

Streptomyces spp. A POTENTIAL BIOCONTROL AGENT AGAINST *Ganoderma boninense* OF BASAL STEM ROT

PEI HUI LIM*; JUALANG AZLAN GANSAU* and KHIM PHIN CHONG**

ABSTRACT

Oil palm industry in Malaysia is suffering from one major disease known as basal stem rot (BSR), caused by *Ganoderma* species. Microbes were isolated from soil samples collected from Crocker Range of Sabah and the percentage inhibition of radial growth (PIRG) against *Ganoderma boninense* was determined. There were 72 types of actinomycetes isolated from 20 soil samples and the most potential actinomycete (A19) showed the highest PIRG with 80.00%. The interaction of A19 with *G. boninense* was observed using scanning electron microscopy (SEM). *G. boninense* was inhibited by anti-fungal effect of A19 due to hyphae damage and A19 was successfully identified as *Streptomyces* spp. Metabolites of *Streptomyces* spp. A19, were extracted and ethyl acetate extract showed the highest inhibition to *G. boninense*. Minimum inhibitory concentration (MIC) of *Streptomyces* spp. A19 ethyl acetate extract was 0.18 mg ml⁻¹. The extracts were then fractionated through solid phase extraction (SPE). SPE fractions (0.02 mg ml⁻¹) showed better inhibition to *G. boninense* compared to crude extracts with the same concentration. Several antimicrobial compounds (ribostamycin, benzylmalic acid, landomycin B and salinomycin) were detected in these samples using Liquid chromatography-mass spectrometry (LC-MS). These metabolites may contribute to the antagonistic effect against *G. boninense*.

Keywords: basal stem rot, biology control, *G. boninense*, oil palm, *Streptomyces* spp.

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INTRODUCTION

Oil palm is the golden crop of Malaysia because it contributes to profitable income for the country and help to reduce poverty in Malaysia. The export from palm oil or palm oil products contributes approximately RM 64.58 billion to Malaysia's revenue in 2016 with the planted area of 5.74 million hectares (MPOB, 2017). However, the oil palm industry in South-east Asia is suffering from one major disease, the basal stem rot (BSR) caused by

Ganoderma species. *Ganoderma* is a white rot fungus and the term 'white rot' derives from the fungus ability to degrade specifically the lignin component of wood while leaving the white cellulose exposed. Many methods have been attempted to control BSR, but to date, no method gives good control of *Ganoderma* infection in established plantations (Chong *et al.*, 2011). Cultural practice such as soil mounding is not effective in controlling BSR and it only helps to prolong the economic life of the yielding palms affected by BSR. Chemical control such as trunk injection of systemic fungicides has also been reported (Chung, 1990; George *et al.*, 1996) and at most, some systemic fungicides managed to prolong the economic life of BSR infected palms, but they failed to give effective control of the disease because treated BSR infected palms will eventually collapse. The difficulties in controlling BSR may be due to the

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failure of planters to recognise the infected palms at the early stage and in addition to this, *Ganoderma* also has several resting stages such as pseudosclerotia (melanised mycelium) and basidiospores (Susanto *et al.*, 2005) that are more resistant to fungicides. The use of biological control agents such as *Trichoderma* spp (Soepena *et al.*, 2000; Alexander and Chong, 2014), *Bacillus* spp. (Alexander and Chong, 2014), *Agrobacterium radiobacter*, *Burkholderia cepacia* and *Pseudomonas syringae* (Maizatun and Idris, 2009) has been reported and showed varied results. The *Streptomyces* genus in actinomycetes group showed a high inhibition percentage against *G. boninense* *in vitro* through antibiosis and the lysis of hyphae resulting in abnormal hyphae growth (Tan *et al.*, 2002). The *Streptomyces* spp. was proven to inhibit the growth of *G. boninense* *in vitro* (Shariffah *et al.*, 2011). However, not many reports on actinomycetes against *Ganoderma* were documented. In this study, the potential of actinomycetes from the rich biodiversity of Crocker Range in Sabah was studied. Actinomycete with the highest percentage inhibition of radial growth (PIRG) to *G. boninense* was identified and the potential bioactive compounds were extracted and identified.

MATERIALS AND METHODS

Soil Sampling and Isolation of Potential Antagonists from Soil Samples

Twenty soil samples were collected randomly from Crocker Range forests area (5.4008° N, 116.1033° E). Soil samples were collected 15 cm in depth from the ground using sterilised pvc pipes (5 x 15 cm). Soil samples were packed in ziplock bags and stored in a container with icepacks during transportation from sampling site to laboratory.

The isolation of microbes of interest was conducted using dilution plate method. A serial dilution of the soil suspension from 10^{-1} to 10^{-8} was prepared. To prepare a soil suspension with 10^{-1} dilution, 10 g of soil was suspended into 90 ml of sterile distilled water in a 250 ml conical flask. The suspension was then serially diluted to 10^{-8} . An aliquot of 100 μ l of each dilution was taken and spread evenly on the surface of actinomycete isolation agar (AIA) (Difco®) using a L-shaped glass spreader. All plates were incubated at room temperature for one to two weeks. Colonies formed on the AIA plates were then sub-cultured on oatmeal agar (OA) to obtain a pure culture.

Dual Culture Assay

The assays were conducted using potato dextrose agar (PDA). *G. boninense* mycelial plug (8 mm) was taken from *G. boninense* pure culture

stock of 10 days old which was previously identified by Chong *et al.* (2011) and placed 30 mm from one side of the PDA plate. After two days, a loopful of the potential antagonist actinomycetes was streaked 30 mm from the *G. boninense* plug. The radial growth of the pathogen in the dual culture was measured after seven days of incubation at room temperature ($28 \pm 1^\circ\text{C}$) and the PIRG was calculated as described by Bivi *et al.* (2010). The formula for PIRG is as follows: $[(R1 - R2) / R1] \times 100\%$ where, R1 is the radial growth of *G. boninense* in the control plate while R2 is the radius of the *G. boninense* colony in the direction towards the antagonist colony. Plates with only *G. boninense*, served as controls. Each assay was repeated thrice. Antagonist with the highest PIRG against *G. boninense* was identified and further studied.

Scanning Electron Microscopy (SEM) Study of the Interaction

The interaction of isolate with the highest PIRG against the pathogen was observed using Zeiss EVO® 10MA SEM. The zone of interaction between *G. boninense* and the isolate in dual culture assay (Day 7) was prepared for observation. *G. boninense* fungal mycelia in the middle of the agar plate were cut to approximately 1.0 x 1.0 cm in size using scalpel. The samples were then air dried in laminar flow at room temperature for about 3 to 4 hr. The samples were mounted onto aluminium stubs using conductive double sided adhesive carbon tabs (Nisshin EM. Co. Ltd). The stubs were then sputter coated with gold-palladium (Emitech K550x carbon coater) from different angles to ensure a complete and uniform film over sample surfaces. Coated surfaces of samples were viewed at 15 kV voltage.

Identification of Microbes

Actinomycete (A19) with the highest PIRG was identified using molecular technique. The method in extracting the DNA and PCR work as described by Latifah *et al.* (2002) with some modifications. Primers used were Eub 518: 5'- ATT ACC GCG GCT GCT GG -3' and Eub 338: 5'- ACT CCT ACG GGA GGC AGC AG -3'. Forward and reverse sequences were assembled in BIOEDIT v.7.2.5.0 and used for BLASTN with NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>). Sequences found in the database with the highest nucleotide similarities (97% or more) were considered as the closest relative of the query sequence.

Anti-microbial Assay of Crude Extracts

Actinomycete with the highest PIRG (A19) was cultured in mannitol peptone broth for five days. The broth culture was then extracted using hexane, ethyl

acetate and acetone respectively. The solvent phase was collected and dried using rotary evaporator (Stuart®) at 37°C for anti-microbial assay (Lakshmi and Selvi, 2013). Agar dilution method (0.20 to 1.0 mg ml⁻¹) was used to test the anti-microbial efficacy of the extracts (dissolved in 1% Dimethyl sulfoxide, DMSO) on *G. boninense* (Chaudhary *et al.*, 2013). All assays were conducted in triplicates, and mean values and standard deviations of *G. boninense* growth diameter were calculated. Mean significance of *G. boninense* diameter for different treatments with different concentrations was subjected to Univariate Analysis of variance (two-way ANOVA) with means compared using Tukey's Range Test. Differences between the mean values were considered significant when $p < 0.05$.

Determination of Minimum Inhibitory Concentration (MIC)

MIC A19 extracts were determined by testing the concentration of the extracts from 0.02 to 0.18 mg ml⁻¹. The stock solutions and agar preparation were carried out as in section 2.5 but with different volume of stock solutions used. Seven days old *G. boninense* mycelial plug was placed in the centre of each petri plate. Diameter of *G. boninense* was measured from day one to day seven for determination of the minimum concentration which caused inhibition to *G. boninense*.

Solid Phase Extraction (SPE)

Two types of cartridges, Phenomenex Strata SI-1 (55 µm, 70 Å) silica-based sorbent 500 mg 6 ml⁻¹ (normal phase) and Phenomenex Strata C18-E (55 µm, 70 Å) silica-based sorbent 500 mg 6 ml⁻¹ (reversed phase) were used. Crude extract samples preparation of the selected isolate was prepared as in section 2.6. For Strata C18-E cartridges, methanol (4 ml) was used for conditioning and activating the sorbent bed of the cartridge. Cartridges were later equilibrated with 2 ml Milli-Q water to prepare the sorbent for optimised interaction with the analyte. Samples (5 ml) with the concentration of 50 mg ml⁻¹ were then loaded into the cartridges, which were washed with 5 ml of 20% (v/v) acetonitrile to remove any impurities from samples, and finally eluted with 5 ml of ethyl acetate. For Strata SI-1 cartridges, methanol (4 ml) was used for conditioning. Cartridges were later equilibrated with 2 ml hexane. Samples (5 ml) with the concentration of 50 mg ml⁻¹ were then loaded into the cartridges, which were washed with 5 ml of 2% (v/v) acetone and finally eluted with 5 ml of 50:50% (v/v); hexane: ethyl acetate. A19 C18 elute fraction [A19 C18(E)], A19 C18 wash fraction [A19 C18(W)], A19 Si-1 elute fraction [A19 Si-1(E)], and A19 Si-1 wash fraction [A19 Si-1(W)] were collected and dried using rotary

evaporator (Stuart®) at 37°C. The final concentration was adjusted to 0.02 mg ml⁻¹. Agar dilution method was used to test the anti-microbial efficacy of the extracts on *G. boninense*. The concentration tested in the anti-microbial activity assay was 0.02 mg ml⁻¹. Seven days old *G. boninense* mycelial plug was placed in the centre of each petri plate. Diameter of *G. boninense* was measured at Day 7. *G. boninense* growth inhibition percentage was calculated.

Liquid Chromatography Mass Spectrometry (LC-MS) Compounds Identification

Identity of potential bioactive compounds from A19 C18(E) and A19 Si-1(W) were identified using LC-MS. The LC-MS analysis was carried out using an Acquity Waters ultra-performance liquid chromatography (UPLC) coupled with synapt high definition mass spectrometer quadrupole-orthogonal acceleration, time-of-flight detector equipped with an electrospray ionisation (ESI) source. The mobile phase used was Acetonitrile: Water + Trifluoroacetic acid 0.1%; 80:20. The identification of the individual compounds were done using METLIN metabolites database (<https://metlin.scripps.edu/>) on the basis of the mass fragments and m/z values of each component.

RESULTS

PIRG of the Potential Antagonists against *G. boninense*

Out of the total of 195 isolates from the 20 soil samples with different morphology, there were only 72 actinomycete isolates. However, only 14 actinomycete isolates showed PIRG greater than 50% against the tested pathogen (Table 1). Actinomycete isolate with the highest PIRG (80%) was given an identity code as A19. The dual culture assay of A19 to *G. boninense* is illustrated in Figure 1.

Observation on Morphological Changes of *G. boninense* during the Interaction with A19

Interaction zone of antagonist (A19) and *G. boninense* in dual culture assays were examined under SEM. Figure 2a is the control *G. boninense* with dense fungal mat. Figure 2b shows *G. boninense* hyphae with the presence of A19. The density of the fungal mat was much lower compared to control *G. boninense* and distorted hyphae were also observed.

Identification of A19

The gel electrophoresis images from crude DNA of A19 is shown in Figure 3a. The gel electrophoresis

TABLE 1. ACTINOMYCETES ISOLATES SHOWING MEAN PIRG GREATER THAN 50% AGAINST *G. boninense*

No.	Actinomycetes isolate	Mean PIRG (%)
1	A19	80.00
2	A16	78.18
3	A72	70.30
4	A14	69.70
5	A42	67.27
6	A3	66.06
7	A48	65.45
8	A46	63.03
9	A59	61.82
10	A20	60.00
11	A44	55.15
12	A56	54.55
13	A34	53.94
14	A43	50.30

Note: 'A' denotes actinomycetes isolates.

PIRG - percentage inhibition of radial growth.

images from PCR amplification of A19 using EUB 338 and EUB 518 primer are shown in *Figure 3b*. The A19 amplicon was about 200 bp. *Figure 4* shows the assembled sequences of A19 that was used for the BLASTN in NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>). Sequences found in the database with the highest nucleotide similarities were considered as the closest relative of the query sequence. The identity of A19 was confirmed using molecular technique as *Streptomyces* spp. (*Table 2*).

Anti-microbial Activity of Crude Extracts of *Streptomyces* spp. A19 against *G. boninense*

Ethyl acetate extract of *Streptomyces* spp. A19 showed the highest inhibition against *G. boninense* and was significantly higher ($p < 0.05$) compared to other extracts. *Figure 5* shows the comparison of *G. boninense* growth inhibition percentage at different concentrations of *Streptomyces* spp. A19 solvent extracts. Ethyl acetate extract (1 mg ml^{-1}) of *Streptomyces* spp. A19 gave 90.59% of inhibition to

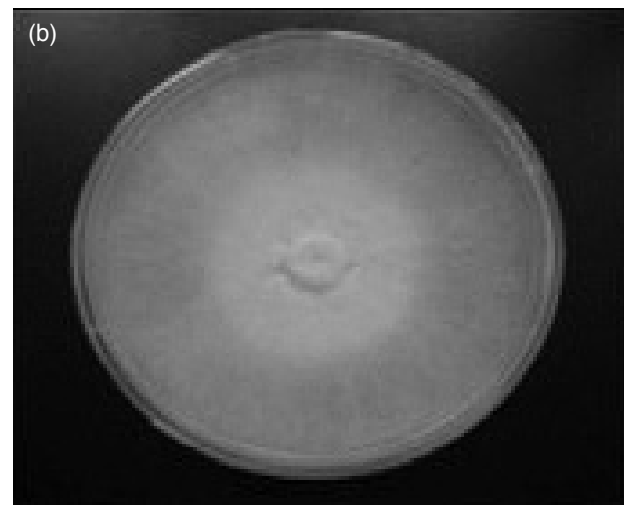


Figure 1. Morphology of potential biological control agent (A19) and G. boninense, a. Actinomycete isolate (A19) and G. boninense (arrowed) with highest percentage inhibition of radius growth (80%); b. Control plate of G. boninense.

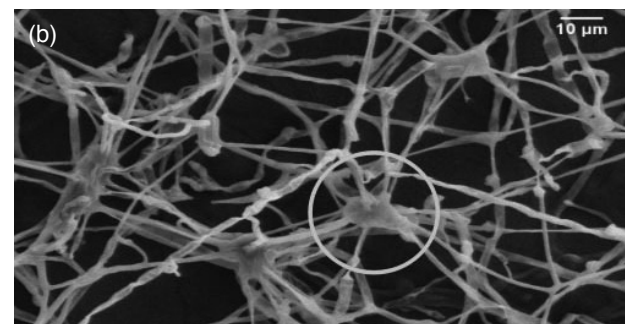
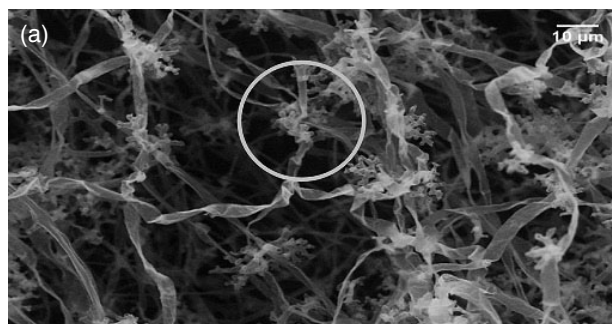


Figure 2. Micrograph of G. boninense under scanning electron microscopy observation, a. Morphology of G. boninense hyphae without interaction with the antagonist (control) at 2000X magnification; b. Morphology of G. boninense hyphae interaction with A19 at 2000X magnification. Note: Low fungal mat density and distorted hyphae were observed for G. boninense with A19. Circles showed the morphology differences between control G. boninense and G. boninense with A19.

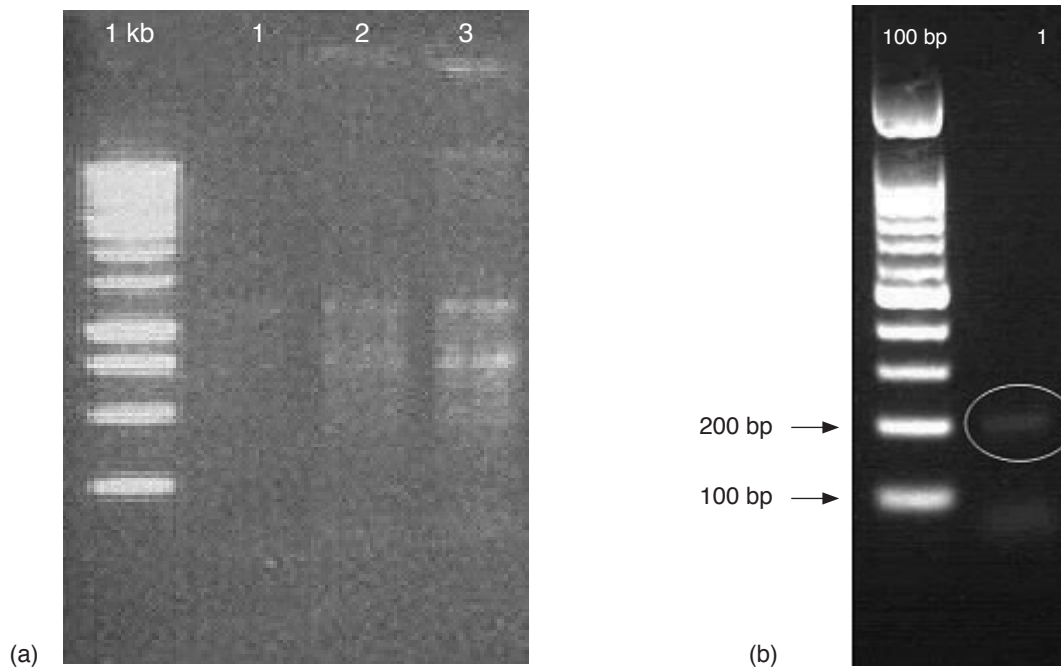


Figure 3. Gel images of A19 genomic DNA and polymerase chain reaction (PCR) product, a. Lane 2 represents genomic DNA; b. PCR amplification using the pairs of primers EUB 518/EUB 338. The DNA PCR product of A19 is circled.

TABLE 2. THE FIRST FIVE CLOSEST IDENTITIES OF A19 FROM NCBI GENBANK DATABASE

Isolate	Accession No.	Description	Max score	Total score	Query value	E value	Identity
A1A4 2A485 3.949	KC928120.1	<i>Streptomyces</i> sp. ZZY-2013 strain TRM46927 16S ribosomal RNA gene, partial sequence	226	226	87%	1e-55	97%
	NR115264.1	<i>Streptomyces sundarbansensis</i> strain MS1/7 16S ribosomal RNA gene, partial sequence	226	226	87%	1e-55	97%
	FJ475362.1	Uncultured streptomycete clone AhedenS22 16S ribosomal RNA gene, partial sequence	226	226	87%	1e-55	97%
	AJ576404.1	Uncultured bacterium partial 16S rRNA gene, clone PeM32	226	226	87%	1e-55	97%
	KC122238.1	<i>Streptomyces</i> sp. vh6 16S ribosomal RNA gene, partial sequence	226	226	87%	1e-55	97%

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GNNNNNCNNGTCTCTCCGGCAGGTCCGTCACCTTGCCTTCTTCCTGGCT
GAAGAGGTTTACAACCCGAAGGGCCGTCATCCCTCACGCGGCGTCGCT
GCATCAGGCTTTCGCCATTGTGCAATATCCCCACTGCTGCCTCCCGTA
GGAGTA
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Figure 4. A19 sequences used for BLAST search at NCBI GenBank database.

G. boninense growth after seven days of incubation, while hexane extract had no inhibition to *G. boninense*. The macroscopic features of *G. boninense* mycelia growth at different concentrations of different extracts are shown in Figure 6.

MIC of *Streptomyces* spp. A19 Crude Ethyl Acetate Extract against *G. boninense*

G. boninense growth treated with different concentrations of *Streptomyces* spp. A19 ethyl acetate crude extracts from Day one to Day seven is

shown in Figure 7. There was no visible growth of *G. boninense* observed at the concentration of 0.18 mg ml⁻¹ to 0.20 mg ml⁻¹. Thus, MIC for *Streptomyces* spp. A19 ethyl acetate crude extract was determined at concentration of 0.18 mg ml⁻¹.

Anti-microbial Activity of Solid Phase Extraction (SPE) Fractions of *Streptomyces* spp. A19 against *G. boninense*

The anti-microbial efficacy of *Streptomyces* spp. A19 SPE wash and elute fractions were tested

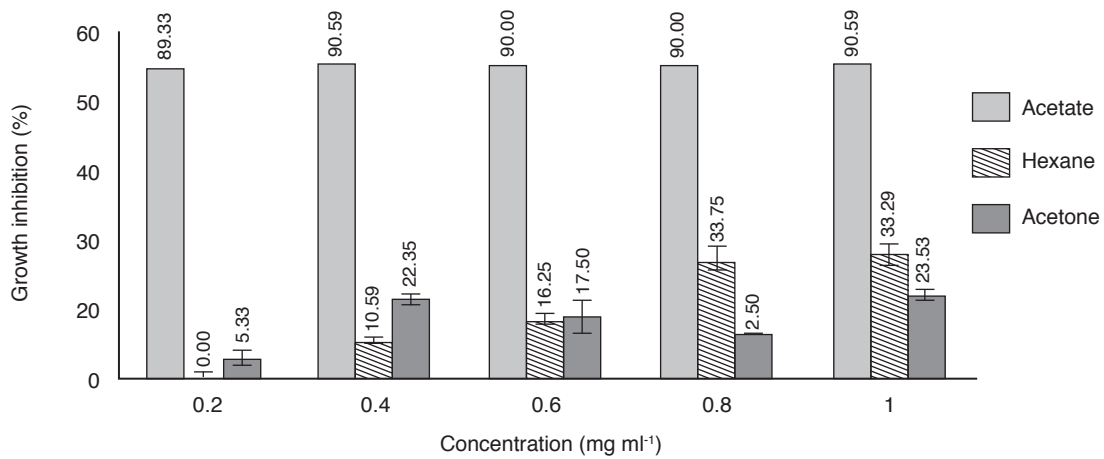


Figure 5. Percentage inhibition of *G. boninense* growth at different concentrations of *Streptomyces* spp. A19 with different solvent extracts.

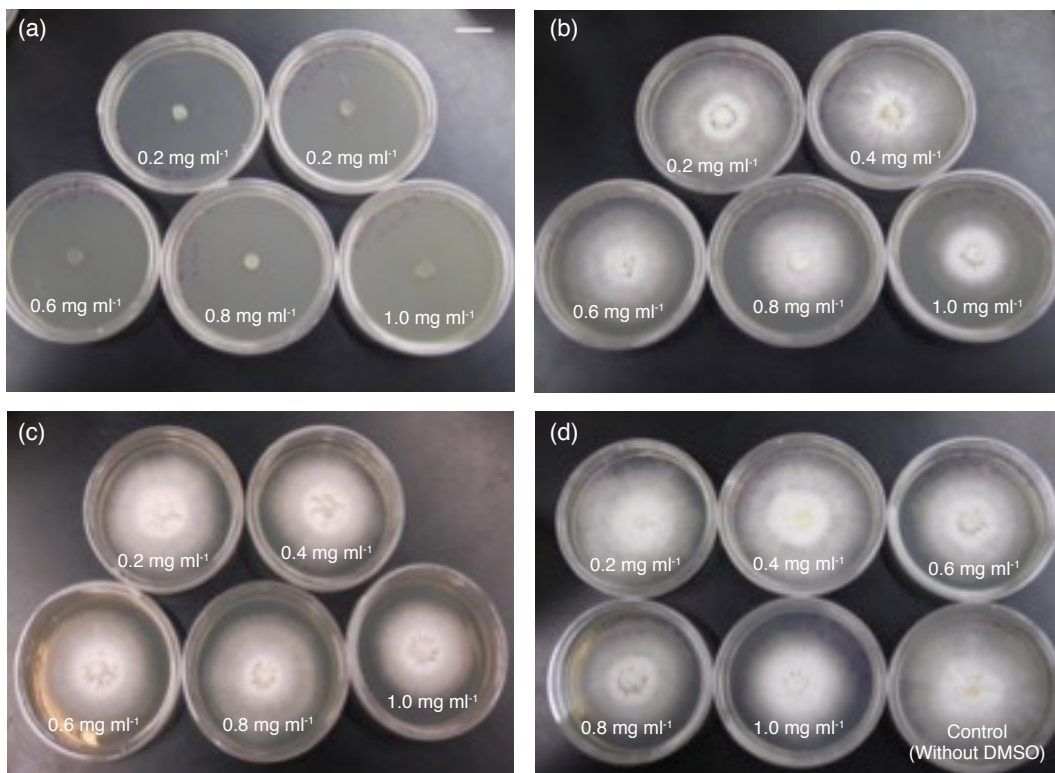


Figure 6. Anti-microbial assays of different concentrations of *Streptomyces* spp. A19 extracts with different solvents. a. Ethyl acetate extract; b. Hexane extract; c. Acetone extract; d. Control. Scale bar: 2 cm, DMSO - dimethyl sulfoxide.

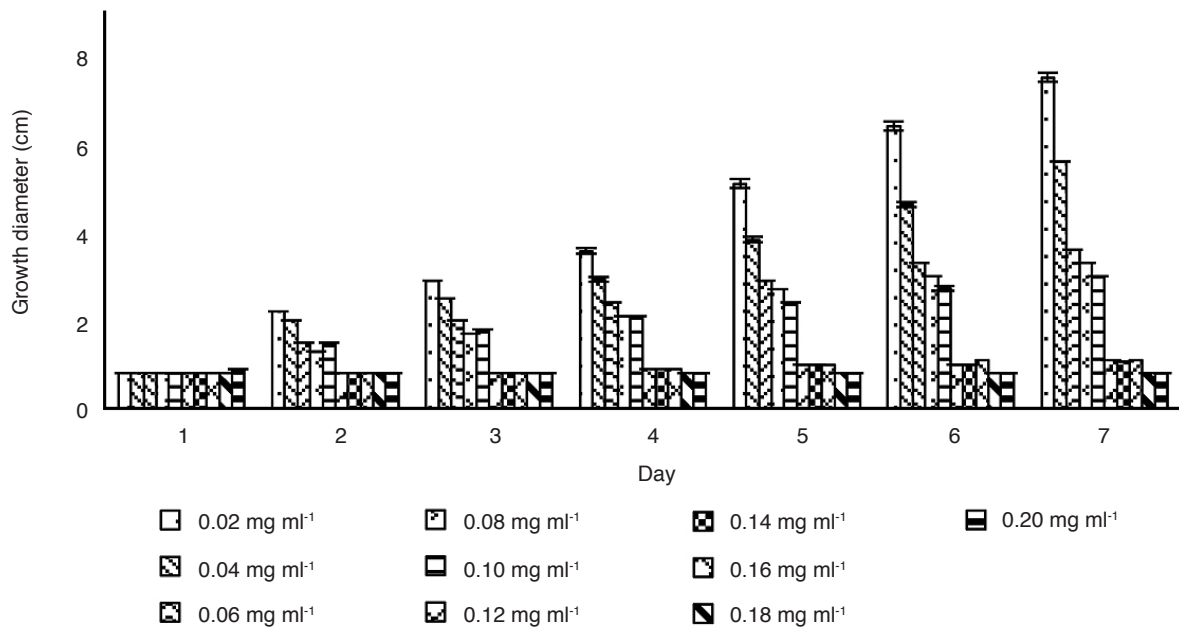


Figure 7. The growth diameter of *G. boninense* at different concentrations of *Streptomyces* spp. A19 ethyl acetate crude extracts.

against *G. boninense*. The lowest mean diameters of *G. boninense* growth was 2.43 cm in A19C18(E) fraction with 71.41% of growth inhibition while the highest mean diameters were 4.70 cm in A19SI-1(E) fraction with 44.71% of growth inhibition (Table 3).

TABLE 3. ANTI-MICROBIAL EFFICACY OF SPE FRACTIONS OF *Streptomyces* spp. A19 AT 0.02 mg ml⁻¹ CONCENTRATION

Samples	Growth of <i>G. boninense</i> (diameter in cm)*	Growth inhibition (%)
C18 (E)	2.43 ± 0.15	71.41
SI-1 (W)	2.90 ± 0.00	65.88
C18 (W)	4.00 ± 0.00	52.94
SI-1 (E)	4.70 ± 0.00	44.71
Control DMSO	8.50 ± 0.00	No inhibition
Control without DMSO	8.50 ± 0.00	No inhibition

Note: Original size of mycelial plug - 0.80 cm, DMSO - dimethyl sulfoxide, E- elute, W - wash.

* Values are mean ± Standard deviation of three replicates.

Identification of Corresponding Compounds of *Streptomyces* spp. A19 SPE Extract against *G. boninense* Using LC-MS

Fractions that gave good inhibitory effect to *G. boninense* were selected for further identification based on published results on their anti-microbial activity (Figures 8 and 9). Potential anti-microbial compound (Table 4) detected in A19SI-1(W) extract was identified as ribostamycin with m/z 437.22 [M+H-H₂O]⁺. Meanwhile, potential anti-microbial compounds detected in A19C18(E) were benzylmalic acid with m/z 242.10 [M+NH₄]⁺, landomycin B with m/z 509.19 [M+2Na]²⁺ and Salinomycin [M+K]⁺ with m/z 789.44.

DISCUSSION

Forest soil has higher microbial population compared to disturbed soil such as plantation soil. The relatively dense growth of plants and higher accumulation of litter on the forest floor and distribution of fine roots in undisturbed forest favor

TABLE 4. POTENTIAL COMPOUNDS CONTRIBUTE TO THE ANTI-MICROBIAL ACTIVITY OF *Streptomyces* spp. A19 SPE EXTRACTS AGAINST *G. boninense*

Sample	Compound		m/z	Formula	References
SI-1 (W)	Ribostamycin	[M+H-H ₂ O] ⁺	437.22	C ₁₇ H ₃₄ N ₄ O ₁₀	Okba <i>et al.</i> (2013)
C18 (E)	Benzylmalic acid	[M+NH ₄] ⁺	242.10	C ₁₁ H ₁₂ O ₅	Tanaka <i>et al.</i> (1984)
	Landomycin B	[M+2Na] ²⁺	509.19	C ₄₉ H ₆₄ O ₂₀	Mulert <i>et al.</i> (2003)
	Salinomycin	[M+K] ⁺	789.44	C ₄₂ H ₇₀ O ₁₁	Lindsay and Blagburn (1995)

Note: E - elute, W - wash.

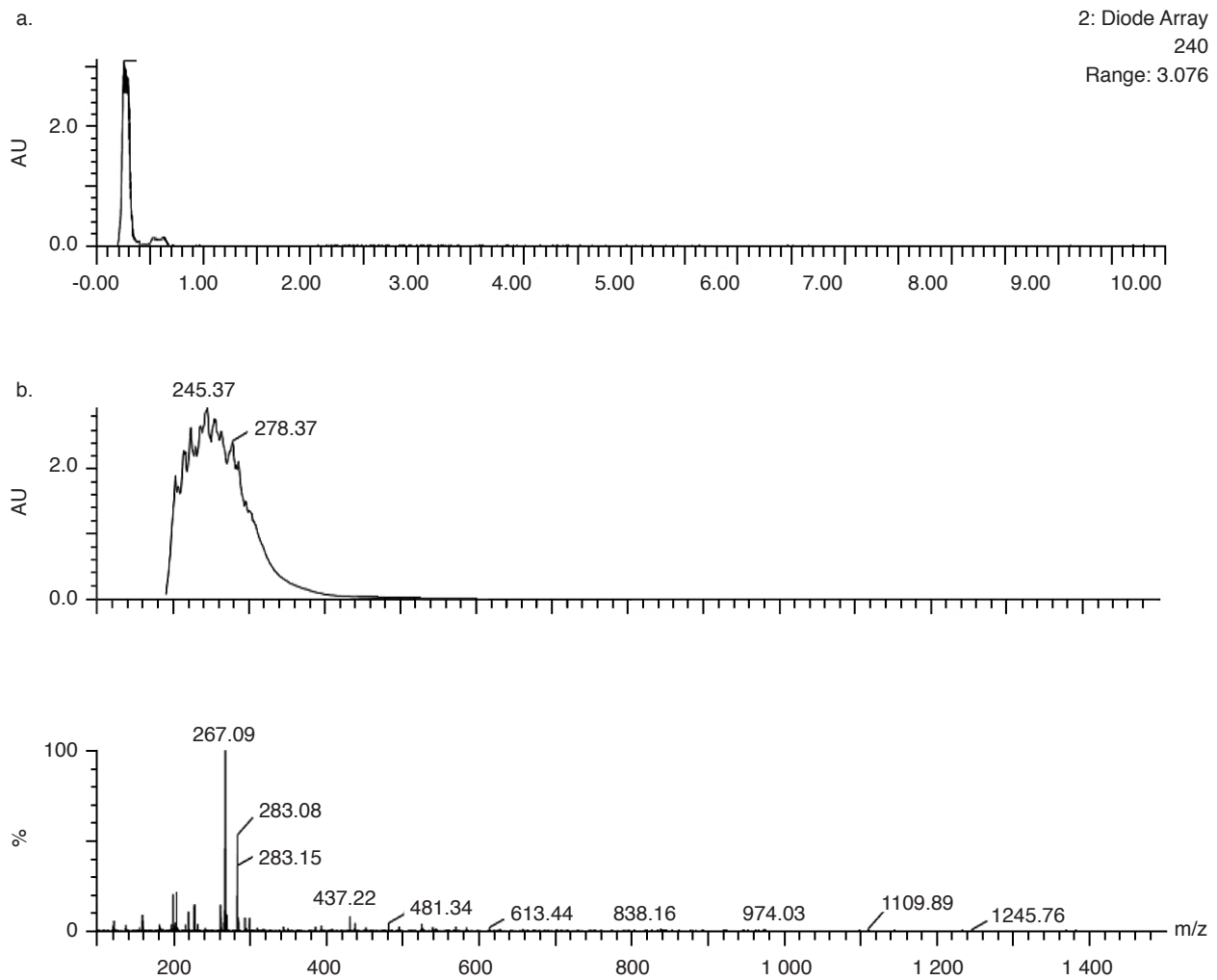


Figure 8. UV chromatogram and mass spectra of A19SI-1(W) extract obtained from liquid chromatography-mass spectrometry (LC-MS) analysis, a. Chromatogram of A19SI-1(W) extract; b. UV and mass spectra at t_R 0.25 min. Potential anti-microbial compound detected in the mass spectra; Ribostamycin $[M+H-H_2O]^+$ m/z 437.22.

the growth of microorganisms (Dkhar *et al.*, 2012). From dual culture assays, the growth of *G. boninense* might be inhibited by bioactive compounds produced by *Streptomyces* spp. A19. SEM (Figure 2b) revealed lower density of *G. boninense* hyphal mat compared to control. Cellular changes in the hyphal morphology of *G. boninense* was also observed. Degradation of cell wall component to some extent resulted in hyphal abnormality. Abnormalities in fungal hyphae are due to morphological changes of impaired growth of fungal hyphal (Suryanto *et al.*, 2012). These might be caused by secondary metabolites secreted from *Streptomyces* A19. The recent isolate of *S. sundarbansensis* from sediments of the Sundarbans mangrove forest in India was reported to produce 2-allyloxyphenol (Arumugam *et al.*, 2011). Hydroxyl and allyloxy groups in 2-allyloxyphenol are responsible for many anti-microbial and antioxidant activities.

The ethyl acetate extract of *Streptomyces* spp. A19 showed effective results against *G. boninense*. This could be related to the presence of bioactive metabolites which are soluble in ethyl acetate and might be a moderately polar to polar in nature. For comparison between SPE fractions and crude extracts, SPE fractions of *Streptomyces* spp. A19 showed better inhibition of *G. boninense* compared to crude extract at the same concentration (0.02 mg ml^{-1}). This is because SPE enables the concentration and purification of analytes from solution by sorption on a solid sorbent and purification of extract after extraction (Żwir-Ferenc and Biziuk, 2006). Hence, SPE process can provide samples that are better of interfering matrix components and concentrated enough for detection.

Ribostamycin is an aminoglycoside-aminocyclitol antibiotic isolated from *S. ribosidificus*, originally identified in a soil sample in Japan.

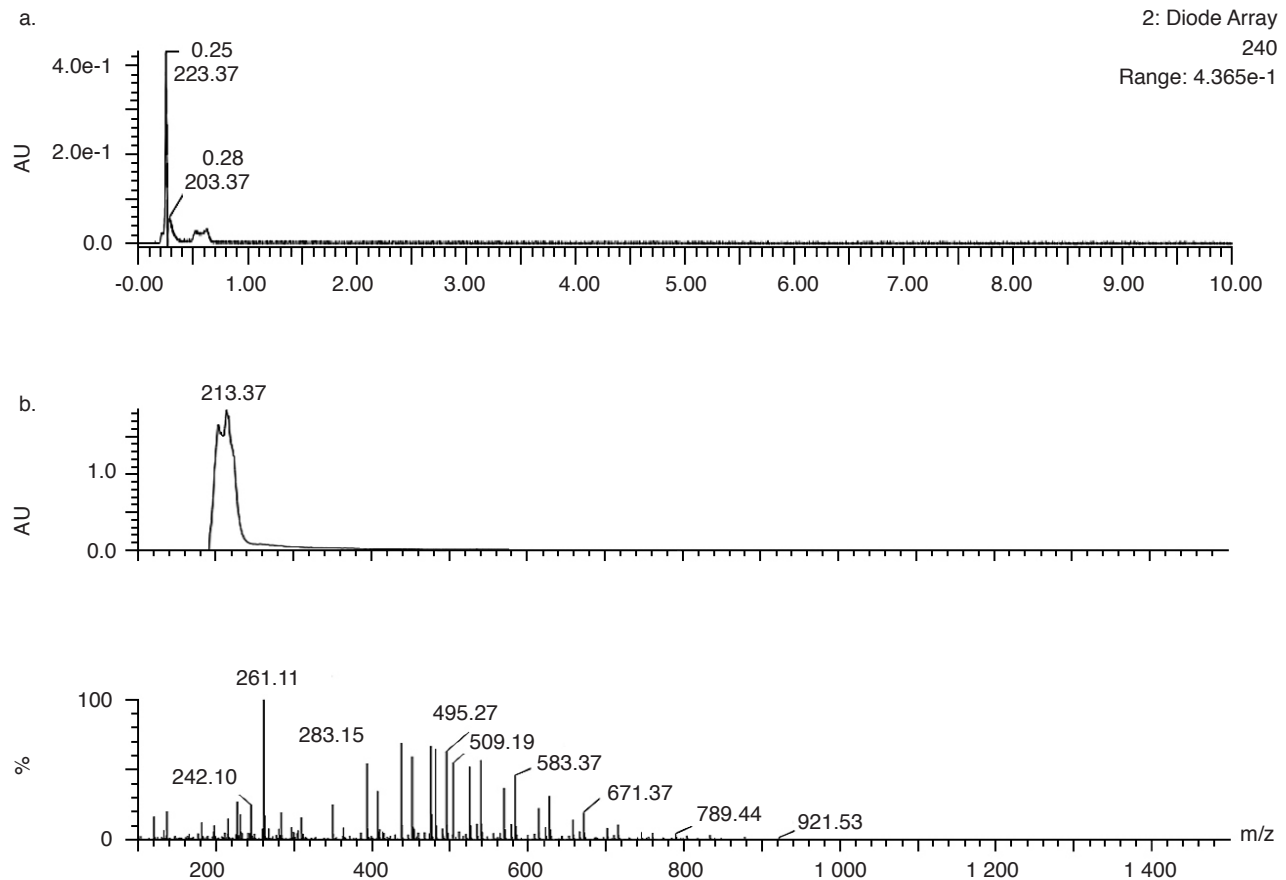


Figure 9. UV chromatogram and mass spectra of A19C18(E) extract obtained from liquid chromatography-mass spectrometry (LC-MS) analysis, a. Chromatogram of A19C18(E) extract; b. UV and mass spectra at t_R 0.25 min. Potential anti-microbial compounds detected in the mass spectra; benzylmalic acid $[M+NH_4]^+$ m/z 242.10, landomycin B $[M+2Na]^{2+}$ m/z 509.19, and salinomycin $[M+K]^+$ m/z 789.44.

Tanaka *et al.* (1984), reported carboxypeptidase A, (S)- α -benzylmalic acid from the culture filtrate of *S. hygroscopicus* inhibitors of carboxypeptidase A. Carboxypeptidase serves as a useful introduction to the biosynthesis (translation, transport, and proteolytic or glycosyl modifications) of many vacuolar proteins (Klionsky *et al.*, 1990). Landomycins belong to the angucycline group of polyketide-derived natural products, the largest group of polyketide-derived natural products. *S. cyanogenus* is the producer of landomycin A (LaA), a polyketide-derived antibiotic with antitumor activity. Salinomycin (SAM) is a fermentation product of *S. albus* (Lindsay and Blagburn, 1995). This compound belongs to monovalent carboxylic ionophorous polyether antibiotic that interfere with the plasma membrane ion transport system especially for alkali ions with relative K^+ selectivity, and also able to interfere with mitochondrial functions.

In general, ribostamycin (aminoglycosides) are known to interfere with translation in prokaryotic ribosomes, as well as benzylmalic acid, inhibitor of carboxypeptidase which serves as a useful introduction to the biosynthesis (translation,

transport, and proteolytic or glycosyl modifications) of many vacuolar proteins. Whereas, landomycin belongs to angucycline group with interesting and diverse biological activities including enzyme inhibitors and cytostatic effects. Many antibiotics such as penicillin and vancomycin inhibit the enzymes that produce and then cross-link the strands of this polymer together (Katz and Caufield, 2003). This causes the cell wall to lose strength and the fungal mycelial malformation. It is therefore suggested that these potential compounds might affect the growth of *G. boninense* in the present study.

CONCLUSION

Streptomyces spp. A19 is a biological control agent isolated from Crocker Range forest of Sabah and has the potential to be further studied as a biological control agent for controlling *G. boninense*. Potential anti-microbial compounds from *Streptomyces* spp. A19 such as ribostamycin, benzylmalic acid, landomycin B and salinomycin might contribute to the inhibitory effect of *G. boninense*.

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