrimp lethality test

The experiment was carried out according to a previously described method (Pisutthanan et al., 2004). Brine shrimp eggs (OSI, USA) were hatched in sea water using a 1,000 ml beaker and then incubated at room temperature (28°C) for 24 h. Nauplii were collected using 100 µl tips after removing 2 mm from the tip ends. One hundred milligrams of the extracts were dissolved in 10% dimethyl sulphoxide (DMSO) (Chempur, US). 2-fold serial dilutions were made in 96-well micro-plates using 100 µl of sea water in triplicate. A suspension of nauplii containing 10-15 organisms (100 µl) was added to each well and incubated at room temperature (28°C) for 24 h. The control sample contained 10% DMSO without extracts. The plates were then examined under a binocular stereomicroscope (Nikon, Japan) and the number of dead nauplii in each well was counted. One hundred microlitres of methanol were added to each well to immobilise the nauplii and the total numbers of nauplii were taken after 15 min. The data analysis was performed using the linear regression of probit method to determine the lethality of LC_{50} value.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate measurements. Probit linear regression analysis was done to analyse the LC_{50} values using statistical package for the social sciences (SPSS) version 16.0 for windows. Analysis of variance (ANOVA) and the mean was compared with least significant difference ($P<0.05$) using Gestate 12.1 program.

RESULTS

Antibiotic resistance test was done on all selected samples of Gram negative bacteria. Isolates showed 100% resistant to Nitrofurantion, Gentamycin and Neomycin and, 100% sensitive to Flumequine (Table 1). The percent yield was 7.0% and the mass of the crude was 0.15 g. Antimicrobial activities of methanolic extract of *E. agallocha* on bacteria were carried out by measuring inhibition zone, using agar well and disc diffusion methods.

The mean zone of inhibition for *E. agallocha* methanol extracts of concentration 100, 300 and 500 (mg/ml) using well technique ranged from 15.67 to 20.33, 18.67 to 20 and 18.67 to 23.67 mm, respectively. When the mean zone of inhibition against all isolates for the flumequine antibiotic ranged from 25.19 to 27.33 mm, there was a significant difference ($P<0.05$) among all concentration and antibiotic against bacteria (Table 2).

The result revealed there are significant difference ($P<0.05$) as compared to the mean inhibition zone for *E. agallocha* methanol extracts of concentration 100, 300 and 500 (mg/ml). Although, the concentration of 500 mg/ml showed the high inhibition zone than 100 and 300

Table 1. Percentage (%) of antibiotic resistance (R), intermediately sensitive (I) and sensitive (S) of the present isolate.

Antibiotic	Disc potency (mcg)	Isolates					Zone of inhibition			
		*1	*2	*3	*4	*5	R	I	S	S (%)
Ampicillin	10	S	R	R	R	R	14	15-16	17	20
Flumequine	30	S	S	S	S	S	15	16-18	21	100
Oleandomycin	15	S	R	R	R	R	12	13-16	17	20
Fosfomycin	50	S	R	R	R	R	13	14-16	17	20
Nitrofurantion	50	I	R	R	R	R	19	20-25	26	0
Spiramycin	100	S	R	R	R	R	12	13-15	16	20
Lincomycin	15	S	R	R	R	R	14	15-20	21	20
Neomycin	10	R	R	R	R	R	12	13-15	17	0
Tetracycline	10	S	R	R	R	R	14	15-18	19	20
Florfenicol	30	S	R	R	R	S	14	15-17	18	40
Amoxicillin	25	S	R	R	R	R	13	14-17	18	20
Deoxycycline	30	S	S	S	R	R	14	15-18	19	60
Erythromycin	15	S	R	R	I	R	13	14-22	23	20
Colistin sulphate	25	S	R	R	R	R	8	9-10	11	20
Novobiomycin	30	I	I	S	I	R	17	18-21	22	20
Oxolinic acid	2	S	S	S	I	R	14	15-17	18	60
Gentamycin	10	R	R	R	R	R	12	13-14	15	0
Polymyxin B	30	S	R	R	R	R	8	9-11	12	20

1(*F. indicum*), 2 (*E. meningoseptica* (kidney), 3 (*E. meningoseptica*(skin), 4 (*C. gleum*), 5 (*C. indologenes*).

Table 2. Comparison of antimicrobial activities of crude methanolic extract of *E. agallocha* on bacterial growth.

Bacteria	Concentration						
	100 mg/ml	300 mg/ml	500 mg/ml	Flumequine		Methanol	
C. gleum	15.67^{fg}	18.67^{cde}	23.33^{b}	26.67	a	0	h
C. indologenes	16.67^{def}	18.67^{cde}	18.67^{cde}	25.19	a	0	h
E. meningosepticum (kidney)	18.67^{cde}	20.33^{c}	23.33^{b}	25.67	a	0	h
E. meningosepticum (skin)	20.33^{c}	18.67^{cde}	23.67^{b}	27.33	a	0	h
F. indicum	14.00^{g}	14.00^{g}	16.33^{efg}	27.33	a	0	h
L.S.D (least significant difference)			2.265				

Different letter show significant differences ($p< 0.05$).

mg/ml concentration but the result revealed there are significant difference ($P<0.05$) as compared to the mean inhibition zone of flumequine (Figure 1).

The present result was confirmed by using disc diffusion assay and showed significant difference ($P<0.05$) in compareson with antibiotics Flumequine and disc of *E. agallocha* methanol extract against individual test bacteria (Figure 2).

The antimicrobial activity of the extracts was quantitatively assessed by determining the MIC and MBC, respectively. The lowest MIC and MBC values for the *E. agallocha* methanol extracts on test bacteria were 3.12 and 6.25 mg/ml (Table 3).

The toxicity of brine shrimp was done dependently on the mortality data from concentration of 50 to 0.08 (mg/ml). It was analyzed by SPSS version 16.0 to obtain the 50% lethal concentration (LC_{50}). The LC_{50} value was calculated by probit method which showed 94.19 mg/ml (Figure 3).

DISSCUSION

Antimicrobial susceptibility data on *Chryseobacterium* spp. remain very limited, since this pathogen has been rarely isolated from clinical specimens (Fraser and Jorgensen, 1997). *Chryseobacterium* spp. is recognized to demonstrate resistance towards aminoglycosides,

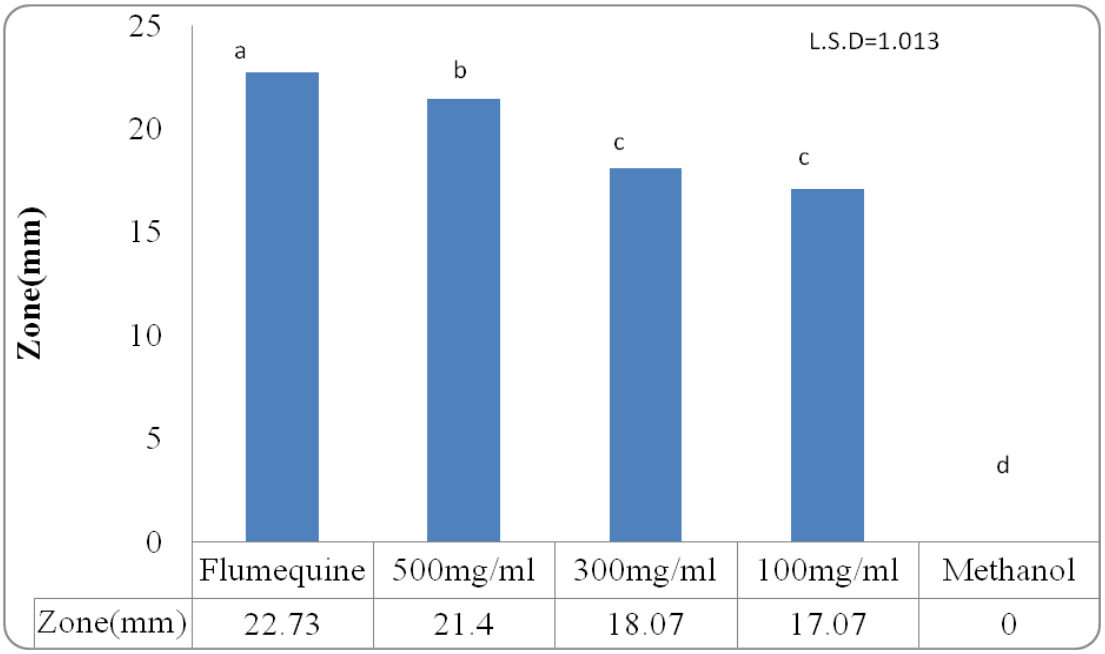

Figure 1. Mean inhibition zone of different concentration of crude methanolic extract of *E. agallocha* and antibiotic. Different letter show significant differences ($p<0.05$).

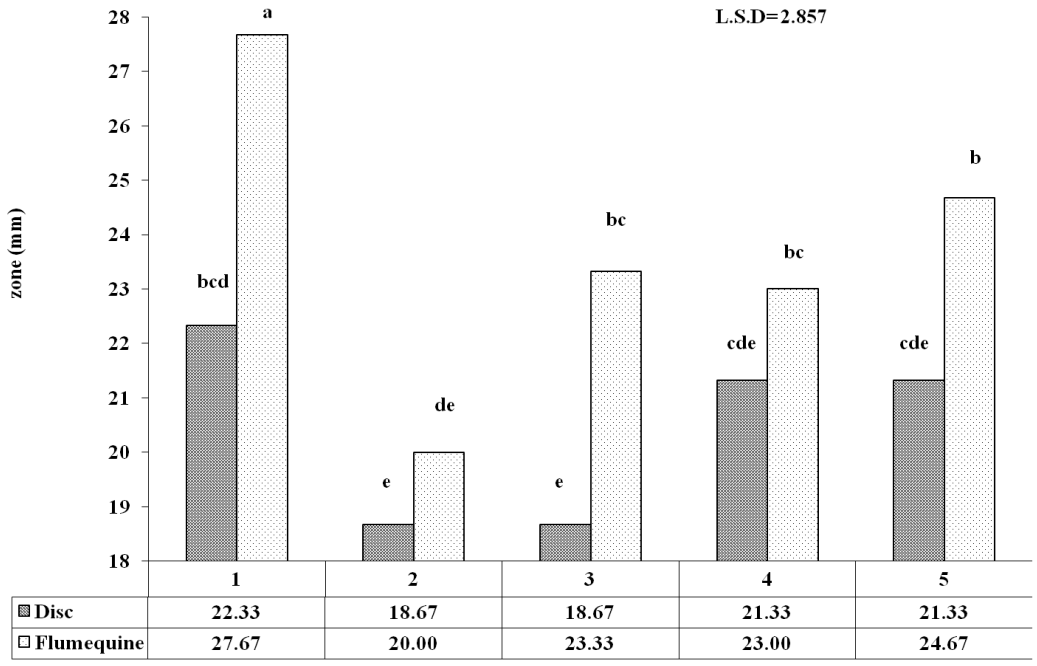

Figure 2. Comparison of disc and antibiotics using Disc Diffusion Assay on the mean inhibition zone of bacterial group. 1 (*F. indicum*), 2 (*E. meningoseptica* (kidney), 3 (*E. meningoseptica* (skin), 4 (*C. gleum*), 5 (*C. indologenes*). Different letters show significant differences ($p<0.05$).

tetracyclines, erythromycin, and chloramphenicol (Hsueh et al., 1997). Therefore, we used this type of bacteria in our study. In another study, Chang (1997) reported more than 90% of *Flavobacterium* spp. isolates showed resistance to amino-glycosides, glycopeptides and macrolides. However, the authors suggested for treating infections

Table 3. MIC, MBC values of active extract on test bacteria (mg/ml).

Bacteria	MIC	MBC	MBC/MIC
F. indicum	6.25[a]	6.25[a]	1.05[b]
E. meningoseptica (kidney)	6.25[a]	6.25[a]	0.99[b]
E. meningoseptica (skin)	6.25[a]	6.33[a]	1.01[b]
C. gleum	6.25[a]	6.25[a]	1.05[b]
C. indologenes	3.12[b]	6.25[a]	2.00[a]
L.S.D (least significant difference)	0.45	0.47	0.12

Different litter show significant differences ($p<0.05$).

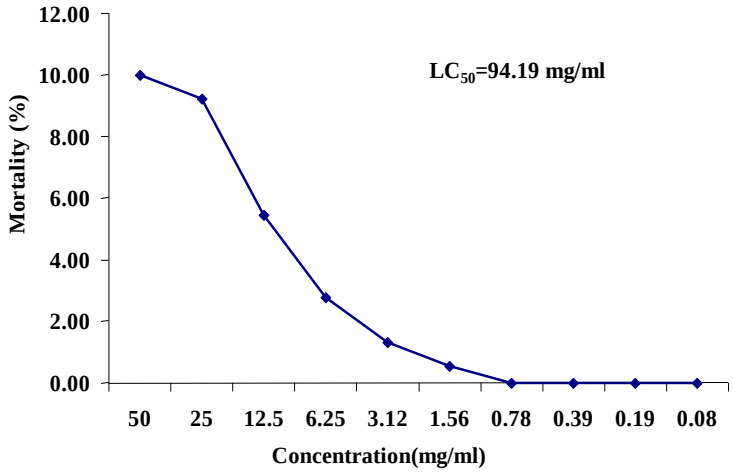

Figure 3. Brine shrimp lethality of *E. agallocha* against *A. salina.*

caused by *Chryseobacterium* spp, that the determination of the MIC for each individual isolate is mandatory.

The antimicrobial susceptibility test performed in our experiment showed that all isolates appeared to be resistant to Nitrofurantion, Gentamycin and Neomycin and sensitive to Flumequine. Although, majority of isolates demonstrated a high level of bacterial resistance towards antibiotics in the present study this finding is in agreement with Maraki (2009) indicating similarities among isolates might be due to the close genetic relatedness.

Products come from a new source of antibacterial agents becoming popular in covering the basic health needs against infective microorganisms. In the meantime, applications of herbs as biomedicine are becoming more important in disease treatment in aquaculture sectors (Citarasu et al., 2010). Mangroves are wide spread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coast lines. They have also been found to contain biologically active antiviral, antibacterial and antifungal compounds (Ravikumar et al., 2009). *Excoecaria agallocha* extract is gaining enormous interest as an alternative to antibiotics. Although, the result from present study showed Flumequine antibiotic had the highest significant effect on bacteria growth which was formally used as drug of choice in the treatment of infection caused by *Flavobacterium* spp. (Sano et al., 1998) but still the value of inhibition zone of *E. agallocha* methanolic extract at concentration 500 (mg/ml) was more closely effective to the flumequine antibiotic.

C. meningosepticum was usually resistant to multiple antibiotics. Otherwise, it was susceptible to fluoroquinolones and trimethoprim-sulfamethoxazole, andvancomycin (Ozkalay et al., 2006). These resistance phenoltypes could be explained by the presence of beta-lactamases, including extended-spectrum beta-lactamases and metallo-beta-lactamases (Vessillier et al., 2002). Previous research on *E. meningoseptica* reported was highly resistant to aminoglycosides, tetracyclines, chloramphenicol, erythromycin, clindamycin and teicoplanin (Hoque et al., 2001; Tekerekoglu et al., 2003; Lin et al., 2004).The antimicrobial resistance mechanisms of *Chryseobacterium* species are still unknown. Efflux pump systems have been established in many bacteria that have shown multiple drug resistances. These systems are accountable for the active and nonspecific removal of foreign substances from the cell, which includes antimicrobial substances (Michel et al., 2005).

Based on disc diffusion results, the antimicrobial activity

of *E. agallocha* methanolic leaves crude extract are in agreement with the result of Suryati and Hala et al. (2002) that the biological activity test of mangrove *E. agallocha* crude extract against *Vibrio mimicus* showed inhibitory activity of 10.35 ± 0.05 mm at 10 µL and 12.6 ± 0.05 mm at 20 µL using diffusion agar methods. Our finding is compatible with previous report of Kumar (2009) who reported that mangrove *E. agallocha* leaf extracts showed maximum inhibitory activity of 18 mm against *Streptoccocus aureus.* The diameters of the zone of inhibition shown by *E. agallocha* methanolic leaves crude extract against the pathogenic bacteria are similar to the study of Chandrasekaran (2006). The present results revealed that the *E. agallocha* minimal inhibition concentration (MIC) value was 3.12 mg/ml against bacteria. This finding are in accordance with the previous study of Patra (2009) who recorded the MIC of *E. agallocha* methanolic leaves crude extract to range from 5 to 7 mg/ml against the following bacteria; *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis*, *Staphylococcus aureus, Vibrio cholera, Shigella flexneri*, *Bacillus licheniformis, Bacillus brevis* and *Escherichia coli.*

Nowadays, demand on medicinal plants has increased for new drugs due to the bacterial resistance to present drug. The low toxicity of medicinal plant play an important role in new drugs. Therefore, brine shrimp larvae have been used as a bioassay for a variety of toxic substances (Ameen et al., 2011). Based on previous study, the brine shrimp assay is a simple and useful tool for the isolation of potentially cytotoxic compounds from plant extracts (Meyer et al., 1982). The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. Our results are in agreement with study of Nusrat (2008) which claimed that the extract of mangrove plant *E. agallocha* showed considerable brine shrimp toxicity (LC_{50} = 20 mg/mL), and the variation in the BSLA results may be due to the difference in the amount and kind of cytotoxic substances such as; tannins, flavonoids or triterpenoids present in the extracts.

Recently, study on the mechanism of antimicrobial activity of plant against *Vibrio cholerae* (Sanchez et al., 2010) and *Vibrio parahemolyticus* and *Vibrio alginolyticus* (Nadirah et al., 2013) concluded that the mechanism of extracts of edible and medicinal plants occurs through the changes in membrane integrity, membrane potential, internal pH and ATP synthesis of cell bacteria and cause damage to the membrane of Vibrios exerting profound physiological changes that lead to bacterial death. Another research done by (Yamasaki et al., 2011) concluded that the potential of natural compounds may have direct inhibition on virulence gene expression in *Vibrio cholerae*. Furthermore, this inhibitory mechanism may be inhibited by the secreted cholera toxin (CT) or the growth of bacteria.

The present results are in accordance with the results of previous research that *E. agallocha* extracts are recognized for their antimicrobial activities (Subhan et al., 2008; Ravikumar et al., 2009; Thirunavukkarasu et al., 2011). The bioactivity properties of *E. agallocha* might be due to presence of a higher relative percentage of antibacterial substances. On the other hand, the present results are in accordance with the results of Agoramoorthy et al. (2007) and Ravikumar et al. (2009) that *E. agallocha* leaf extract display antimicrobial activity.

Conclusion

Based on the screening results, *E. agallocha* has showed promising antibacterial potential to combat the fish pathogenic bacteria. Determination of bioactive compound of the mangrove plants will be carried out in the near future.

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