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Abstract. Many infectious diseases caused by *Escherichia coli* and *Staphylococcus aureus* which turned into a resistant pathogen. A symbiotic fungi of marine sponge *Axinella* sp., *Aspergillus sydowii* from the waters of Riung, East Nusa Tenggara, Indonesia showed antibacterial activity, cultured on the four media, MEB (ST), Noni Juice Media (MG), avocado leaves media (AL), and Soursop leaves media (SR). The symbiotic fungi was cultured for 14 days on each media. The largest weight of symbiotic fungi biomass on ST media 138,95gr and at least 99,12gr of AL media. Purification of bioactive compound is carried out using separatory funnel, and column chromatography. The highest rendemen of extracts on SR media was 3,67%, while the lowest in ST media was 1,22%. The bioactive test used diffusion agar method. Fungi extracts from four mediums have bioactivity against, *E. coli* and *S. aureus*. The biggest inhibition zone obtained from the extract of MG KN-15-3-1-3, with inhibition zone 10.71mm and 10.98mm against *E. coli* and *S. aureus*.
Keywords: antibacterial, sponge, symbiotic fungi, *Aspergillus sydowii*

1. Introduction

Axinella sp. is one of marine sponge that have many biological activities [1], such as antifungi, anti-microbial, aniviral, insecticidal activity, cytotoxicity, and antioxidant [2-7]. Axinella is also source of bioactive compounds [1]. Some compounds from marine sponge *Axinella* sp. are Axinastatin, Cyclonellin, and Daminin [8-10].

Only few of them passed to the preclinical and clinical stages [11]. Supply is a major problem for drug development. Therefore, another alternative is required, by utilizing microorganisms such as fungal symbionts [12-13]. Kelecom (2001) [14] mentioned that symbiotic microorganisms like fungi symbiont produce secondary metabolites that are similar to those produced by its host. Fungi symbiont can be isolated and cultured [15].

Growth media is one of the major factors in isolating and growing microorganisms. This is because it can affect the content of compounds in these microorganisms [16-17]. Atalla et al. (2008) [18], stated that the formation of a secondary metabolite in micro-organisms will be affected by conditions and growth media.

This paper describes the effect of growth media on the biomass and antibacterial activity of symbiotic fungi of marine sponge *Axinella* sp., *Aspergillus sydowii* from the waters of Riung, East Nusa Tenggara, Indonesia.

2. Materials and methods

2.1. Sample collection

Symbiotic Fungi of Marine Sponge *Axinella* sp., *Aspergillus sydowii* was sample collection of Natural Product Laboratory, Faculty od Fisheris and Marine Science, Diponegoro University, Semarang.

2.2. Antibacterial screening

The MDR bacteria, *Escherichia coli* and *Staphylococcus aureus*, was obtained from Microbiology Laboratory, Kariadi Hospital, Semarang. Each MDR bacteria was cultured in the logarithmic phase, be mixed in Zobell soft agar medium (1% v/v), and poured on the agar surface that previously inoculated with fungi symbiont. The inhition zone shown after 24 h incubation and the isolates active as antibacterial [19]. (the data were not shown in this paper).

2.3. Isolation and Cultured of Fungi

Isolation and cultured of fungi symbiont *Aspergillus sydowii* conducted with modification methods of Trianto *et.al.* [20]. There are four mediums that used for mass cultured, Malt Extract Broth/MEB (ST), Noni Juice Media (MG), Avocado leaves media (AL), and Soursop leaves media (SR). Symbiotic fungi *Aspergillus sydowii* was cultured on growth media for 7 days or until the maximum growth at ambient temperature.

2.4. Extraction

The media was filtered to obtain the micellium that was extracted with methanol at room temperature. The solvent was filtered using filter paper and then dried with rotary evaporator [21].

2.5. Purification

2.5.1. Separatory funnel

The solvents used in the separatory funnel method are water and ethyl acetate with a ratio of 1: 3, and dried with rotary evaporator [22].

2.5.2. Thin layer chromatography (TLC)

Thin layer chromatography were performed on silica-gel 60 TLC plate and developed using different solvent systems to obtain best separation. There were two methods to visualize the compound. Using UV-light and *vanilin sulfuric acid* that sprayed at plat TLC and heated on hot plate until the color changes on the TLC plate [23].

2.5.3. Open Column chromatography (OCC)

Purification by column chromatography conducted with modification methods of Jafarzade *et al.* [24]. The solvents used were n-hexane (1), chloroform (2), chloroform: ethyl acetate (1: 1) (3), ethyl acetate (4), and ethyl acetate: methanol (2:1) (5).

2.6. Bioassay

Bioassay based on the method proposed by Safaeian *et al.* [25] with slight modification. The extracts were tested against *S.aureus* and *E.coli* using difussiun agar method at concentration 100 and 50 µg/disk with two times repeated. The pathogenic bacteria were inoculated by spread method on MEA media, after 30 minutes incubation, the paper disks contained the extract were placed on the agar surfaced.

3. Result and discussion

Fungi of marine sponge, *Aspergillus sydowii*, code KN-15-3 is isolate collection of Natural Product Laboratory, Faculty of Fisheris and Marine Science, Diponegoro University, Semarang. The isolate was be morphology and molecular identify. Further studies were done after antagonistic test (The process was not performed in this paper).

The isolate fungi was isolated dan cultured on four liquid mediums, Malt Extract Broth/MEB (ST), Noni Juice Media (MG), Avocado Leaves Media (AL), and Soursop Leaves Media (SR) for 14 days (on optimum growth). Then be extracted with methanol.

The result of isolation and mass cultured, known that the largest wet weight of fungi symbionts were 138.95gr on ST medium, and the least were 99.12gr on AL medium with filtrate volume 750mL. The largest rendement extract from SR medium (Table 1).

Table 1. Crude extracts of symbiontic fungi on four growth media

Isolate	Media	Weight wet of fungi (gr)	Filtrate volume (mL)	Crude extract (gr)	Rendement (%)
KN 15-3	ST	138.95	750	1.707	1.23
	AL	99.12		3.122	3.15
	SR	103.07		3.783	3.67
	MG	126.59		4.244	3.35

The crude extract were separated again with separatory funnel. From the separation, fraction of ethyl acetate AL medium had the highest amount, and the lowest was ST medium (Table 2). The result of separation was checked with TLC plate (Figure 1), and bioassay test result on Figure 2.

Table 2. Separatory funnel (fraction extract)

Isolate	Media	Crude extract (gr)	Fractions	Weight of fraction extract (gr)
KN-15-3	ST	1.7068	Ethyl acetate	0.5384
			Water	1.1684
	AL	3.1221	Ethyl acetate	0.9569
			Water	2.1652
	SR	3.7834	Ethyl acetate	1.9192
			Water	1.8642
	MG	4.2442	Ethyl acetate	0.8461
			Water	3.3981

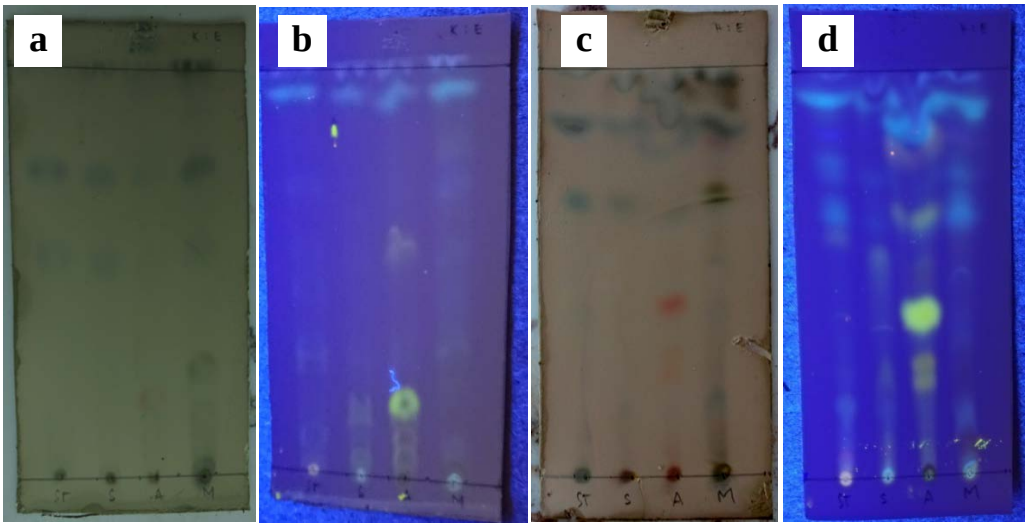

Figure 1. Visualization of TLC, vanilin sulfuric and UV rays (left: ST, SR, AL,and MG)
(a, b) Kloroform : Etil Asetat (4 : 1); (c, d) N-Heksan : Etil Asetat (1 : 1).

The visualization of TLC plate showed the spot change color and and the spots formed on each media were almost identical. The Spot colors on the TLC plate are dominated by blue, green, and red (Figure 1). The blue green spot on the TLC plate indicated fraction containing steroid and red spot were indicating terpenoid compounds [26].

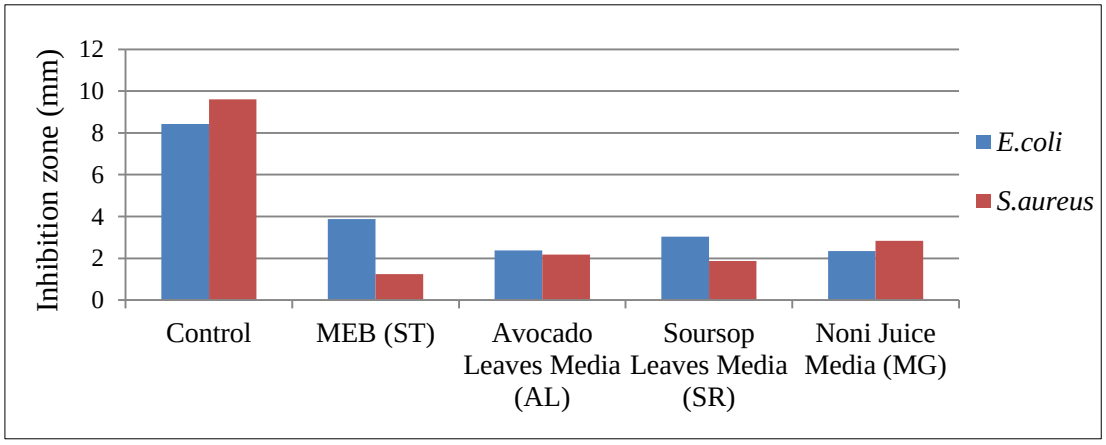

Figure 2. Diagram Inhibition zone of *separatory funnel* ethyl acetat fractions (KN-15-3-1).

The biggest *E.coli* inhibition zone was ST-KN-15-3-1 of 3.88mm, and the biggest *S.aureus* inhibition zone was MG-KN-15-3-1 of 2.83mm (Figure 2). All of the ethyl acetate fractions were separated again using open column chromatography (Table 3). The largest weigth was MG-KN-15-3-1-3 of 383.8mg and the smallest was ST-KN-15-3-1-4 of 34mg.

The different between wet weight and rendement extract on four growth were influenced by the nutrient contents in each growth medium (Table 1-3). The nutrient contents on each growth media affected the growth quantity of microorganisms [27].

Table 3. Open Column Chromatography (fraction extract)

Isolate	Media	Ethyl acetate fraction (separatory funnel) (gr)	Fraction (OCC)	Weight of the OCC fraction extract (mg)
KN-15-3-1	ST	0.5384	1	120.9
			2	75.7
			3	183.8
			4	34
			5	116.1
	AL	0.9569	1	157.4
			2	103.9
			3	329.8
			4	35.6
			5	64.3
	SR	1.9192	1	210
			2	245.9
			3	168.9
			4	262.2
			5	148.4
	MG	0.8461	1	120.1
			2	178.4
			3	383.8
			4	47.1
			5	105.2

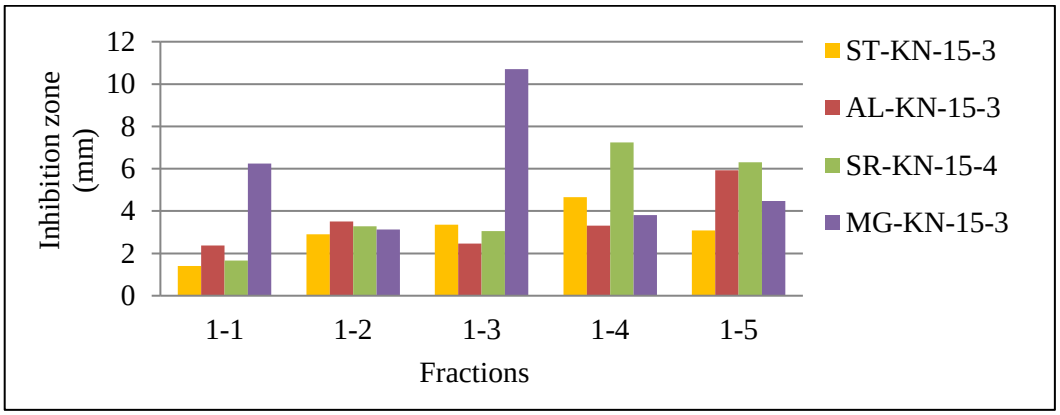

Figure 3. Diagram inhibition zone of Open Column Chromatography against *E.coli*.

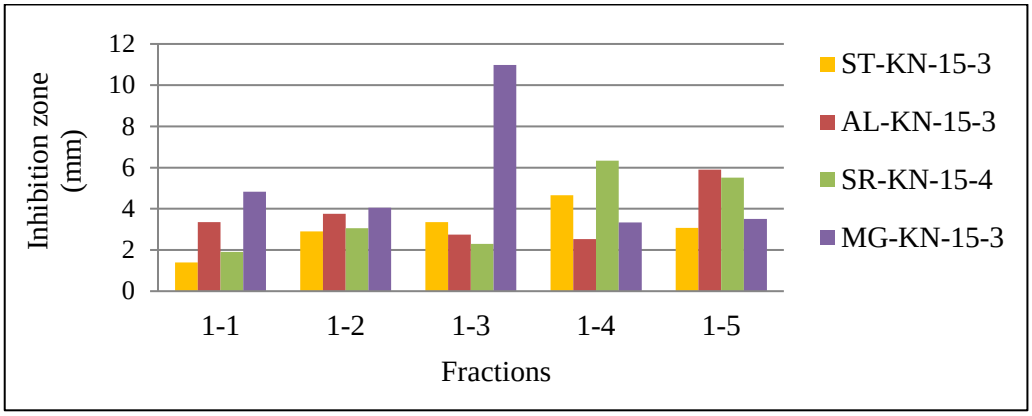

Figure 4. Diagram inhibition zone of Open Column Chromatography against *S.aureus*.

The biggest *E.coli* and *S.aureus* inhibition zone were MG-KN-15-3-1-1 of 10,71mm and 10,98mm (Figure 3-4). Growth media also affected the quality and ability to inhibit pathogenic bacteria, *E.coli* and *S.aureus*, extracts of symbiotic fungi of marine sponge *Axinella* sp., *Aspergillus sydowii* (Figure 2-4). The content of nutrients in growth media affected the content of bioactive compounds from microorganisms [28-29].

4. Conclusion

Symbiotic fungi of marine sponge *Axinella* sp., *Aspergillus sydowii* could be grown on four media, Malt Extract Broth/MEB (ST), Noni Juice Media (MG), Avocado leaves media (AL), and Soursop leaves media (SR). Growth media affected the quantity and quality of the symbiotic fungi extract, *Aspergillus sydowii* and its ability to inhibit pathogenic bacteria, *E.coli* and *S.aureus*.

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