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Investigation on antibacterial ability of acetone extract from leaves of *Avicennia alba* Blume growing in the Can Gio Mangrove Biosphere Reserve, Vietnam

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Abstract

The study aims were to investigate the antibacterial ability of *Avicennia alba* Blume leaves collected in An Nghia, Dan Xay, Long Hoa belonging to Can Gio Mangrove Biosphere Reserve, Vietnam. Sample leaf powder of *A. alba* was used to make acetone extracts. Investigation on antibacterial ability of the extracts was performed by using agar well diffusion method. The results showed that acetone extracts from *A. alba* leaves exhibited antibacterial activity against Gram (+) bacteria: *Bacillus cereus* and *Staphylococcus aureus* but no inhibitory effects on Gram (-) bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*. Antibacterial strength of extract increased with increasing extract concentration. Leaf extracts of *A. alba* growing An Nghia had higher inhibitory effect of B. cereus than two others, while leaf extracts of *A. alba* growing Dan Xay was more effectively inhibitory against *S. aureus* than the others. Only extract of *A. alba* growing An Nghia at concentrations of 900 mg mL^{-1} and 1000 mg mL^{-1} and *A. alba* growing Dan Xay at concentration of 1000 mg mL^{-1} had moderately antibacterial strength against *B. cereus* and *S. aureus*. The other tested extracts of *A. alba* had antibacterial activity at weak level.

Keywords: Acetone extract; Antibacterial activity; *Avicennia alba* Blume; Can Gio Mangrove Biosphere Reserve

1. Introduction

Avicennia alba Blume is a common mangrove plant belonging to Avicenniaceae, widely contributes in estuaries and coastal regions through the tropical and subtropical areas of the World. In Southeast Asia countries, *A. alba* is able to be found in Bangladesh, Indonesia, Malaysia, Brunei, Myanmar, Singapore, Thailand, the Philippines, and Vietnam [1, 2]. *Avicennia alba* Blume contains a variety of phytocompounds such as alkaloids, flavonoids, glycosides, steroids, tannins and phenol compounds. In traditional medicine, *A. alba* is used against different types of conditions such as ulcers, skin diseases, contraception and snake bites, asthma, rheumatism, scabies [3].

Presently, some studies have found *A. alba* inhibited many types of pathogenic bacteria [2]. Methanol and chloroform extracts of plant *A. alba* were active against different types of Gram (+) and Gram (-) bacteria including *Streptococcus mutans*, *Lactobacillus acidophilus*, *Rhizoctonia solani*, *Pseudomonas marginales*, *Erwinia carotovora*, *Acremonium strictum* [4]. Using agar well and disc diffusion methods, the antibacterial activity of leaf and bark extracts of *A. alba* in different solvents were studied against eight Gram (+) and six Gram (-) bacteria. The acetone, benzene, chloroform, ethyl acetate, hexane and methanol leaf and bark extracts of the plant were reported to have antibacterial activity against *S. entrica*, *A. prop-tophormiae*, *A. tumefaciens*, *P. mirabilis*, *P. aerugi-nosa*, *R. rhodochrous*, *B. subtilis, P. vulgaris, S. mutans, S. aureus* and *A. faecalis*, in comparison with standard tested bacterial antibiotics [5].

In Vietnam, the genus *Avicennia* includes 3 species: *A. marina* (Forssk) Vierh., *A. oficinalis* L. and *A. alba* Blume, distributed mainly in the coastal mangrove forests, including the Can Gio Mangrove Biosphere Reserve (MBR) [6].

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However, investigation on antibacterial ability of *Avicennia* species, especially *A. alba* in Vietnam has been still limited. Therefore, to understand more about its importance in medicine field, the study was conducted with the aims of screening the antibacterial ability of acetone leaf extract from *A. alba* Blume collected from different buffer zones of Can Gio MBR, Vietnam.

2. Material and methods

2.1. Collection and Preparation of *A. alba* Blume leaves

A. alba Blume leaves were collected in three sites: An Nghia, Dan Xay and Long Hoa belonging to buffer zones 5a, 10c and 17 of Can Gio MBR and preserved according to the instructions of the Ministry of Health of Vietnam [1]. In laboratory, the leaves were washed with water to remove sand and dust, dried at room condition for one - two days and then dried in oven at 50 °C until their mass were remained constant. The dried leaves were grounded into powder preserved for further uses [7].

2.2. Preparation of acetone extract of *A. alba* Blum leaves

The *A. alba* leaf powder was soaked in a solution of 70^{o} acetone with a ratio of 1:10 (100 g of fine powder in 1000 ml of acetone). After 2 days, the mixtures were collected by filtration with filter paper. The remaining residues were further immersed with the solvent according to the above procedure, repeated 3 times. All extracts of leaf powder were dried with a Yamato Scientific RE801 rotary evaporator, at 90 rounds per minute, 130 Pa pressure and 50 °C; evaporated at room temperature. The filtrates were stored at 4 °C for further uses [7].

2.3. Preparation of bacterial suspension

Test bacteria were Gram (+) bacteria: *Bacillus cereus* and *Staphylococcus aureus* and Gram (-) bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*. The bacteria were cultured in liquid LB media and incubated on a thermostatic shaker at 37 °C, 120 rpm. After 48 hours of culture, the suspension of each bacterium was collected and measured optical density with a spectrophotometer at wave length of 600 nm and adjusted to the McFarland standard 0.5, cell density of 1.5×10^{8} CFUmL^{-1} [8]. This standard cell density was used for all later experiments.

2.4. Antibacterial activity screening

Agar well diffusion method was used to screen antibacterial potential of extracts of *A. alba* Blume leaves [9]. Test medium was sterilized solid nutrient agar (NA). Bacterial suspension was spread on surface of petri dishes containing 0.2 cm of NA thick. Using a sterile round glass pipe (Φ = 5 mm) created four wells on each medium plate. 20 µL of acetone leaf extracts with each concentration of 100 mg mL^{-1}, 200 mg mL^{-1}, 300 mg mL^{-1}, 400 mg mL^{-1}, 500 mg mL^{-1}, 600 mg mL^{-1}, 700 mg mL^{-1}, 800 mg mL^{-1}, 900 mg mL^{-1} and 1000 mg mL^{-1} were applied in wells of each medium plate, respectively. Medium plates' wells containing 20 µL of acetone 70° were used as negative controls and medium plates' wells containing 10 µL of aqueous 1.0 mg mL^{-1} solution of tetracycline or gentamicin were used as positive controls. The dishes were incubated at 5 °C for three hours and at 37 °C for one – two days.

Antibacterial activity of the extracts produced clear zones of inhibiting tested bacteria growth. The greater the diameter of the clear zone, the higher potential of antibacterial activity of the extracts. The rating scale of Manuanza's antibacterial zones was applied to measure degrees of bacterial growth inhibition of the extracts [10].

2.5. Experiment design and data analysis

Experiments were set up in RCRD type with three replicates. Extract concentration effects on bacterial growth inhibition was analysed by using one - way ANOVA. Effects of sample collection sites and concentration in combination on bacterial growth inhibition was analysed by using two-way ANOVA. Comparison of means was performed according to Duncan's test at the 5% confidence level. ANOVA analyses was carried out with the help of IBM SPSS Statistics software version 20.0.

3. Results and discussion

3.1. Antibacterial effects of acetone extract of *A. alba* Blume leaves on *Bacillus cereus*

In general, acetone extract obtained from *A. alba* Blume at three sites An Nghia, Dan Xay and Long Hoa showed antibacterial activity against *Bacillus cereus* (Table 1 and Figure 1). The antibacterial potential of extracts increased with

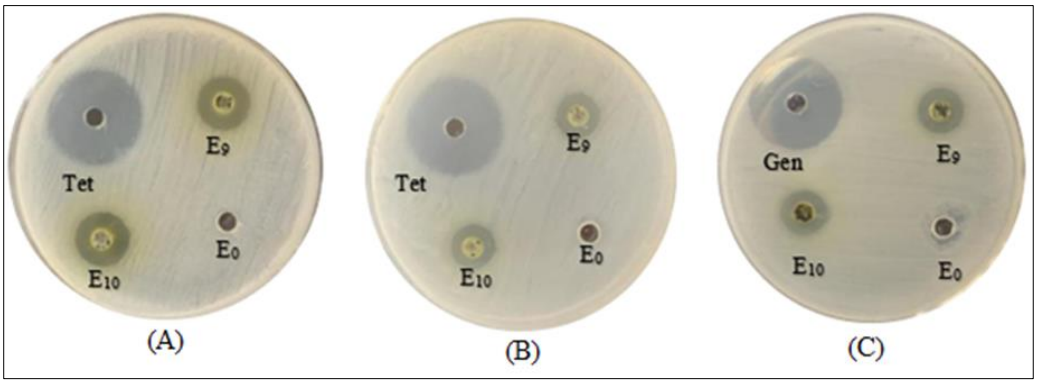
the increase in the tested concentration and assessed from weak to moderate levels. However, there was difference in the antibacterial activity of extracts of *A. alba* Blume leaves collected from three sampling sites. At the same concentration, acetone extract of *A. alba Blume* leaves collected at An Nghia had higher antibacterial activity than samples collected at Dan Xay and Long Hoa. Both extract concentration and sample collecting sites or their interaction were statistically significant effect on the diameter of bacterial inhibition zone (Sig. = .00).

Table 1 Antibacterial effects of acetone leaf extract on *Bacillus cereus*

Extract concentration	Diameter (mm) of bacterial inhibition zone		
	An Nghia	Dan Xay	Long Hoa
E_0	0.00	0.00	0.00
E_1	1.31 ± 0.05^a	1.34 ± 0.09^a	0.00
E_2	2.12 ± 0.13^b	2.2 ± 0.19^b	0.00
E_3	3.55 ± 0.16^c	2.62 ± 0.11^b	1.77 ± 0.11^a
E_4	4.36 ± 0.53^d	3.14 ± 0.14^c	2.32 ± 0.05^a
E_5	5.22 ± 0.90^e	3.72 ± 0.12^d	3.01 ± 0.19^b
E_6	6.23 ± 0.49^f	4.57 ± 0.40^e	3.97 ± 0.31^c
E_7	6.56 ± 0.22^f	5.11 ± 0.17^f	4.51 ± 0.20^c
E_8	7.95 ± 0.21^g	5.54 ± 0.07^g	5.72 ± 0.12^d
E_9	9.45 ± 0.40^h	6.27 ± 0.20^h	6.36 ± 0.82^e
E_{10}	10.31 ± 0.40^i	6.96 ± 0.18^i	7.39 ± 0.18^f
Gen	21.43 ± 0.25^j	20.69 ± 0.68^j	20.25 ± 0.69^g
Tet	22.60 ± 0.22^k	22.55 ± 0.10^k	21.90 ± 0.22^h

(E_0): negative control. (E_1): 100 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 300 mg mL^{-1}; (E_4): 400 mg mL^{-1}; (E_5): 500 mg mL^{-1}. (E_6): 600 mg mL^{-1}; (E_7): 700 mg mL^{-1}; (E_8): 800 mg mL^{-1}; (E_9): 900 mg mL^{-1}; (E_{10}): 1000 mg mL^{-1}. Gen (Gentamicin) and Tet (Tetracycline): positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test. Two extract concentration and sample collecting sites or their interaction were statistically significant effect on the diameter of bacterial inhibition zone (Sig. = .00).

For *Avicennia alba* Blume collected in An Nghia, the acetone extract from the leaves showed activity against *Baclilus cereus* at all tested concentrations from 100 mg mL^{-1} (E_1) to 1000 mg mL^{-1} (E_{10}), corresponding to the antibacterial ring diameters of 1.31 ± 0.05 mm to 10.31 ± 0.40 mm (Table 1). The inhibitory effect of the extract was weak to moderate and gradually increased with increasing concentration of the applied extract. Specifically, the applied extract concentration at 900 mg mL^{-1} (E_9) and 1000 mg mL^{-1} (E_{10}) created sterile ring diameters of 9.45 mm and 10.31 mm, respectively and reached moderately antibacterial potential against *Baclilus cereus* (Figure 1 A).



(E_0): negative control, (E_9): 900 mg mL^{-1}; (E_{10}): 1000 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; (A); An Nghia; (B): Dan Xay; (C): Long Hoa

Figure 1 Antibacterial effects of some acetone leaf extracts on *B. cereus*

Acetone extract extracted from *Avicennia alba* Blume leaves collected at Dan Xay also showed activity against *Bacillus cereus* at all tested concentrations and increased gradually from 100 mg mL^{-1} (E_1) to 1000 mg mL^{-1} (E_{10}). The antibacterial levels were only weak at corresponding to diameters from 1.34 ± 0.09 mm to 6.96 ± 0.18 mm (Table 1 and Figure 1 B). With *Avicennia alba* Blume leaves collected in Long Hoa, leaf extract had active antibacterial reactivity against *Bacilus cereus* at extract concentrations ranging from 300 mg mL^{-1} (E_3) to 1000 mg mL^{-1} (E_{10}) corresponding to diameters from 1.77 mm ± 0.11 to 7.39 mm ± 0.18 (Table 1 and Figure1 C). When extract concentrations at 100 mg mL-1 and 200 mg mL-1 were applied, no antibacterial activity was found.

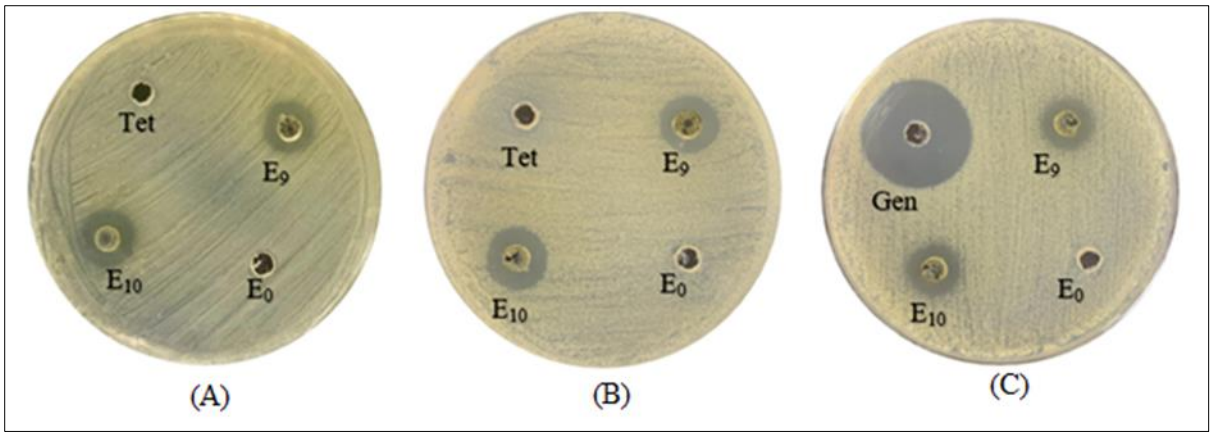
3.2. Antibacterial effects of acetone leaf extract on *Staphylococcus aureus*

Acetone extract obtained from *Avicennia alba* Blume leaves at three locations An Nghia, Dan Xay and Long Hoa showed antibacterial activity against *Staphylococcus aureus* (Table 2 and Figure 2). The antibacterial ability of extracts expressed at concentration of 200 mg mL^{-1} (E_2) and increased with rise in the tested concentration. The antibacterial degrees against *S. aureus* of extract were weak to moderate. At the same concentration, acetone extract from *Avicennia alba* Blume leaves collected at Dan Xay had higher antibacterial activity than samples collected at An Nghia and Long Hoa. The positive control (Tetracycline) had no effect on the above test strain. The extract concentration and sample collecting sites or their interaction had statistically significant effect on bacterial inhibition capacity (Sig. = .00).

Table 2 Antibacterial effect of acetone leaf extract on *Staphylococcus aureus*

Extract concentration	Diameter (mm) of bacterial inhibition zone		
	An Nghia	**Dan Xay**	**Long Hoa**
E_0	0.00	0.00	0.00
E_1	0.00	0.00	0.00
E_2	1.94 ± 0.56[a]	3.21 ± 0.11[a]	1.72 ± 0.98[a]
E_3	2.47 ± 0.23a	3.84 ± 0.07[b]	2.8 ± 0.13[b]
E_4	4.31 ± 0.35b	4.68 ± 0.08[c]	3.79 ± 0.09[c]
E_5	5.13 ± 0.39 [c]	5.15 ± 0.28[d]	4.26 ± 0.21[d]
E_6	5.48 ± 0.33[c]	6.18 ± 0.10[e]	5.49 ± 0.36[e]
E_7	6.16 ± 0.34[d]	6.50 ± 0.29[e]	5.64 ± 0.07[e]
E_8	6.33 ± 0.56[d]	7.38 ± 0.09[f]	6.32 ± 0.14[f]
E_9	7.42 ± 0.30[e]	8.31 ± 0.07[g]	7.01 ± 0.21[g]
E_{10}	8.37 ±0.20[f]	9.66 ± 0.18[h]	7.46 ± 0.33[h]
Gen	22.5± 0.34[g]	23.44 ± 0.66[i]	22.01 ± 0.71[i]
Tet	0.00	0.00	0.00

(E_0): negative control; (E_1): 100 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 300 mg mL^{-1}; (E_4): 400 mg mL^{-1}; (E_5): 500 mg mL^{-1}; (E_6): 600 mg mL^{-1}; (E_7): 700 mg mL^{-1}; (E_8): 800 mg mL^{-1}; (E_9): 900 mg mL^{-1}; (E_{10}): 1000 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test. Two extract concentration and sample collecting sites or their interaction were statistically significant effect on the diameter of bacterial inhibition zone (Sig. = .00).



(E_0): negative control; (E_9): 900 mg mL^{-1}; (E_{10}): 1000 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; (A): An Nghia; (B): Dan Xay; (C): Long Hoa.

Figure 2 Antibacterial effects of some acetone leaf extracts on *S. aureus*

Acetone extract from *Avicennia alba* Blume leaves collected in An Nghia showed antibacterial ability against *Staphylococcus aureus* at concentrations from 200 mg mL^{-1} (E_2) to 1000 mg mL^{-1} (E_{10}). Diameters of halo ring ranged from 1.94 ± 0.56 mm – 8.37 ± 0.20 mm (Table 2 and Figure 2 A). Thus, antibacterial degrees of the extracts were just equivalent to weak level. The similar results attained when studying antibacterial potential of the extracts of *A. alba* leaves from Long Hoa. The antibacterial ability only reached weak impact level because the diameters of bacterial inhibition rings ranging from 1.72 ± 0.98 mm - 7.46 ± 0.33 mm (Table 2 and Figure 2 C). While *A. alba* leaves extracts from Dan Xay caused higher diameters of bacterial inhibition rings ranging from 3.21 ± 0.11 mm - 9.66 ± 0.18 mm compared to both An Nghia and Long Hoa. However, antibacterial activity of the extracts of 100 mg mL^{-1} to 900 mg mL^{-1} was also achieved at weak level, except for 1000 mg mL^{-1} which was achieved at moderate degree with diameter of antibacterial halo zone of 9.66 mm (Table 2 and Figure 2 B).

3.3. Antibacterial effects of acetone leaf extracts on *Pseudomonas aeruginosa and Escherichia coli*

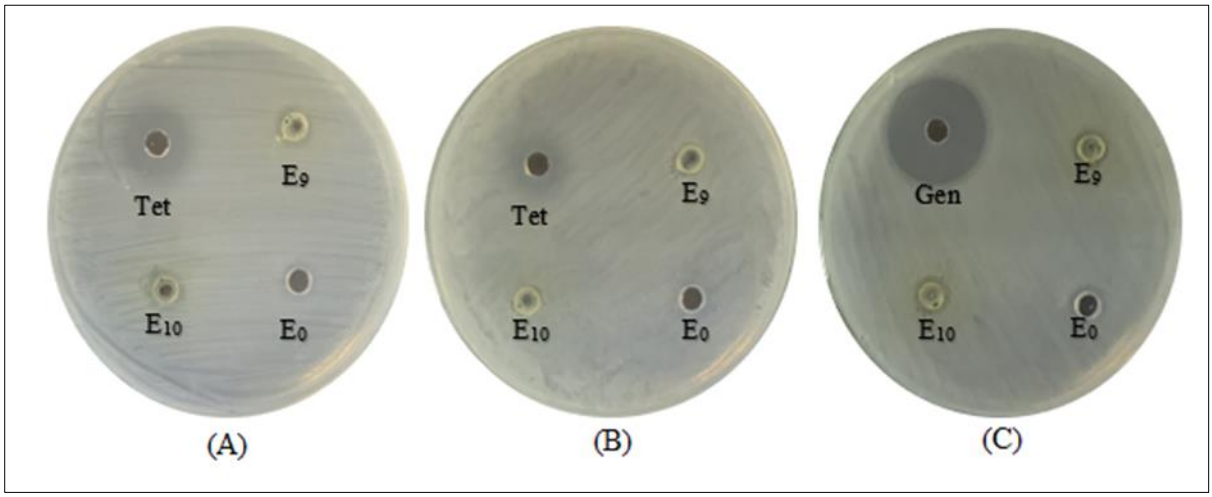
The acetone extract from *Avicennia alba* Blume leaves collected in An Nghia, Dan Xay and Long Hoa did not express antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* (Table 3 and Figure 3)

Table 3 Antibacterial effects of acetone leaf extract on *P. aeruginosa* and *E. coli*

Extract concentration	*Tested bacteria*	Diameter (mm) of bacterial inhibition zone		
		An Nghia	Dan Xay	Long Hoa
E_0	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_1	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_2	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_3	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_4	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_5	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_6	*P. aeruginosa.*	-	-	-

	E. coli	-	-	-
E_7	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_8	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_9	*P. aeruginosa.*	-	-	-
	E. coli	-	-	-
E_{10}	*P. aeruginosa.*			
	E. coli			
Gen	*P. aeruginosa.*	20.24 ± 1.82^{b}	20.15 ± 0.16^{b}	19.39 ± 0.78^{b}
	E. coli	14.66 ± 1.74^{a}	14.99 ± 0.82^{a}	14.46 ± 1.22^{a}
Tet	*P. aeruginosa.*	10.19 ± 0.93^{a}	8.51 ± 0.84^{a}	9.35 ± 1.10^{a}
	E. coli	20.27 ± 0.78^{a}	20.57 ± 0.22^{b}	21.08 ± 0.36^{b}

(E_0): negative control; (E_1): 100 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 300 mg mL^{-1}; (E_4): 400 mg mL^{-1}; (E_5): 500 mg mL^{-1}; (E_6): 600 mg mL^{-1}; (E_7): 700 mg mL^{-1}; (E_8): 800 mg mL^{-1}; (E_9): 900 mg mL^{-1}; (E_{10}): 1000 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.



(E_0): negative control. (E_9): 900 mg mL^{-1}; (E_{10}): 1000 mg mL^{-1}. Gen (Gentamicin) and Tet (Tetracycline): positive controls; (A); An Nghia; (B): Dan Xay; (C): Long Hoa.

Figure 3 Antibacterial effects of some acetone leaf extracts on *E. coli* and *P. aeruginosa*

The results of the present study were similar to some investigations on antibacterial activity of *A. alba* extract with different solvents reported. With the same concentration, the methanol extracts from *A. alba* leaves showed significant inhibitory zones against *P. aeruginosa* (antibacterial zone diameters of 3 - 9 mm), *Bacillus subtilis* (antibacterial zone diameters of 2 - 3.46 mm) and *S. aureus* (antibacterial zone diameters of 7 - 12.8 mm) while acetone extracts produced antibacterial zones with diameters of 0.73 – 3 mm, 1.33 - 2.73 mm, 0.03 - 5.76 mm against *P. aeruginosa*, *B. subtilis* and *S. aureus*, respectively [11]. Purba et al. [12] investigated antibacterial activity of *A. alba* leaf extracts with methanol against *P. aeruginosa, A. salmonicida* and *S. aureus*. The results showed that leaf extracts had the highest inhibitory activity at concentration of 100% with average antibacterial ring diameters of 4.37 - 5.13 mm. The highest inhibition ability was against *S. aureus* with antibacterial ring diameters of 5.13 mm and the lowest inhibition potential was against *A. salmonicida* with antibacterial ring diameters of 4.37 mm. The lowest inhibition against tested bacteria was at concentration of 12.5% with average antibacterial zone ring diameters of 0.45 - 0.67 mm. The strength of antibacterial activity of *A. alba* leaf extract was weak level. Vadlapudi [13] studied antimicrobial activity of plant extracts of *A. alba*

against some important pathogens the results showed that chloroform and methanol extracts exhibited different degrees of inhibition against tested bacteria. Chloroform extracts of *A. alba* showed antimicrobial activity against tested microbial strains with diameters of antimicrobial zones varied from 9 mm to 17 mm while diameters of antimicrobial zones of methanol extracts arranged from 11 to 28 mm at 100 mg mL^{-1} concentration.

Another study of antimicrobial activities of some mangrove plants from Sundarbans Estuarine Regions of India found that methanol, ethanol, ethyl acetate, hexane and chloroform extracts of *A. alba* leaves inhibited Gram (+) *Bacillus subtilis*, *Bacillus coagulans* but had no effects on Gram (-) *Escherichia coli*, *Enterobacter sakazakii* [14]. The difference in sensitivity to antibiotics between Gram (+) and Gram (-) bacteria is caused by morphological difference in their cell wall. The outer phospholipid membrane of Gram (-) bacteria possesses the structural lipopolysaccharide components which is a protective and a unique feature distinguishing Gram (-) bacteria from Gram-positive bacteria. This structure prevents lipophilic solutes from penetrating into the cell. Moreover, the outer consists of proteins called the outer membrane proteins forming porins allowing small molecules like amino acids and small saccharide. Most antibiotics have to go through the outer membrane to get their targets. The outer membrane helps Gram (-) bacteria resist to a wide range of antibiotics. The Gram (+) bacteria lack an outer membrane containing lipopolysaccharide, so are more susceptible to the drugs [15].

4. Conclusion

In this research, acetone extracts from *A. alba* leaves collected in An Nghia, Dan Xay and Long Hoa in Can Gio Mangrove Biospheres Reverse exhibited antibacterial activity against Gram (+) bacteria: *B. cereus, S. aureus* but no inhibitory effects on Gram (-) bacteria: *P. aeruginosa* and *E. coli* by using agar well diffusion method. Antibacterial strength of extracts increased with increase in extract concentration. Leaf extracts of *A. alba* growing An Nghia had higher inhibitory effect of *B. cereus* than two others, while leaf extracts of *A. alba* growing Dan Xay was more effectively inhibitory against *S. aureus* than the others. Only extract of *A. alba* growing An Nghia at concentrations of 900 mg mL^{-1} and 1000 mg mL^{-1} and growing Dan Xay at concentration of 1000 mg mL^{-1} had moderately antibacterial strength against *B. cereus* and *S. aureus*. The other tested extracts of *A. alba* leaves had antibacterial activity at weak level.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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