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Potency of Antimicrobial, Antioxidant and Cytotoxic Activities from Fractions *Streptomyces* MA02

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ABSTRACT

Streptomyces sp. MA02 is potential to produce various biological activities. In previous research, its extract shows antimicrobial activities. In this study, extract prepared from *Streptomyces* sp. MA02 stored at -80 ^{o}C was re-evaluated the antimicrobial activity on various medium and incubation time to find out storage effect toward the antimicrobial activity. We also evaluated n-hexane and ethyl acetic fractions toward the antimicrobial, antioxidant and toxicity activities. The extract *Streptomyces* sp. MA02 still inhibit tested microorganisms eventhough it decreases 50% than the extract prepared from *Streptomyces* sp. MA02 without -80 ^{o}C storage. Antimicrobial activity was only exhibited by the methanolic fraction *Streptomyces* sp. MA02. The methanolic and n-hexane fractions showed cytotoxic eventhough it was not for antioxidant activity in the linoleic acid system with ferrothiocyanate reagent (FTC).

INTRODUCTION

Secondary metabolites are pivotal compounds for humankind due to biological activities which can be used for drugs and pharmaceutical. Most scientists have explored microorganisms to get potential secondary metabolites, for instance, *Streptomyces* which shows the highest contribution in production bioactive compounds especially antimicrobial. Terrestrial *Streptomyces* contributes around 80% of the total antibiotic. Apart from antimicrobial activity, *Streptomyces* also exhibit other activities. *Streptomyces* spp. colony KR-5 exhibit cytotoxic activity against the growth of human breast cancer cell line (Sateesh *et al.*, 2011).

Microorganisms will generally produce secondary metabolites at stationary phase. It is induced by depletion of nutrient in their growth medium so that microorganisms compete with other microorganisms to get nutrients which it creates a stressful condition for them. Differences in the composition of growth mediums also affect type of secondary metabolites produced by microorganisms. For instance, antimicrobial activity of Streptomyces sp. MA03 cultivated M2^{+} broth medium is better than that of Streptomyces sp. MA03 cultivated M13 broth medium (Nofiani et al., 2012). Some secondary metabolites may be not biosynthesized all time eventhough their cultivations have conducted with similar condition. Biosynthesis of secondary metabolites of micro-organisms are affected many factors. For instance, sponge-associated microorganisms can produce a spesific secondary metabolite compound but it probably can not re-biosythesized while microorganisms can not associated with sponge. *Fusarium solani* RT2A F1 associated with coral secreted red pigment. Unfortunately, it can not produced the pigment after 5 times of cultivation at laboratory (unpublished data).

In the previous research, *Streptomyces* sp. MA02 has showed antimicrobial activities which inhibit growth 12 of tested microorganisms (Nofiani *et al.*, 2012). In this study, we re-evaluated the antimicrobial activity on various medium and incubation time to find out storage effect toward the antimicrobial activity. We also evaluated n-hexane and ethyl acetic fractions toward the antimicrobial, antioxidant and toxicity activities.

MATERIALS AND METHODS

Enumeration of *Streptomyces* sp. MA02.

Streptomyces sp. MA02 which was kept in -80^{o}C storage was re-enumerated on M2^{+} agar and incubated on 30 ^{o}C for 3 days.

Cultivation and Extraction of *Streptomyces* sp. MA02

Medium and incubation time for *Streptomyces* sp. MA02 production was carried out by various medium (M_2^+, 1/10M_2^+, ZoBell, 1/10 ZoBell and M_{13}) and incubation (4, 6 and 9 days). The seeds was prepared by inoculation of 1 colony in 10 mL of liquid

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medium and incubated on shaker incubator at 150 rpm for 3 days. Furthermore, the seeds were inoculated 1 L of liquid medium and incubated on shaker incubator at 130 rpm for certain time. Finally, the culture was harvested and extracted the secondary metabolites using ethyl acetate as solvent which ratio between the culture and solvent is 1:1. The crude extract was concentrated using rotary evaporator.

Partition of the Crude Extract

The crude extract redissolved with methanol then was added the same volume with n-hexane. The mixture was shaked and let it form 2 layers. The layers were separated and each layer was concentrated using rotary epavorator so that found 2 extract namely n-hexane dan methanol.

Antimicrobial Assay

Antimicrobial assay was performed according to well-difusion agar method. The well was made on nutrient agar medium which was inoculated with culture of microorganism tests. Approximately 20 µL of extract was put into well and let it dry then incubated at 37 ^{o}C for 24 h. Furthermore, diameter of inhibition zone measured from the edge of the colony to the edge of the clear zone. This procedure was also determined antimicrobial activity using fungal test, *C. albicans* but the medium assay was potato dextrose agar (PDA).

Antioxidant Assay

Antioxidant assay was conducted based on the linoleic acid system with ferrothiocyanate reagent (FTC) (Kumar *et al.*, 2008). Approximately 10 µL of sesame oil was mixed with 1 mL of ethanol and 10 µL of extract. After incubated at dark room and room temperature for 24 hours, the mixture was added 20 µL of $FeSO_4$ 0,014 M and 20 µl of KCNS 30% then the mixture measured antioxidant activity using microplate reader at 490 nm. Ascorbic acid was used as positive control. Percentage inhibition of linoleic acid oxidation was calculated as follows:

$$\text{Percentage of Inhibition} = 100 - \left[\frac{\text{Absorbans sample}}{\text{Blank Absorbans}}\right] \times 100$$

Toxicity

Toxicity was determined according to *Brine Shrimp Lethality Test (BSLT)* (Meyer *et al.*,1982). Before the test, larvae of *Artemia salina* Leach were prepared by hatching of *A. salina* Leach eggs using an aerated bottle containing sea water for 2 days at ambient temperature. Furthermore, 10 the larvae were added the extract in certain concentratrion and seawater until 1 mL of final concentration. After 24 hours, the total of live larvae was counted and recorded.From the data, LC_{50} value was determined by probit analysis using SPSS version 17.

RESULTS AND DISCUSSION

Antimicrobial Activity of Crude Extract

The extract MA02 prepared by cultivation of MA02 on various medium and incubation time to evaluate this effect toward antimicrobial activity according to the number of inhibited microorganism tests.

The result is all of the crude extract cultivated on 5 media did not inhibit growth *P. aeruginosa, V.vara, V.cholerae, S. aureus* dan *E. coli* but inhibit *B. subtilis), K. pneumoniae, C. freundii, A. hydrophila* and *C. albicans* (Table 1). However, the best antimicrobial activity can been seen on *Streptomyces* sp. MA02 cultivated on $M2^+$ with 6 days incubation time, 1/10 $M2^+$ with 4 and 6 days incubation time, 1/10 ZoBell with and 6 days incubation time because they inhibited 7 microorganism tests.

According to Table 1. medium and incubation time plays a pivotal role in secondary metabolites productions especially antimicrobial activity. Decrease of nutrional value from $M2^+$ to 1/10 $M2^+$ did not increase the number of inhibited microorganism test although is not for Zobell medium. The crude extract produced in poor nutritional ZoBell medium (1/10 ZoBell broth) with incubation 6 days showed it can inhibit growth more microorganism tests compared with that of rich nutrional ZoBell medium (ZoBell broth). Based on the result, limited nutrition on cultivation of *Streptomyces* is not guaranted to get the best antimicrobial compounds from its extract.

Streptomyces sp. MA02 used in this study was found from -80^{o}C storage for 18 months. Generally, the extract *Streptomyces* sp. MA02 prepared by various media and incubation time can only active against around 6 of 12 microorganism tests (Table 1). On the basis the results, there are decrease significantly in the inhibition of tested microorganism total compared with the extract prepared from *Streptomyces* sp. MA02 without -80 ^{o}C storage (Nofiani *et al.*, 2012). It means that the extract prepared from *Streptomyces* sp. MA02 isolated from its habitat and use directly to make an extract.

Antimicrobial, Antioxidant and Cytotoxic Activities of *Streptomyces* sp. MA02 Fractions

The crude extract was partitioned using n-hexane as a solvent so that was found two fractions namely n-hexane and methanol. Both fractions were tested antimicrobial activities against 2 tested microorganisms. On the basis antimicrobial activity, the fraction exhibiting antimicrobial activity is only methanol (Table 2) so antimicrobial compounds is probably polar compounds. Microorganisms produce various secondary metabolites which are probably showing various biological activities. To find out other biological activities, both fractions were conducted antioxidant and cytotoxic test. Both fractions showed no antioxidant activity in the linoleic acid system with ferrothiocyanate reagent (FTC). LC_{50} value for cytotoxic activity based on BSLT was 90.90µg/mLfor methanolic fraction and 256.44 µg/mL for n-hexane fraction. The methanolic fraction showed more cytotoxic effects than those of n-hexane fraction. However, the value of both fractions was higher than that of umbelliferon which the LC_{50} is 377.02 ppm (Mert *et al.*, 2008).

Table. 1: Antimicrobial activitity of extract *Streptomyces* MA02 no barious medium and incubation time.

Microorganism Test	Average diameter of clear zone (mm) of extract *streptomyces* MA02														
	M_2^+ broth			$1/10M_2^+$ broth			ZOBELL broth			1/10 Zobell broth			M13 broth		
	4 days	6 days	9 days	4 days	6 days	9 days	4 days	6 days	9 days	4 days	6 days	9 days	4 days	6 days	9 days
B. subtilis	3.58	7.82	6.13	7.24	6.14	3.70	5.64	4.54	4.08	6.52	6.36	5.54	4.20	3.04	4.85
K. pneumoniae	8.95	10.25	8.,54	9.12	9.81	9.26	10.10	9.56	9.48	8.65	8.00	7.89	10.02	9.12	8.14
A. hydrophila	3.58	5.48	4.22	5.09	4.71	4.00	3.74	5.,00	4.17	5.43	3.59	2.52	4.16	4.06	2.74
Salmonella sp.	4.79	7.77	5.56	-	5.06	-	-	-	-	5.65	3.52	-	-	4.28	-
C. freundii	-	7.57	3.04	-	8.64	-	8.15	-	-	8.74	5.00	4.68	2.74	-	6.36
V. harveyii	-	5.58	-	7.24	8.37	8.32	8.74	7.25	5,79	9.63	8.48	7.96	9.36	8.63	7.95
S. aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. cholerae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. vara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	3.00	6.98	4.18	5.10	3.98	4.80	2.94	2.04	4.58	4.69	4.74	4.21	3.05	2.54	2.92

(-) No antimicrobial activity.

Table. 2: Antimicrobial compounds of *Streptomyces* SP. MA02 fractions.

Microorganism Tests	Average Diameter of Clear Zone (MM)	
	Methanolic Fraction	N- Hexane Fraction
B. subtilis	10.62	-
K. pneumoniae	9.56	-

-: No antimicrobial activity.

CONCLUSION

Streptomyces sp. MA02 stored -80 ^oC exhibited instability of antimicrobial activity especially for tested microorganisms which it decreases around 50% than *Streptomyces* sp. MA02 without -80 ^oC storage. However, the fractionated extract (methanolic and n-hexane fraction) has various biological activities such as antimicrobial and citotoxic. In addition, both fractions are not active as antioxidant in the linoleic acid system with ferrothiocyanate reagent (FTC).

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