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Abstract. Inner Ambon bay, Ambon Island, Maluku Province, is a good place for mangrove to growth. Some mangroves have been known as a source of germplasm that has antimicrobial properties. This research goals to study the antibacterial activity of various extract of *Rhizophora apiculata* from the coast of the Inner Ambon Bay, against several pathogenic bacteria. The experimental design was complete random design with two factors, firstly, the type of extract solvent was extract namely methanol, ethyl acetate and n-hexane, and the second four strains of bacteria consist of Gram positive strains: *Bacillus cereus* and Gram negative strains: *Escherichia coli, Salmonella typhi Staphylococcus aureus.*The highest antibacterial activity was resulted by ethyl acetate extracts with an average value of inhibition zone was 18.64±3.88b mm, then followed by methanol extract 15.02±5.17b mm and n-hexane extract 8.48±1.25a mm. The strongest extract against the bacterial pathogen was ethyl acetate extract. The most susceptible bacteria to the three extracts tested with an inhibition zone was (18.02±7.49b) mm, followed by *B. cereus*, *E. coli* and *S. aureus* with the inhibition zones were (13.48±4.50a) mm, (12.94±4.69a) mm and (11.74±3.90a) mm, respectively.

1. Introduction

Inner Ambon bay, Ambon Island, Maluku Province, is a good place for mangrove to growth. Mangrove in Ambon Bay are generally dominated by 4 genera namely *Avicennia officinalis, Bruguiera cylindric, Rhizophora apiculata,* and *Sonneratia alba*. *Rhizophora apiculata* and *Sonneratia alba* were the most abundant species with a height of trees can reach 30 m with a diameter reaching > 41 cm [1].

The primary purpose of the mangroves is to prevent the coastal from abrasion so that it stays in balance and beautiful. Mangroves are also home to a variety of wildlife such as various of mollusks, echinoderms, fish, crustaceans, birds, epiphytic plants and other various of biota. Mangroves are also known as spawning areas, nursery ground, and feeding ground for several types of marine biota. Besides protecting the land, mangrove have functionate as a wildlife conservation area and sedimentation prevention. Mangroves can also be used for commercial purposes such as the source of wood for exported, leather for tannins, charcoal, paper materials, medicines and food [2]. Beside above mention, some species of mangrove have known potentially to use as medicinal purpose. Some mangroves have been known as a source of germplasm that has antimicrobial properties. Many mangrove plants especially *Rhizophoraceae* species show a strong antimicrobial properties [3]. It has been reported that some extracts of *Rhizophora* species have wide varieties of pharmacological properties like antifungal, antibacterial, antiseptic, anti-inflammatory, antiulcer and efficacy in wound healing [4].

[5] reported the extract of *R. apiculata* and *Bruguiera gymnorrhiza* from the coast of Burmanallah, South Andaman, India, has an antimicrobial and anti-fungal activity against *Aspergillus niger, Klebsiella pneumoniae, Escherichia coli, Shigella flexneri, Salmonella typhi* and it has been explored the active fraction named tannin. While [6] reported that antibacterial isolates from endophytic fungi from

mangrove *R. apiculata* L. and *Bruguiera gymnorrhiza* L. (L.) Lamk showed an antibacterial properties against *Salmonella typhi.*

According to [7] revealed that n-hexane and chloroform extracts of the leaves of *Rhizophora mucronata* showed a great inhibition against *Bacillus subtilis,Staphylococcus aureus, Candida albicans, Aspergillus fumigatus* and *Aspergillus niger* and a moderate inhibition against *Bacillus subtilis, Staphylococcus aureus, Candida albicans, Aspergillus fumigatus* and *Aspergillus niger*. [8] reported the results of chemical and biological characterization studies of leaves, roots, where the best extracts produced by leaves came from secondary metabolites of flavonoids and tannins. [9] reported the roots, stems, and leaves endophytic antibacterials *of Rhizophora mucronata Lam* come from the coastal area of Lombok Island (Gili Sulat) had an antibacterial activity from moderate (8-12mm) to strong (> 12mm) against *B. cereus* and *P. aeruginosa*, while [10] reported the presence of antibacterial activity of endophytic fungi of *Rhizophora mucronata* from Segara anakan has a moderate to low activity against *Escherichia coli* and *Staphylococcus ureus*.

[11] reported Actinomycetes isolate originated from *Rhizophora apiculata rhizosphere*, were able to inhibit the growth of *Escherichia coli,Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus sp*. [12] reported there was an antibacterial activity of mangrove leaf extract, *Rhizophora mucronata, Sonneratia alba and Exoecariaagallocha* from Chorao Island, Goa against human pathogenic bacteria *Staphylococcus aureus, Streptococcus sp, Salmonella typi, Proteus vulgaris* and *Proteus mirabilis*, phytochemical components found in human pathogenic bacteria *Staphylococcus aureus, Streptococcus sp, Salmonella typi, Proteus vulgari*s and *Proteus mirabilis*. saponins, glycosides, tannins, flavonoids, phenols and essential oils in mangrove leaves.

Because of the great antimicrobial potential of mangroves in various places in Indonesia and even in the world, it has inspired writers to study the antibacterial properties of mangrove leaf extract (*Rhizopora apiculata*) originated from Inner Ambon Bay, Maluku Indonesia. This research goals to study the antibacterial activity of various extract of *Rhizophora apiculata* from the coast of the Inner Ambon Bay, against several pathogenic bacteria.

2. Materials and Method

2.1. Description of the study sites

Location of sampling encompass the coastal area of the Inner Ambon Bay waters, Ambon Island, Maluku Province (Figure 1), where there many species of manggrove growth, especially *R. apiculata*. In Ambon Bay, the forest of mangrove were an important ecosystem supporting the development and protection of Ambon City.

2.2. Sample extraction preparation

The leaf of *R. apiculata* were carried to the Laboratory of Fisheries Products Technology, Pattimura University Ambon. The mangrove leaves were shade dried and powdered, then as much as 50 g leaf powdered soaked with a solution of methanol, ethyl acetate and n-hexane as much as 200 ml each, then macerated for 48 hours. The extract was then filtered by using Whatman paper filter No. 41. The filtrate is concentrated by using a vacuum evaporator at 50ºC. The extract was weighed, stored in a bottle in the refrigerator at 5ºC and the yield percentage was calculated using the following formula: Yield extract (Yield)% = R / S 100, where R = extracted weight by leaf residue and S = sample weight.

Figure 1. Location of Inner Ambon Bay, of District of Baguala Ambon Bay, Indonesia.

2.3. Preparation of bacterial inoculums

All tested bacteria strains were subcultured overnight on nutrient agar Mueller-Hilton at 37°C. Using 5 ml of sterile salt water, each of the bacteria tested was harvested and measured by using spectrophotometer at 580 µm and diluted to reach a decent cell count of 10^7 CFU / ml.

2.4. Preparation of paper disk

Paper disk used for screening the antibacterial activity were Whatmann No.1 filter paper 6 mm diameter size. The extract of *R. apiculata* mixed with 1 ml of 5% Dimethyl sulfoxide (DMSO). The discs were impregnated by n-hexane, ethyl acetate and methanol extract then screen their antibacterial activity. The positive control used was amoxillin (100 mg/ml) and the blind control was 5% DMSO.

2.5. Antibacterial assay

Method proposed by [13] was used to determine the antibacterial activity of *R. apiculata*. Muller Hinton agar was poured to the dish as much as 20 ml and it allowed to jell, for the use in susceptibility test against pathogenic bacteria. Bacterial inoculums suspension as much as 0.1 ml was poured and spreaded uniformly. Extract impregnated paper dish were placed on the surface of the Muller Hinton Agar. Positive controlled using an amoxillin (100 mg/ml) and the 5% DMSO was used as a blind control. The Petri dish were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each test in these experiments was repeated three times for concordance.

2.6. Statistical analysis

The experimental design was complete random design with two factors, firstly, the type of extract solvent, namely: N-Hexane, Ethyl acetate and Methanol, and secondly, the types of pathogenic bacteria consist of: *E. coli*, *B cereus*, *S. thipy* and *S. aureus* the experimental replicated 3 times. Then the variance was analyzed to determine the influenced between treatments. If there is an influenced then continued by the Least Significant Difference test (LSD) to determine the differences between treatments.

3. Result and Discussion

The yield of n-hexane, ethyl acetate and methanol extract of *R. apiculata*is is shown in Table 1. The yield was range of 5.98 – 29.86%. The highest yield was found in methanol extract with an amount of 29.86%, followed by ethyl acetate extract and n hexane extract 6.36 and 5.98% respectively.

Table 1. The yield of leaf extract of *R. apiculata.*

Solvent	Yield %
n-Hexane	5.98 %
Ethyl Acetate	6.36 %
Methanol	29.86 %

The results of antibacterial test in Table 2 showed that the inhibitory zones of extracts (n-hexane, ethyl acetate and methanol) of *R. apiculata* leaf was in the range of 7.77 ± 0.23 to 23.72 ± 2.94 mm. [14] had classified the zone of inhibition based on their antibacterial strength as follows: <5 mm is called a weak inhibition power, 5-10 mm a moderate inhibition power and 10-20 mm strong inhibition power. According to that classification, the extract of *R. apiculata* was classified from moderate to strong power.

Analysis of variance resulted that the treatment of extract types (n-hexane, ethyl acetate and methanol) and types of bacteria *E. coli* (B1), *B. cereus* (B2), *S. thypi* (B3) and *S. aureus* (B4) have very significantly influenced the zone of inhibition (at p> 0.05). This means that there is at least one type of extract or bacterial treatment that has a zone of inhibition different from the others.

Table 2. Zone of inhibition of leaf extract of *R. apiculata* against pathogen bacterias.

Bacteria strain	Zone of Inhibition (mm)			The average zone of inhibition of Bacteria (mm)	The zone inhibition of Positive controlled (Amoxillin) (mm)
	n-Hexane	Ethyl acetate	Methanol		
E. coli	7.77±0.23	17.60±3.73	13.47±0.98	12.94±4.69a	15.1
B. cereus	8.03±0.06	16.73±1.88	15.67±3.17	13.48±4.50a	17.9
S. thypi	10.12±1.75	23.72±2.94	20.23±7.95	18.02±7.49b	54.6
S. aureus	8.00±0.00	16.50±2.08	10.73±0.40	11.74±3.90a	22.4
The average of Zone of Inhibition of extracts (mm)	8.48±1.25a	18.64±3.88b	15.02±5.17b		

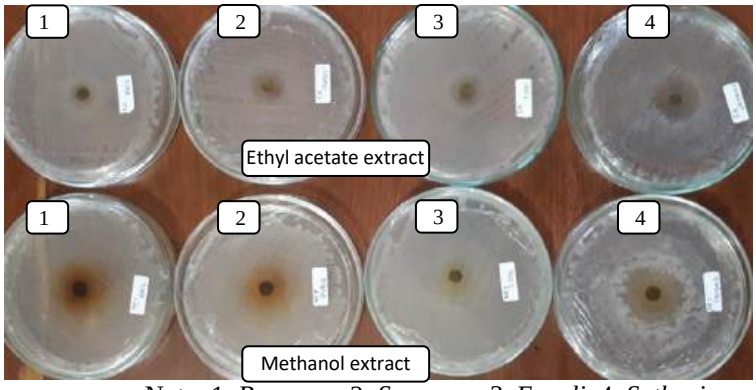
Note: Numbers followed by the same letters indicate no difference between treatments.

The result of LSD test of extracts treated (n-Hexane, Ethyl acetate and Methanol) against the 4 species bacteria tested showed that there was a difference between n-hexane extract with methanol extract and ethyl acetate extract. The highest antibacterial activity was resulted by ethyl acetate extracts with an average value of inhibition zone was 18.64±3.88b mm, then followed by methanol extract 15.02±5.17b mm and n-hexane extract 8.48±1.25a mm. The strongest extract against the bacterial pathogen was ethyl acetate extract, statistically it was not significantly different from methanol extract but with n-hexane extract. [15] reported that extracts of n-hexane, ethyl acetate and methanol of *R. apiculata* against *S. aureus* produced inhibition zones 8 ± 0.5, 10 ± 0.3 and 11 ± 03 respectively. There was not a significant difference between the zone of inhibitory of n-hexane extract and the zone inhibitory of methanol, but the inhibition zone of ethyl acetate was very different, between 10 ± 0.3 with 16.50 ± 2.08 mm from the results of this study. [16] reported that the inhibition zone of methanol extract

of *R. apiculata* leaves came from the waters of Tanjung Api-Api, South Sumatra against *S. aureus* and *E.coli* bacteria were 14 mm and 21.9 mm, respectively. [17] reported methanol extracts from *R. stylosa* zone of inhibition against *E. coli* and *S. aureus* bacteria 9.7 ± 0.6 mm and 8.7 ± 0.3 mm respectively. Secondary metabolites such as alkaloids, phenolics, steroids, terpenoids, tannins, flavonoids, saponins and quinons have been characterized from mangrove plants for toxicology and pharmacological purposes [18][19][20].

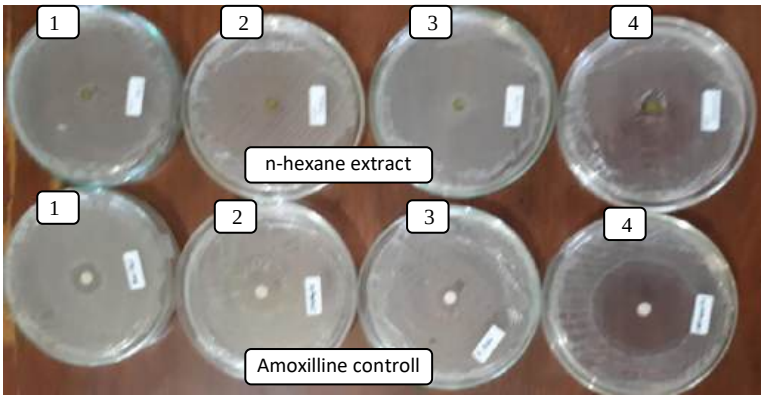
The result of LSD test on the bacterial species treated showed that the zone of inhibition of bacteria *S. thypi* was significantly different from the others, where the *S. thypi* bacteria was the most susceptible bacteria to the three extracts tested with an inhibition zone was (18.02±7.49b) mm, followed by *B. cereus*, *E. coli* and *S. aureus* with the inhibition zones were (13.48±4.50a) mm, (12.94±4.69a) mm and (11.74±3.90a) mm, respectively (Table 2.). The results of this study indicated that extractof *R. apiculata* leaf is very effective to inhibit the growth of bacteria *S. thypi*. In contrast to the results of [21] *S. typhi* was the most resistant strain to plant extracts followed by *E. coli* and *S. aureus*.

The results of the positive control amoxillin against 4 species bacteria tested showed that when compared to the extract of *R. apiculata* leafs, an amoxillin only effective in killing *S. thypi*, while compared to other bacteria there is seems not significantly differences, especially for *E. coli* extract, ethyl acetate it could be seen that *R. apiculata* ekstract more effective than amoxillin (Table 2, Figures 2 and 3). This means that because of *E. coli* has a resistant possibility to amoxillin, so that, an optimization and more in-depth studies of *R. apiculata* extract against various types of bacteria need to be done.



Note: 1. *B. cereus*, 2. *S. aureus*, 3. *E. coli*, 4. *S. thypi*

Figure 2. Zone of inhibition of ethyl acetate, and methanol extract against *B. cereus*, *S. aureus*, *E. coli*, and *S. thypi* bacterial.



Note: 1. *B. cereus*, 2. *S. aureus*, 3. *E. coli*, 4. *S. thypi*

Figure 3. Zone of inhibition of n-hexane extract and amoxilline control against *B. cereus*, *S. aureus*, *E. coli*, and *S. thypi* bacterial.

In addition to research on mangroves, many marine plants and animals that have a potential antibacterial. [22] reported traditional plant extracts have an antibacterial activity against pathogenic bacteria of *Tilapia sp*. [23] reported the sponge associated fungi showed inhibition zone against various types of vibriosis bacterial of shrimp growth disturbing.

According to [24], the seaweed extract of *Phorphyra sp* shows a good inhibition against 3 kinds of bacteria namely *E. coli, Staphylococcus aureus* and *S. thypi*, they mentioned the inhibition zones of methanol, ethyl acetate and n-hexane extracts from *Phorphyra sp* ranged from 6.21 - 15.41 mm, the largest inhibition zone was 15.41 mm resulted by ethyl acetate extract against *E. coli*.

[25] reported the active components of methanol extracts of *R. mucronata* leaf were tannins, saponins, flavonoids, phenol hydroquinone, triterpenoids, and alkaloids. Methanol extract has the greatest antibacterial activity with inhibition zones ranging from 3-12 mm.

The inhibitory zone of ethyl acetate extract from the seaweed species *Sargassum sp* was the most active extract against *P. aeruginosa* and *M. luteus* bacteria [26], while the methanol extract *Sargassum sp* was the most active extract against *S. epidermidis* bacteria. The most dominant bioactive compound from seaweed extract is steroid.

4. Conclusion

The results of this study indicated that extractof *R. apiculata* leaf is very effective to inhibit the growth of bacteria *S. thypi*, and the highest antibacterial activity was resulted by ethyl acetate extracts followed by methanol extract and n-hexane extract. An amoxillin only effective in killing *S. thypi*, while compared to other bacteria there is not significantly differences, especially for *E. coli* extract. Ethyl acetate ekstract of *R. apiculata* more effective than amoxillin. It needs more effort and in-depth studies of *R. apiculata* extract against various types of bacteria, in order to find the new and best antibacterial as a substitute for resistant antibacterial.

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