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Screening of Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Mangrove Plant *Rhizophora mucronata* Lam

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Abstract

Research on the screening antimicrobial and cytotoxic activities of ethyl acetate extract of endophytic fungi from leaves, bark and roots of mangrove *Rhizophora mucronata* Lam that derived from Jambak Beach, West Sumatera has been done. Isolation of endophytic fungi using direct planting with growing medium Sabouraud Dextrose Agar (SDA) and obtained 14 isolates of the fungus. The isolates were cultivated on rice medium for 3-8 weeks, and extracted using ethyl acetate solvent. The 14 ethyl acetate extracts were screened phytochemicals and tested for antimicrobial activity by disc method against test bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Cytotoxic activity of the extracts was tested by MTT assay using T47D cell and Vero cell. The results of this study indicate that nine (64.3%) isolates of endophytic fungi can inhibit the growth of test bacteria and fungi. Four isolates of endophytic fungi from the roots, four from the bark, and one from the leaves of mangrove *Rhizophora mucronata* Lam had antimicrobial activity. While 12 extracts (85.7%) were cytotoxic (cell viability < 50%) against T47D cells. This study concluded that the endophytic fungi of *Rhizophora mucronata* Lam can be developed as a new source of antimicrobial and anticancer compounds.

Keywords: Endophytic fungi, mangrove, *Rhizophora mucronata* Lam, antimicrobial activity, cytotoxic activity

1. Introduction

Mangrove forests are typical forest types along the coast or river estuaries that are affected by tides. The mangrove forest ecosystem is complex, because its ecosystem is influenced by mangrove vegetation as well as the habitat of various animals and aquatic biota (Nybakken, 1992).

Endophytic fungus is a fungus contained in plant tissue systems that do not cause symptoms of disease in host plants. Endophytic fungus can produce potent antibacterial compounds as biological control agents (Purwanto, 2011). Endophytic fungi can be isolated from plant roots, stems and leaves (Dwilestari *et al*., 2015).

Antimicrobials are among the most commonly used drugs. The unavoidable consequence of the widespread use of antimicrobial compounds is the emergence of antibiotic-resistant pathogens, leading to an increase in the need for new drugs as well as the rising cost of treatment (Hardman *et al*., 2007). Increased use of antibiotics in overcoming various diseases caused by bacteria began to cause new problems, especially since most of the antibacterial ingredients used are harmful chemicals and are not safe for health (Rashid, 2012).

Research on the potential of mangrove has been widely promoted, this is because the various bioactive compounds produced by endophytic fungi that have unique structures and have a high potential for exploitation

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(Saad *et al*., 2012). *Rhizophora mucronata* Lam is called the black mangroves (Orwa *et al*., 2009). The skin, roots, leaves, fruits and flowers of *Rhizophora mucronata* Lam have been used in traditional medicine in South Asian countries including India to treat diabetes, diarrhoea, hepatitis, inflammation, wounds and ulcers (Sur *et al*., 2015). Trichoderma endophytic fungus derived from *Rhizophora mucronata* Lam as a potential source for the discovery of antioxidant compounds (Kandasamy & Kathiresan, 2014). Endophytic fungi originating from *Rhizophora mucronata* are also able to inhibit the growth of bacteria that cause diarrhoea (Tarman *et al*., 2013). The ethanol extract from *Rhizophora mucronata* leaves can inhibit the growth of *Bacillus subtilis*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* (Kusuma *et al*., 2011). Setiawan's research proves that *Rhizophora apiculata* Bl has an antibacterial activity, in which four ethyl acetate fractions and two butyl alcohol fractions have antibacterial activity against *Escherichia coli* test bacteria, four ethyl acetate fractions and one butyl alcohol fraction having antibacterial activity against *Staphylococcus aureus* test bacteria.
Researches related to potential of mangrove as a producer of medicinal compounds have been much carried out. In continuation of our work on natural substances of terrestrial and marine origins (Handayani and Artasasta, 2017; Handayani and Aminah, 2017; Handayani, *et al*., 2016, Handayani, *et al*., 2015), we examined the endophytic fungi from the mangrove *S. griffithi* Kurz in producing antibacterial compounds (Handayani, *et al*., 2017). Based on these potentials, continuous research on screening for antimicrobial and cytotoxic activities of other *Sonneratia* species, such as *S. alba* Sm has been performed (Handayani *et* al., 2018). Screenings of antimicrobial and cytotoxic activities were performed on fungal extract isolated from leaves, bark, and roots of *S. alba* Sm collected from West Sumatra, Indonesia. Thus, research needs to be done about the potential of *Rhizophora mucronata* Lam such as antimicrobial and cytotoxic activities of endophytic fungal metabolites isolated from leaf, bark and root of *Rhizophora mucronata* Lam grown in West Sumatra, Indonesia.

2. Materials and Methods

2.1 Equipment and materials

Standard laboratory equipment were needed among other scales (Precise), cotton (Paramedic), gauze (Paramedic), spirit light, Erlenmeyer flask (Pyrex), hotplate (Chimeric), use needle, incubator (Mamet), paper disc (Advantech), rotary evaporator (Hahnvapors model HS-2361N5), laminar air flow (Innotech), autoclave (Model 25x-2 Wisconsin Aluminium Foundry Co., Inc.), TLC silica gel 60 F254 (Merck), vortex (Vortex Mixer model VM-1000) and tools that support the research.
Materials were used in the research among other *Rhizophora mucronata* Lam, distilled water (Brataco), tissue, 70% ethanol (Brataco), ethyl acetate (Merck), 0.9% sodium chloride (Otsuka), spirit (Brataco), sodium hypochlorite (Brataco), tetracycline hydrochloride (PT Phapros), ketoconazole (PT Kimia Farma), dimethylsulfoxide (DMSO), nutrient agar (NA) (Merck), sabouraud dextrose agar (SDA) (Merck), and rice, ferric chloride (Merck), hydrochloric acid (Merck), bismuth sub nitrate (Merck), potassium iodide (Merck), nitric acid (Merck), magnesium powder (Merck), acetic acid anhydrous (Merck), sulphuric acid (Merck), chloroform (Merck), suspension of test bacteria consisting of *Escherichia coli* and *Staphylococcus aureus*, and *Candida albicans* test fungi.

2.2 Isolation, cultivation and extraction of secondary metabolites from endophytic fungi

The stages of the research method for isolation and cultivation of endophytic fungi has been carried out as written in the research that we have done before (Handayani, *et al*., 2017; 2018).

2.3 Identification of secondary metabolite content

2.3.1 Alkaloid Test

The extract sample was dissolved in 2 ml of hydrochloric acid, heated 5 minutes and filtered. The obtained filtrate plus 2-3 drops of Dragendorff reagent (bismuth sub nitrate-potassium iodide solution). The presence of alkaloid compounds is indicated by red sediment (Tiwari, *et al*., 2011).

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2.3.2 Flavonoid Test
A total of 2 mL of extract is added with enough hot water, then boiled for 5 minutes then filtered. The filtrate of 5 mL was added 0.05 mg of Mg powder and 1 mL of concentrated HCl, then shaken vigorously. The positive test is shown by the formation of red, yellow or orange.

2.3.3 Triterpenoid and Steroid Test
The testing is using Lieberman-Burchard reagent (acetic acid anhydrous-sulphuric acid). A total of 1 mL of sample plus 1 ml of chloroform, then added five drops of anhydrous acetic acid and 1 mL of concentrated sulphuric acid by the tube. The presence of steroid compounds is indicated by the formation of dark blue or greenish black and terpenoid formation of purple (Sreedhar & Christy, 2015).

2.3.4 Phenolic Test
A total of 2 mL of sample was dissolved in 10 mL distilled water, heated 5 minutes and filtered. The added filtrate is added 4-5 drops of $FeCl_3$. The presence of phenol is indicated by the formation of dark blue or dark green.

2.4 Screening of antimicrobial activity
Screening of antimicrobial activity of the ethyl acetate extracts of endophytic fungi was performed by the disk diffusion method (Bauer *et al.*, 1966). The testing was done against pathogenic bacterial and fungal such as *Staphylococcus aureus, Escherichia coli*, and *Candida albicans.* The ethyl acetate extract was prepared to the concentration (in DMSO) 5, 3 and 1 mg/mL. Tetracycline HCl and Ketoconazole as a reference compound were prepared at 300 µg/ml and 20 mg/ml in distilled water, respectively. Each 10 µL of above reference and extracts were dropped onto 6 mm sterile paper disk on the surface of the medium containing bacteria and fungi test strain. Each plate was incubated at 37 ºC for 24 hours for bacteria and at a temperature of 25 ºC - 27 ºC for 5-7 days for fungi. Inhibition zones were measured and recorded. Screening of antibacterial activity was experimented in triplicate, and mean value ± standard deviation was also determined (Handayani *et al.*, 2018).

2.5 Screening of cytotoxic activity
The cell line of T47D (human ductal breast epithelial tumour) and Vero (normal cell) have been prepared for cytotoxic assay using MTT. All cell lines were obtained from Laboratory of Parasitology at UGM. T47D was cultured in RPMI 1650 and Vero was cultured in M199 Medium. All cells were sub cultured after mild trypsinization with trypsin-EDTA (Sigma-Aldrich, USA), and then determined the cell number and viability. The cells were seeds in 96-well plates at density $6x10^3$ cells/well in 100 µL medium and incubated overnight. All media were supplemented with 10% with foetal bovine serum (Gibco) and streptomycin and penicillin (2%, Sigma-Aldrich, USA). The cell line was kept at 37ºC, 98% relative humidity with 5% CO_2 atmosphere (Handayani *at al.*, 2018).
A stock solution was prepared by dissolving the samples in DMSO and was given 100.000 ppm concentration. Cells that had been incubated 24 hours, then divided into several groups, namely treatment, positive control, cell control and media control (blank). Removed medium and washed using PBS sterile which each well was added 100 µL PBS. Then, 100 µL of each material (extract) added to each well with one concentration (100 ppm). As control positive was used with doxorubicin. Then it was incubated for 24 hours in an incubator at 37 ºC, 5% CO_2 (Handayani *et al.*, 2018)
Cells that had been treated and incubated 24 hours later dumped throughout the medium and washed using sterile PBS. Then in each well was added 100 mL of MTT (5mg/mL) followed by 4-hour incubation in an incubator at 37ºC, 5% CO_2. To each well was added 100µl of 10% SDS to dissolve the formazan crystals formed and incubated one night at room temperature. The plates were then read by ELISA reader at 540 nm (Permanasari *et al.*, 2016).
Per cent of cell viability then was calculated by the equation as follow:

$$\%\,\text{Cell viability} = \frac{\text{OD of treatment} - \text{OD of blank}}{\text{OD of comtrol} - \text{OD of blank}} \times 100$$

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3. Results and Discussion

3.1 Isolation, cultivation and extraction of secondary metabolites from endophytic fungi

Isolation of antimicrobial active compound from endophytic fungus symbiotic with mangrove *Rhizophora mucronata* Lam begins with *Rhizophora mucronata* Lam sampling at Pasir Jambak Beach, West Sumatra. Plant samples were identified first in Herbarium ANDALAS, Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University, Padang City, West Sumatera. This sample specimen is stored in the ANDALAS Herbarium. The mangrove plant used in this study is shown in Figure 1. Weight of ethyl acetate extracts of endophytic fungal isolated from leaves, bark and roots of *Rhizophora mucronata* Lam were presented in Table 1.

Figure 1: Mangrove Plant *Rhizophora mucronata* Lam

Table 1: Ethyl acetate extracts of endophytic fungal isolates from leaves, bark and roots of mangrove plants *Rhizophora mucronata* Lam

No	Sample Code	Weight of ethyl acetate extract (mg)
1	$RmDa_1$	161
2	$RmDa_2$	821
3	$RmKB_1$	1425
4	$RmKB_2$	204
5	$RmKB_3$	67
6	$RmKB_4$	504
7	$RmKB_5$	225
8	$RmKB_6$	472

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9	$RmAk_1$	581
10	$RmAk_2$	342
11	$RmAk_3$	481
12	$RmAk_4$	212
13	$RmAk_5$	80
14	$RmAk_6$	194

3.2. Identification of secondary metabolite content
Results of secondary metabolite test from ethyl acetate extracts of *Rhizophora mucronata* Lam were showed in Table 2 as follow.

Table 2: Secondary metabolite test results from ethyl acetate extract of endophytic fungi

Sample Code	Phytochemical Test	Reagent	Result	Conclusion
$RmKB_5$	Alkaloid	Dragendorff	No orange formation	Negative
	Flavonoid	Mg + HCl concentrated	No red / orange formation	Negative
	Triterpenoid and steroid	Lieberman-Burchard	No green-blue / purple formation	Negative
	Phenolic	$FeCl_3$	The formation of a greenish black colour	Positive
$RmAk_3$	Alkaloid	Dragendorff	The formation of red sediment	Positive
	Flavonoid	Mg + HCl concentrated	No red / orange formation	Negative
	Triterpenoid and steroid	Lieberman-Burchard	No green-blue / purple formation	Negative
	Phenolic	$FeCl_3$	No greenish black colour formation	Negative
$RmAk_5$	Alkaloid	Dragendorff	No orange formation	Negative
	Flavonoid	Mg + HCl concentrated	The formation of red colour	Positive
	Triterpenoid and steroid	Lieberman-Burchard	No green-blue / purple formation	Negative
	Phenolic	$FeCl_3$	No greenish black colour formation	Negative

3.3 Testing of antimicrobial activity
Morphological colonies and antimicrobial activity test of endophytic fungi from *Rhizophora mucronata* Lam are shown in Tables 3 and 4.

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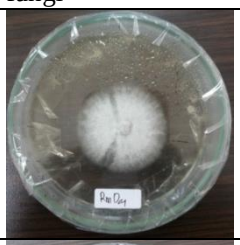
Table 3: Morphological colonies of endophytic fungal isolates from *Rhizophora mucronata* Lam

No	Isolates of endophytic fungi	Sample code	Morphology
1		$RmDa_1$	White colour like cotton, smooth and thick, bumpy edge
2		$RmDa_2$	Colour green as smooth cotton, yellow surface, flat edge
3		$RmKB_1$	Colour grey, white surface, bumpy edge
4		$RmKB_2$	White with black spots spread evenly, bumpy edges
5		$RmKB_3$	Pink like smooth cotton, white surface, bumpy edge
6		$RmKB_4$	White, furry surface, flat edge

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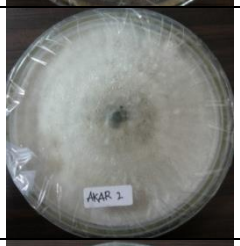
7		$RmKB_5$	White, thin cotton-shaped surface
8		$RmKB_6$	Colour beige, cotton-shaped, white surface, bumpy edge
9		$RmAk_1$	Red, middle grey with pink cylindrical edges
10		$RmAk_2$	White, cotton-shaped, flat edges
11		$RmAk_3$	White, greenish surface, cotton-shaped, flat edges
12		$RmAk_4$	White with the centre of grey, cotton-shaped on its surface

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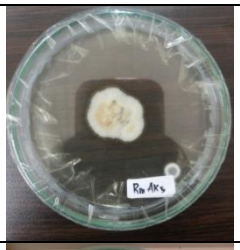
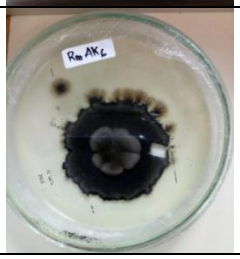
13		$RmAk_5$	Colour yellow, cotton-shaped, wavy white edges
14		$RmAk_6$	Black grey, black-edged striped edges, centred mounted, velvety shaped

Table 4: Result of antimicrobial activity test of endophytic fungal isolates from *Rhizophora mucronata* Lam

No	Sample code	Extract	Concentration	Average inhibitory diameter (mm) (twice repeat)		
				SA*	EC*	CA*
1	$RmAk_1$	Ethyl acetate	1 %	8.92	6.93	12
			3 %	12.35	10.76	15.30
			5 %	14	13.77	20.25
2	$RmAk_2$	Ethyl acetate	1 %	6	5.50	-
			3 %	6.40	5.80	-
			5 %	6.65	6.50	-
3	$RmAk_3$	Ethyl acetate	1 %	7.15	6.30	18.20
			3 %	9.40	10.10	27
			5 %	12.58	12.40	27.60
4	$RmAk_4$	Ethyl acetate	1 %	-	-	-
			3 %	-	-	-
			5 %	-	-	-
5	$RmAk_5$	Ethyl acetate	1 %	11.97	14.56	14.30
			3 %	12.68	15	15
			5 %	17.24	15.80	15.50
6	$RmAk_6$	Ethyl acetate	1 %	7.27	8.64	-
			3 %	12.25	11	9.40
			5 %	15.90	12	13.50
7	$RmKB_1$	Ethyl acetate	1 %	-	-	8
			3 %	7	-	8
			5 %	7.05	-	8.55
8	$RmKB_2$	Ethyl acetate	1 %	11.27	11.49	9
			3 %	13.55	13.80	13.9
			5 %	15.78	15.49	15
9	$RmKB_3$	Ethyl acetate	1 %	6.20	8	-
			3 %	11	12.55	11
			5 %	13.50	13.13	15

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10	$RmKB_4$	Ethyl acetate	1 %	6.63	8.35	-
			3 %	8.78	9.63	8
			5 %	11.43	14.13	11
11	$RmKB_5$	Ethyl acetate	1 %	12.60	10.13	8
			3 %	14.98	16.13	12.60
			5 %	16.58	16.65	14
12	$RmKB_6$	Ethyl acetate	1 %	8.65	7.43	-
			3 %	8.88	12	-
			5 %	11.30	14.88	-

*Note: SA = *Staphylococcus aureus*; EC = *Escherichia coli*; CA = *Candida albicans*

The *Rhizophora mucronata* Lam samples were sterilized stratified prior to planting on the agar medium in a petri dish. The sample was washed with running water for 10 minutes and cut to a size of approximately 1 cm. The sample piece was sterilized surface by stirring it into 70% alcohol for 1 minute, putting it into sodium hypochlorite (NaOCl 5.3%) for 5 minutes, and dipping it again into 70% alcohol for 30 seconds. The sterile sample of bark and root shoots is then split into two parts and the leaves are scraped off the surface, and then placed in an inner way facing the solidified SDA media inside the petri dish. This sterilization process is carried out in the laminar air flow (Kumala & Fitri, 2008).

Sterilization in stratified is done so that the fungus that grows around the sample is a fungus that only comes from the sample (Purwanto, 2005). The growth of endophytic fungi incubated at 27-30 ºC began to appear on days 5-7, and then the fungus was observed daily growth.

The purification process is carried out by removing any fungus that grows around the sample on the new medium. The macroscopic colony observations were performed on the basis of criteria: colour, surface, and the edge of the colony. The same criteria are considered to be the same isolates, and the criteria showing differences are considered to be different isolates (Kumala & Fitri, 2008). This is done to facilitate the identification stage.

Isolation of endophytic fungi from leaves, roots and bark of *Rhizophora mucronata* Lam resulted in fourteen isolates of endophytic fungi, where the fungal isolates were coded according to the organ of fungal origin. Endophytic fungi that have become a single isolate are first cultivated in the rice medium for about 3-8 weeks or the rice is completely covered by endophytic fungi. The growth of endophytic fungi in rice can be due to favourable environmental conditions and the availability of nutrients in rice such as starch (80-85%), protein, vitamins, minerals and water (Pakki, 2005).

During the cultivation period there are several factors that influence the growth of microorganisms in the fermentation medium, including the size of the inoculum, the nutrients contained in the substrate, moisture content, pH, and growth temperature. The growth of microorganisms in cultures begins when inoculation begins in the lag phase of adaptation, followed by a logarithmic phase (exponential phase), which is the period of growth, followed by a stationary phase where vegetative growth stops and then enters the phase of death. During the stationary phase, some microorganism cultures can synthesize certain compounds known as secondary metabolites. It is also known that not all microorganisms can form secondary metabolites. Examples of secondary metabolites are antibiotics, steroids, kojic acid, polymers and polymers (Djamaan, 2011).

The cultivated endophytic fungal isolates were then extracted using 100 mL ethyl acetate solvent with 3 repetitions. From this process the solvent is evaporated using a rotary evaporator resulting in a viscous extract. From the extraction results obtained by weight of ethyl acetate extract ranged from 67 mg to 1425 mg.

The antimicrobial activity test of this ethyl acetate extract used three concentrations of 1%, 3%, and 5%. From the extract that has been made various concentrations and then tested its antimicrobial activity by using the technique of "paper disc diffusion technique" that is by extracted piping as much as 10 µL then dripped on sterile disc paper then placed on medium containing test microbes and clear areas indicate the growth barrier microorganisms.

In this study, positive controls were used as a comparison of tetracycline with a concentration of 0.3% and ketoconazole with 2% concentration. Positive controls for bacteria exhibited a 28 mm inhibit zone, and positive

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controls for fungi exhibited a 26 mm inhibit zone. Dimethylsulfoxide (DMSO) as a negative control does not provide inhibitory zones of bacteria and test fungi. The use of dimethylsulphoxide (DMSO) was reported to have no effect on cell proliferation so as not to interfere with the observed results of antibacterial activity testing by the agar diffusion method, besides dimethylsulfoxide (DMSO) is one solvent which can dissolve almost all polar and non-polar compounds (Handayani et al., 2009; Maryati & Sutrisna, 2007).
Antimicrobial activity of ethyl acetate extract of endophytic fungus was tested against Gram-positive bacterial pathogen *Staphylococcus aureus*, Gram negative bacterial bacteria *Escherichia coli* and *Candida albicans* pathogenic fungi. The results of the antimicrobial activity test showed that eleven extracts of ethyl acetate from endophytic fungus were active against *Staphylococcus aureus* and ten extracts were active against *Escherichia coli* and ten extracts were active against *Candida albicans*. While there are nine extracts that are active against the three microbes test.

3.4 Testing of cytotoxic activity
The cytotoxic activity screening of all extracts on T47D and Vero cell lines was evaluated as presented in Figure 2. The results revealed that 12 out of 14 extracts tested (85.7%) were cytotoxic and exhibited a percentage of viability cell value ≤ 50%. Of these, 9 extracts (64.3%) from all extracts were found to have a percentage of viability cell value ≤ 20%. Fungi extract with the lowest percentage of viability (≤ 20%) especially against the T47D cancer cells are extracted DoXo, RmKb1, RmKb2, RmKb4, RmKB5, RmKB6, RmAK1, RmAK2 and RmAK4 (Figure 2). The percentage of viability extracts against T47D cancer cells is highly variable and some of which are not toxic to the normal cells (Vero).

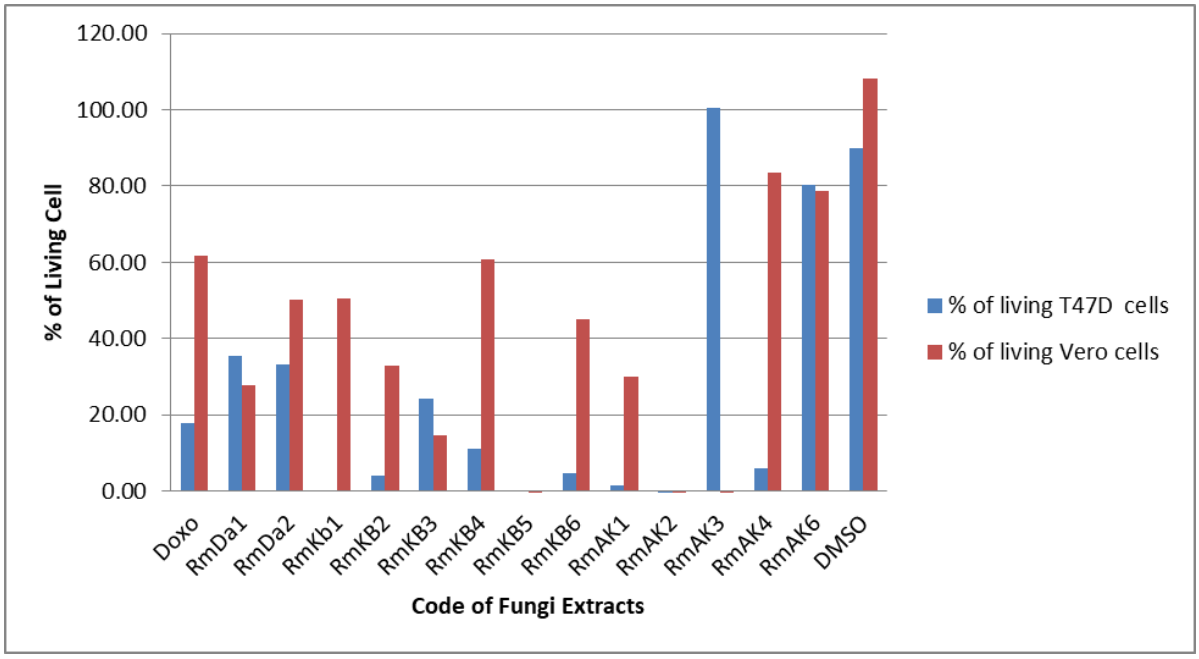

Figure 2: Cytotoxic activity of endophytic fungi extracts of *Rhizophora mucronata* on T47D and Vero cells

4. Conclusion
The results showed that 14 endophytic fungal isolates were obtained from mangrove *Rhizophora mucronata* Lam. Screening of antimicrobial activity of ethyl acetate extract from endophytic fungi from mangrove *Rhizophora mucronata* Lam showed that as much as 64.3% of ethyl acetate extract of endophytic fungus had activity on test microbes of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Four endophytic fungal isolates derived from roots, four from bark, and one from the leaves of *Rhizophora mucronata* Lam

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mangrove have antimicrobial activity. Amount of 12 extracts (85.7%) were cytotoxic (cell viability < 50%) against T47D cells. This study concluded that the endophytic fungi of *Rhizophora mucronata* Lam can be developed as a new source of antimicrobial and anticancer compounds.

Conflict of Interests

The authors declare that no conflict of interest is associated with this work.

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References

[1] Djamaan, A. (2011). Konsep Produksi Biopolimer P (3HB) dan P (3HB-ko-3HV) secara Fermentasi, Padang: Andalas University Press

[2] Dwilestari, Awaloei, H., Posangi, J., & Bara, R. (2015). Uji Efek Antibakteri Jamur Endofit pada Daun Mangrove *Sonneratia alba* terhadap Bakteri Uji *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal e-Biomedik*, *3*(1). 394–398.

[3] Handayani, D., Rivai, H., Mulyana, R., Suharti, N., Rasyid, R., & Hertiani, T. (2018). Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Mangrove Plant *Sonneratia alba* Sm. *Journal of Applied Pharmaceutical Science*, 8(02), 49-53.

[4] Handayani, D., Rivai, H., Hutabarat, M., & Rasyid, R. (2017). Antibacterial Activity of Endophytic Fungi Isolated from Mangrove Plant *Sonneratia griffithii* Kurz. *Journal of Applied Pharmaceutical Science*, *7*(04), 209-212.

[5] Handayani, D., & Artasasta, M. A. (2017). Antibacterial and cytotoxic activities screening of symbiotic fungi extract isolated from marine sponge *Neopetrosia chaliniformis* AR-01. *Journal of Applied Pharmaceutical Science*, *7*(05), 066-069.

[6] Handayani, D., & Aminah, I. (2017). Antibacterial and cytotoxic activities of ethyl acetate extract of symbiotic fungi from West Sumatra marine sponge *Acanthrongylophora ingens*. *Journal of Applied Pharmaceutical Science*, *7*(02), 237-240.

[7] Handayani, D., & Ornando, R. (2016). Rustini, Antimicrobial Activity Screening of Symbiotic Fungi from Marine Sponge *Petrosia nigrans* collected from South Coast of West Sumatera Indonesia. *International Journal of Pharmacognosy and Phytochemical research*, *8*(4), 623-625.

[8] Handayani, D., Ahdinur, R. F., & Rustini, R. (2015). Antimicrobial activity of endophytic fungi from marine Sponge *Haliclona fascigera*. *Journal of Applied Pharmaceutical Science*, *5*(10), 154-156.

[9] Harborne, J. B. (1987). Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan Edisi Kedua. Penerjemah: Padamawinata, K. & Soediro, I., *Bandung: Penerbit ITB*.

[10] Hardman, J. G., Lee, E. L. & Alfred, G. G. (2007). *Goodman & Gilman Dasar Farmakologi Terapi* (Edisi 10). Penerjemah: Aisyah, C., Elviana, E., Syarief, W. R., Hanif, A. & Manurung, J. Jakarta: Penerbit Buku Kedokteran EGC.

[11] Ibrahim, M. N. M. (2014). *Uji aktivitas antimikroba ekstrak n-heksan daun ketepeng cina (Cassia alata linn) terhadap jamur dan bakteri*. (Skripsi). Gorontalo: Universitas Negeri Gorontalo.

[12] Kandasamy, S., & Kandasamy, K. (2014). Antioxidant activity of the mangrove endophytic fungus *Trichoderma* sp. *Journal Coastal Life Medicine*, *2*(7), 566-570.

[13] Kumala, S., & Fitri, N. A. (2008). Penapisan Kapang Endofit Ranting Kayu Meranti Merah (*Shorea balangeran* Korth.) sebagai Penghasil Enzim Xilanase. *Jurnal Ilmu Kefarmasian Indonesia*, *6*(1), 1-6.

[14] Kusuma, S., Kumar, P. A., & Boopalan, K. (2011). Potent antimicrobial activity of *Rhizophora mucronata*. *Journal of Ecobiotechnology*, *3*(11), 40-41.

[15] Mulyani, Y., Bachtiar, E., & Agung, M. U. K. (2013). Peranan Senyawa Metabolit Sekunder Tumbuhan Mangrove terhadap Infeksi Bakteri *Aeromonas hydrophila* pada Ikan Mas (*Cyprinus carpio* L.). *Jurnal Akuatika*, *4*(1), 1-9.

[16] Nybakken, J. W. (1992). Biologi Laut: Suatu Pendekatan Ekologis. Diterjemahkan oleh HM Eidman, Koesoebiono, DG Bengen, M. Hutomo dan S. Subarjo. Jakarta: PT Gramedia Pustaka Utama.

[17] Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Simons, A. (2009). Agroforestry database: a tree species reference and selection guide version 4.0. *World Agroforestry Centre ICRAF, Nairobi, KE.* (http://www.worldagroforestry.org/sites/treedatabase.asp).

[18] Pakki, S. (2005). Epidemiologi dan pengendalian penyakit bercak daun (Helminthosporium sp.) pada tanaman jagung. *Jurnal Litbang Pertanian*, *24*(3), 101.

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[19] Pelczar, M. J. & Chan, E. C. S. (2006). *Dasar-dasar mikrobiologi*, (Edisi I). Penerjemah: Ratna, S. H., Teja I., Sutarmi, S. T. & Sri, L. A. Jakarta: Penerbit Universitas Indonesia.
[20] Permanasari, P., Hertiani, T., & Yuswanto, A. (2016). Immunomodulatory Effect of massoia bark extract and the cytotoxicity activity against fibroblast and Vero cells in vitro. *Int J Pharm Clin Res*, *2016*(8), 326-330.
[21] Prihanto, A. A. (2012). Perbandingan Aktivitas Antibakteri *Penicillium notatum* ATCC 28089 dengan *Penicillium* sp. R1m yang Diisolasi dari Mangrove *Sonneratia caseolaris*. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *15*(1), 66-70.
[22] Purwanto. (2005). *Isolasi dan identifikasi senyawa penghambat polimerisasi hem dari fungi endofit tanaman Artemisia annua* L. (Tesis). Yogyakarta: Universitas Gadjah Mada.
[23] Rasyid, A. (2012). Identification of Secondary Metabolites Compounds, Antibacterial and Antioxidant Activities on the Methanol Extract of Sea Cucumber *Stichopus hermanii*. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, *4*(2), 360 – 368.
[24] Saad, S., Taher, M., Susanti, D., Qaralleh, H., & Awang, A. F. I. B. (2012). In vitro antimicrobial activity of mangrove plant *Sonneratia alba*. *Asian Pacific journal of tropical biomedicine*, *2*(6), 427-429.
[25] Sastrohamidjojo, H. (1991). *Kromatografi*. Yogyakarta: Universitas Gadjah Mada.
[26] Sreedhar, S., & Christy, P. H. (2015). Phytochemical analysis of *Rhizophora mucronata*. *Scrutiny International Research Journal of Agriculture, Plant Biotechnology and Bio Products (SIRJ-APBBP)*, *2*(2), 40-43.
[27] Sur, T. K., Hazra, A. K., Bhattacharyya, D., & Hazra, A. (2015). Antiradical and antidiabetic properties of standardized extract of Sunderban mangrove *Rhizophora mucronata*. *Pharmacognosy magazine*, *11*(42), 389 – 394.
[28] Suriawiria, U. (1985). *Pengantar mikrobiologi umum*. Bandung: Angkasa Bandung.
[29] Tarman, K., Safitri, D., & Setyaningsih, I. (2013). Endophytic fungi isolated from *Rhizophora mucronata* and their antibacterial activity. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, *8*(2), 69-76.
[30] Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), 98-106.
[31] Xiaoling, C., Xiaoli, L., Shining, Z., Junping, G., Shuiping, W., Xiaoming, L., ... & Yongcheng, L. (2010). Cytotoxic and topoisomerase I inhibitory activities from extracts of endophytic fungi isolated from mangrove plants in Zhuhai, China. *Journal of Ecology and The Natural Environment*, *2*(2), 017-024.

