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The extraction for the different polarity fractions of the endophytic fungi of Arthrinium sp L1 from Pleioblastus amarus and biological activity

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ABSTRACT

This essay researches into the extraction of the different polarity parts of the endophytic fungi of Arthrinium sp L1 from Pleioblastus amarus and its biological activities like bacteriostatic activity, antioxidant activity and cytotoxic activity. separate and purify the endophytic fungi from the plant bitter bamboo, and determine it as Arthrinium sp L1; after fermenting it, extract the following different parts respectively, the petroleum ether, ethyl acetate and n-butyl alcohol. Establish the slanting-tube method to evaluate how the different parts of Arthrinium sp L1 affect the in-vitro bacteriostasis of the phytopathogens through determining the value of MIC; determine the in-vitro antioxidant activity of the different parts of Arthrinium sp L1by using the following three methods DPPH, ABTS＋, and FRAP, have a preliminary screening for the cytotoxic activity of the different parts of Arthrinium sp L1 by using larva as the objects and the fatal-to-prawn bioactivity experiment. The different parts of Arthrinium sp L1 all have certain bacteriostatic activity, antioxidant activity and cytotoxic activity, and they all have obvious dose-effect relationship. Among them, the n-butyl alcohol extract has the strongest bacteriostatic activity for Fusarium graminearum and Sclerotinia sclerotiorum, and the ethyl acetate extract has the strongest antioxidant and cytotoxic activities.

KEYWORDS

Pleioblastus amarus; Arthrinium sp L1; Bacteriostatic activity; Bantioxidant activity; Cytotoxic activity.

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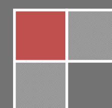

INTRODUCTION

Pleioblastus amarus (Keng) Keng f. is a species under the pleioblastus genus of the grass family, which is mainly distributed in the southern provinces of China[1]. The endophytic fungi of grass family plants refer to the fungi living in the organs of the grass family plants during a certain period of time without causing any obvious disease symptoms for the plants. The research shows that the endophytic fungi living in the plants for a very long time will produce the secondary metabolites similar to and even the same as those in the host plants. These secondary metabolites have very important practical value in the areas of medicine, agriculture, and the prevention of the plant disease and insect pests[2,3]. *Pleioblastus amarus* is an excellent timber of dual purposes. Its shoots have the medical effects of clearing heat, relieving restlessness, dehumidification, and alleviating water detention, which are mainly used in curing fever, polydipsia, jaundice with damp-heat pathogen, difficult urination, and dermatophytosis[4]. If properly utilized, the *Pleioblastus amarus* can produce very good economical effects for the local farmers. This research uses the *Pleioblastus amarus* on the campus of Zhejiang Agriculture and Forestry University as the raw materials to get a fungus of *Arthrinium sp L1* by separation, and studies the biological activities of the crude extracts in the secondary metabolites of this endophytic fungi and its three extract parts.

In recent years, the researches at home and abroad show that the extracts of bamboos have many biological activities like bacteriostasis, antioxidation, pest-killing, and antitumor, etc. At present, there have been no reports on the researches of the bacteriostasis, antioxidation and cytotoxic activity of Arthrinium sp L1 in *Pleioblastus amarus.* Through determining the MIC value, this research uses the slanting test-tube method to evaluate the in-vitro bacteriostatic activities of the methanol crude extracts and the three extract parts for the 8 phytopathogens in the Arthrinium sp L1 of *Pleioblastus amarus,* use Trolox as the contrast and the three different methods DPPH, $ABTS^{+}$, FRAP to have the determination and evaluation for the four samples of the Arthrinium sp L1 in *Pleioblastus amarus*; use prawn larva as the research objects and adopt the lethal-to-prawn bioactivity experiment to make a preliminary screening of cytotoxic activity for the Arthrinium sp L1 in *Pleioblastus amarus*, which will provide a reference data for the further development of the endophytic fungi of *Pleioblastus amarus.*

EXPERIMENTAL

Instruments and materials: Infinite M 200 Microplate Reader(Swiss Tecan); UV-2102 PCS Ultraviolet Visible Spectrophotometer (Shanghai Unica Instrument Corp. Ltd); 101-3 Electro-heating Blast Constant Temperature Drying Oven (Hangzhou Blue-sky Assaying Instrument Corp. Ltd); KQ-250B Supersonic Cleansing Device(Kunshan Supersonic Instrument Corp. Ltd); R201B Rotary Evaporator(Shanghai Shensheng Biotechnology Corp. Ltd); Vacuum Film Concentration Device(self-made); SHZ-D(Ⅲ) Circulating Water Vacuum Pump(Made in Gongyi Yingyu Yuhua Instrument Factory); PL203 Millesimal Electronic Scales(made by METTLER TOLEDO); Diaion HP-20 Column Chromatography. Prawn Ova (Artemia salina L.egg, made in: Tianjin Fengnian Aquiculture Corp. Ltd); Fe^{3+} Tripyradyl triazine,TPTZ, 1,1-Diphenyl-2 -picryl-hydrazyl, DPPH 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfpnic acid, $ABTS^{+}$), 6-Hydroxy-2,5,7,8- tetramethychroman-2-carboxylic, and Trolox are all purchased from Sigma Corp.; Absolute ethyl alcohol, methanol, ferric trichloride and DMSO are all China-made Analytical Reagents.

The separation, purification, fermentation and extraction of Arthrinium sp L1 in *Pleioblastus amarus: The Separation of Hypha:* First, make a pretreatment for the samples of *Pleioblastus amarus*; pick up a healthy *Pleioblastus amarus* and cut off its root, stem and leaves, and then wash them clean by water; put the leaves in the cup in case they wither; slice the root and stem for future use; choose potato, glucose, agar and water to prepare into PDA, and disinfect the prepared PDA respectively by wet-method. Cut off a little from around the spare root, stem, and leaves inside the super-clean workbench sterilized by ultraviolet light and make them into small cubes of 0.5cm×0.5cm. Firstly, use 0.1% mercuric bichloride to sterilize them for 1~2min, and rinse them for 2 to 3 times by sterile water, and

then put 75% ethyl alcohol to sterilize them for 10 s, and then rinse again for 3 to 5 times by sterile water, and finally suck-dry them by sterile filter paper and then put them on the fresh PDA plate-culture medium, and put the culture dish in the incubator with the temperature of 28°C to cultivate for 3 to 7 days. After all the above preparations, observe and make notes carefully everyday.

The purification of the culture

When the hyphas grow at the cuts, we will shift them in time to the fresh PDA culture medium to cultivate continuously by top-end-hypha-purification method. Repeat the above steps to have a gradual purification and number these bacterial strains. After purifying them, we then shift them to the PDA slanting test-tube and cultivate for 5 to 7 days in the incubator with the temperature of 28°C. Make sure there is no pollution and put them into the refrigerator with the temperature of 4°C for safe keeping. Choose some hyphas and shift them onto the fresh PDA culture media and observe their daily growth. If they are not pure, we have to choose the hyphas continuously to repeat the grafting until they shift onto another fresh culture media. After several repetitions, we will see whether they can reach purer fungi by lineate checking.

The appraisal of the endophytic fungi

Take the Direct Card-making method to observe and record the characteristics of the hypha, individual development, conidial fructification and the spore morphology during the cultivating process of the endophytic fungi, and determine the taxonomic unit of the culture according to the literature[5,6]. First determine the obtained purified fungi and then make a molecular evaluation. Finally, we verified it as Arthrinium sp L1 after making a morphological observation and a molecular determination of the endophytic fungi in *Pleioblastus amarus.*

The fermentation of the bacterial strain

Split Arthrinium sp L1 equally in the culture dish and put them into the fermentation flask, and then put the culture dish into the culture room for ferment cultivation. Make sure the fermentation is at the suitable temperature, humidity, oxygen and lighting, that is, the endophytic fungi should be in dark place, at 28°C and in aerobic environment. Shake the flask three times daily at early morning, noon, and evening to avoid the hypha sticking to the flask walls.

The extraction of the different parts of arthrinium sp L1

Firstly, make a supersonic extraction by adding the petroleum ether into the methanol concentrated liquor, and we’ll get the petroleum ether extract liquor. Then use the ethyl acetate to make the supersonic extraction and we’ll get ethyl acetate extract liquor. Finally, use the n-butyl alcohol to make the supersonic extraction for getting the n-butyl alcohol extract liquor. These extract liquors will be turned into extract cream respectively by the rotary-evaporation concentration.

The determination of the bacteriostatic activity

*The Tested Strains:*The 8 kinds of fungi such as *Fusarium graminearum*, *Fusarium graminearum* Schwabe, *Fusarium oxysporum*, *Exserohilum turcicum*, *Rhizoctonia solani* Kühn, *Botrytis cinerea*, *Rhizoctonia solani* AG1-IA, *Sclerotinia sclerotiorum* are all provided by the Forestry-protection Laboratory, Zhejiang Agriculture and Forestry University.

The preparation of the solution

Make the four samples of methanol extract, the extracting part of petroleum ether, the extracting part of acetic ether and the extracting part of n-butyl alcohol of the endophytic fungi in the *Arthrinium sp L1* of *Pleioblastus amarus* into the extract diluents with the following gradient concentrations by using DMSO as the solvents 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, 1.953, 0.977, 0.488 μg· mL^{-1}, the highest concentration being 250 μg · mL^{-1}, and the lowest being 0.488 μg· mL^{-1}. First use the highest concentration to screen them, and after establishing the scope, screen them one by one.

The determination process of the bacteriostatic activity

Determination by Slanting Test-tube method: choose potatoes, glucose, agar, and tap water to prepare into PDA. Sterilize the prepared PDA and the centrifuge tubes by wet method respectively. Channel each 2 mL PDA into the 5 mL centrifuge tubes on the workbench when they are still warm, and put them slantingly for the sake of making them into bevel-plane, after they cooled and solidified, they will become the slanting culture medium. Use the pipette to transfer separately the prepared samples onto the surfaces of PDA in the centrifuge tubes, with the sample- feeding amount in each centrifuge tube being 40 μL, and shake them evenly so that they can uniformly touch the surfaces of PDA. Only add DMSO onto the PDA in the negative control group, while in the blank group, add nothing to the surfaces of PDA. Repeat the experiment in each group three times and average the experiment results.

Put the 8 kinds of plant pathogenic fungi into the centrifuge tubes of the above prepared sample solutions through punching them in the culture dish; after cultivating them four 48 h, observe the fungi growth and regulate the concentration of the volatile oil according to the fungi growth. It is observed that there is a very good fungi growth of each concentration gradient in the blank group and the negative control group. Use the lowest solution concentration of the fungi-free volatile oil as the minimal inhibitory concentration MIC[7-12]. In this experiment, we judge the bacteriostatic ability of different samples through comparing their MIC values, and the smaller the MIC value, the stronger the bacteriostatic ability.

The Determination of the Antioxidant Activity : *The Determination of DPPH Free Radical Scavenging* Ability

DPPH method is the most commonly used method in testing the free-radical scavenging ability. It has been widely used in evaluating the antioxidant activity of all kinds of natural plant extracts[13-14]. We use the 96-hole micro-plate as well as the micro-plate quantification method of the microplate reader, MQ. Weigh precisely 20.5 mg of the DPPH reagent and dissolve it with the absolute ethyl alcohol, and transfer it into 100 mL flask quantitatively, and then use the absolute ethyl alcohol to make it into the constant volume; shake it evenly, we'll get the DPPH stock solution with the concentration of 205.0 mg · L^{-1}, and then put them into the refrigerator for future use. Before using them, use the absolute ethyl alcohol to dilute the DPPH stock solution into the diluent liquid with the concentration of 82.0 mg · L^{-1}. Add respectively the sample solution of 200 μL and the 50 μL test solution of the 82.0 mg · L^{-1} DPPH into the 96-hole ELISA plate. Shake for 30 s after adding the samples. Keep them at 24°C for 30 min, and determine their absorption value (Ap) at 517 nm wavelength, and at the same time, determine the blank absorption value (Ac) without adding DPPH samples, and the absorption value (Amax) with the addition of DPPH but without adding samples (50 μL, using the absolute ethyl alcohol instead of the sample). Use the Trolox as the positive control, and Trolox (X) as the x-coordinate, and free-radical scavenging ability (Y) as the Y-coordinate to make a standard curve, and we will get the regression equation $Y=2.3413X+0.1688$, $R^2=0.9999$. The antioxidant ability of the sample is shown by TEAC(trolox equivalent antioxidant capacity), and the free radical scavenging rate = 1- (Ap-Ac)/Amax ×100%.

The determination of the FRAP antioxidant ability

Yen[15] and Siddhuraju.P[16] think that there is a certain relationship between the reducing ability of the antioxygen and its antioxidant activity.

The determining method: *The preparation of the FRAP reagents*[18]: Take respectively 0.l mol/L buffer solution of acetic acid (pH 3.6), 10 mmol/L of TPTZ (dissolved in the 40 mmol/L hydrochloric acid) and 20 mmol/L of the ferric trichloride solution; mix them by the mixing rate of 10:1:1; take 300 μL of the FRAP reagent and 10 μL of the extracting solution, and let them react for 10 minutes and then calculate their absorption value at 593 nm. Add the FRAP working solution into the absolute ethyl alcohol instead of the sample, and treat it as the blank; calculate the antioxidant activity according to the absorption power and it is known that the higher the FRAP, the stronger the antioxidant power, and the higher the absorption value, the stronger the antioxidant activity. Use the Trolox solution dissolved into

the methonal as the positive control, and Trolox (X) as the x-coordinate, and the absorption value (Y) as the Y-coordinate to make a standard curve, and we will get the regression equation Y=3.1545X+0.1784, R^2=0.9994. The antioxidant ability of the sample is shown by TEAC (trolox equivalent antioxidant capacity).

The determination of $ABTS^+$ free radical scavenging ability

The principle of $ABTS^+$(2,2'-Azinobis (3-ethylbenzothiazoline 6-sulphonate)[19] is that we will get the stable positive ion free radical. Determining method[20]: take the potassium persulphate solution (140 mmol/L) 440 μL and $ABTS^+$ solution 25 mL(7mmol/L) and mix them to react for 12~16 h. Before using it, dilute the $ABTS^+$ solution with ethyl alcohol until its absorption value reaches 0.7±0.002, and then take 300 μL $ABTS^+$ solution and10 μL extracting liquid to react for 10 min and calculate its absorption value at 734 nm, meanwhile, calculate the sample's blank absorption values without adding any $ABTS^+$ (Ac), and adding $ABTS^+$ but without adding $ABTS^+$ (using 50 μL absolute ethyl alcohol instead of the samples) (Amax). Use the Trolox as the positive control, and Trolox (X) as the x-coordinate, and free-radical scavenging ability (Y) as the Y-coordinate to make a standard curve, and we will get the regression equation Y=3.6455X+0.1783, R^2=0.9993. The antioxidant ability of the sample is shown by TEAC(trolox equivalent antioxidant capacity), and the free radical scavenging rate = 1- (Ap-Ac)/Amax ×100%.

The processing methods for the data

Repeat all the above data in parallel for 3 times, and the results can be shown as the average value ± standard deviation. The correlation analysis of the experiment data is processed by SPSS13.0, and adopt OriginPro7.5 to draw the graphs.

The Bioactivity Determination for the Dying Prawn: *The hatching of the brine shrimp larva:*Add 2.0 g NaCl and 2.0 g $NaHCO_3$ in 1 L distilled water to produce the artificial sea water, and then add 2.0 g brine shrimp eggs to be made into the mature tested organism after cultivating for 24 h at the room temperature in dark with good ventilation.

The preparation of the sample solution

Weigh precisely 0.02 g of the extracted samples, and use DMSO to dissolve until the constant volume of 10 mL, we'll get the sample solution with the concentration of 2 mg · mL^{-1}. Then prepare them by using DMSO into the following 5 sample solutions with different concentration gradient, 10, 50, 100, 500, 1000 μg · $mL^{-1.}$

The determination of the LC_{50} value

Take about 30 sample solutions and prawn larva with different concentrations and experiment respectively on the 96-hole microplate. Only add DMSO in the control group, and put the 96-hole microplate in the dark for 24 h and record the number of the prawns, and later, calculate the number of the prawn died in each trough under the microscope. Finally calculate the fatality rate of the prawn larva according to the following equation: M= (A-B-N)/(G-N)×100%.

RESULTS AND DISCUSSION

The Determination Results of the Bacteriostatic Activity: The minimal inhibitory concentrations (MIC) that the four extract parts of the endophytic fungi *Arthrinium sp L1* in the *Pleioblastus amarus.* have for the 8 kinds of fungi are shown in TABLE 1. It is shown in the experiment that the 4 samples all have certain inhibitory activity on the bacterial strains of the 8 plant pathogenic fungi. Among the four extract parts of the endophytic fungi *Arthrinium sp L1* in the *Pleioblastus amarus*, the MIC value of n-butyl part is relatively the lowest, and thus has the strongest bacteriostatic activity for the 8 plant phytopathogens. Also, it is known that n-butyl part has the strongest bacteriostatic activity for the two

bacteria of *Fusarium graminearum* and *Sclerotinia sclerotiorum*, with the smallest MIC value of only 1.953 μg · mL^{-1}.

TABLE 1 : The antifungal activity of the 8 samples from the *Arthrinium sp* L1 as MIC

Sample	MIC of the 8 samples on different fungal (μg · mL^{-1})			
	Mthanol extract	Petroleum ether fraction	EtoAc fraction	n-BuOH fraction
Botrytis cinerea	125	250	15.625	15.626
Exerohilum turcicum	62.5	125	62.5	31.25
Mucor	15.625	125	31.25	7.813
Fusarium graminearum	31.25	62.5	15.625	1.953
Sclerotinia sclerotiorum	15.625	31.25	3.906	1.953
Rhizoctonia solani AG1-IA	31.25	62.5	15.625	3.906
Rhizoctonia solani Kühn	62.5	125	3.906	31.25
Fusarium graminearum Schwabe	62.5	125	15.625	3.906

The Determination Results of the Free Radical Scavenging Ability: In the DPPH evaluating system, the following four samples, the crude extracts of *Arthrinium sp L1* and the 3 different extract parts have some differences in the scavenging capacity for the DPPH free radicals. IC_{50} value is an indicator in evaluating the anti-oxygen ability. It refers to the needed concentration that the antioxidant should have in scavenging 50% of the DPPH free radicals, and that the smaller its value, the stronger their scavenging abilities, and the stronger the antioxidant activity of the corresponding tested samples. TEAC value refers to the fact that how much standard substance of Trolox (mg) can equal the antioxidant activity of 1 g dried sample. The smaller the MIC value, and the bigger the TEAC value, the stronger their scavenging abilities, and the stronger the antioxidant activity of the corresponding tested samples. It is shown in Figure 3 that the 4 samples of *Arthrinium sp L1* all have certain scavenging activity on the DPPH free radicals. With the increasing of the concentration of the tested samples, they have bigger and bigger scavenging ability. So there is a positive correlation between the free radical scavenging rate and the concentration of the samples. Among them, the TEAC value of the petroleum ether is the smallest, only about 9.65±0.21 mg TE/g DW, and this shows that it has the minimal free radical scavenging rate, and it also has the weakest antioxidant ability. While the TEAC value in the ethyl acetate extract part is the highest, reaching 31.19±0.18 mg TE/g DW, which shows that it has the strongest free radical scavenging ability and the strongest antioxidant activity.

In the FRAP evaluating system, different tested samples all show certain antioxidant ability, but there are some differences in the antioxidant activity. Calculate the antioxidant activity according to the absorption values, we will know that the higher the absorption value, the stronger the antioxidant activity. Therefore, in this experiment, we still choose TEAC value as the indicator, that is, when the absorption degree reaches 0.5, the tested substance corresponds to the content of Trolox. The higher the TEAC value, the stronger its reducing ability, and this can be seen in Figure 2. From this Figure 2, we know that the TEAC value in the petroleum ether is the smallest, only about 13.96±0.74 mg TE/g DW, which shows that it has the worst antioxidant ability. The TEAC value in the ethyl acetate extract part is the biggest, reaching 28.80±1.63 mg TE/g DW, that is, 1 g of the dried sample amounts to the antioxidant ability of the Trolox standard of 28.80±1.63) mg. And it also shows that the ethyl acetate extract part has the strongest antioxidant ability. In the $ABTS^{\cdot+}$ evaluating system, we still choose TEAC value as the reducing ability of the substances. From TABLE 2, we know that different tested samples have different scavenging abilities in removing the $ABTS^{\cdot+}$ free radicals. Among them, ethyl acetate extract part has the strongest scavenging ability for free radicals, its TEAC value reaching 48.35±3.63 mg TE/g DW, while the petroleum ether part has the weakest scavenging ability and the worst antioxidant power, its TEAC value being the smallest, only about 26.72±1.16 mg TE/g DW. Through evaluating the scavenging abilities by 3 kinds of methods DPPH, FRAP, and $ABTS^{\cdot+}$, it is shown that there is a consistency among them, that is, in the four samples, the crude extract of *Arthrinium sp L1* and

the 3 different kinds of extraction parts, the ethyl acetate part has the strongest scavenging ability and the antioxidant power, while the petroleum ether part has the worst scavenging ability and the weakest antioxidant activity.

TABLE 2 : The comparison of the free radical scavenging abilities between different parts of *Arthrinium sp* L1

Sample	DPPH(TEAC)	FRAP(TEAC)	$ABTS^{\cdot+}$(TEAC)
Mthanol extract	21.74±0.06	21.25±0.45	39.55±1.25
Petroleum ether fraction	9.65±0.21	13.96±0.74	26.72±1.16
EtoAc fraction	31.19±0.18	28.80±1.63	48.35±3.63
n-BuOH fraction	18.34±0.25	19.56±2.11	41.57±3.15

Note: TEAC data as Mean±SD, n=3. Unit: mg TE/g DW.

The Result Analysis for the Cytotoxic Activity: The fatal-to-prawn biological assaying is often used in America to test the residues of the insecticide and the analysis of the mycotoxin, etc. The research shows[21] that nearly all the natural bioactive compounds have some toxicity when taken in large doses. There is a correlation between their fatality rate for the simple animal like prawn and their inhibitory rate for the cancer cells. Thus, we have achieved a satisfactory result when we use Fatal-to-Prawn Bioactivity Testing Method to screen the anti-cancer medicine. The LC_{50} value of the half-death concentration refers to the needed concentration of the sample when the death rate is 50%. The smaller the LC_{50} value, the stronger the cytotoxic activity. According to the methods recorded in literature[22], after hatching the prawn eggs into larva, divide them into different groups with 30 larva and put them in the prepared groups with different concentrations(the samples are solubilized with 1% DMSO, and use the artificial sea water solution added with DMSO as the blank control). According to the reports, when the LC_{50} value of the plant crude extracts is smaller than 500 $\mu g \cdot mL^{-1}$, and the LC_{50} value of the monomeric compound is smaller than 50 $\mu g \cdot mL^{-1}$, it shows that the tested samples have relatively strong cytotoxic activity.

From TABLE 3, the fatal-to-prawn experiment, it shows that the following four samples, the crude extracts of *Arthrinium sp L1* and the 3 different extract parts have some cytotoxic activity. Among them, the ethyl acetate extract part of the *Arthrinium sp L1* has the strongest cytotoxic activity, and the smallest LC_{50} value is about 176.875 $\mu g \cdot mL^{-1}$. While the petroleum ether part has the worst cytotoxic avtivity, and the LC_{50} value is 347.5 $\mu g \cdot mL^{-1}$. There is no death in the control group, which shows that the endophytic fungi *Arthrinium sp L1* has relatively strong cytotoxic activity, and the preliminary bioactive screening for the prawn shows that it also has relatively strong anti-cancer activity.

TABLE 3 : The Test-results of the biological activity of the lethal-to-prawn larva

Samples	Death rate in different concentration					LC_{50} $\mu g \cdot mL^{-1}$
	10 $\mu g \cdot mL^{-1}$	50 $\mu g \cdot mL^{-1}$	100 $\mu g \cdot mL^{-1}$	500 $\mu g \cdot mL^{-1}$	1000 $\mu g \cdot mL^{-1}$	
Mthanol extract	14%	41%	53%	92%	100%	189.375
Petroleum ether fraction	10%	21%	44%	75%	98%	347.5
EtoAc fraction	10%	41%	60%	94%	100%	176.875
n-BuOH fraction	6%	32%	56%	83%	100%	263.75

CONCLUSIONS

In this experiment, we separated, extracted and purified the endophytic fungi from the *Pleioblastus amarus,* and from this experiment we determined that it is Arthrinium sp L1. After fermenting the Arthrinium sp L1, we extracted it and got the petroleum ether part, the ethyl acetate extract, and the n-butyl part. Then we made the testing and evaluation of bacteria-inhibiting,

antioxidation, and cytotoxicity for the following four samples of Arthrinium sp L1, the crude extracts, the petroleum ether part, the ethyl acetate extract part and the n-butyl part.

The experiment of the bacteriostatic activity shows that in the four extracts of the *Arthrinium sp L1,* the MIC value of the n-butyl part is the smallest, and it has the relatively strong bacteriostatic activity for the 8 plant phytopathogens. And the n-butyl part has the strongest bacteriostatic activity for the two strains of *Fusarium graminearum* and *Sclerotinia sclerotiorum*, and its MIC value is the lowest, only about 1.953 $\mu g \cdot mL^{-1}$.

The antioxidant activity experiment shows that the *Arthrinium sp L1* shows the ability to remove the free radicals and the reducing ability, and it shows a clear dose-effect relationship. It is known that the three methods of removing the free radicals DPPH, FRAP, and ABTS all have certain uniformity, and among them, the ethyl acetate extract part has the strongest antioxidant activity, while the petroleum ether part has the weakest antioxidant activity.

Through the fatal-to-prawn experiment, it shows that the four samples of the crude extracts of *Arthrinium sp L1* and the 3 different extracting parts all have certain cytotoxic activity. And the ethyl acetate extract part has the strongest cytotoxic activity with the LC_{50} value being the lowest, about 176.875 $\mu g \cdot mL^{-1}$. The petroleum ether part has the weakest cytotoxic activity and the LC_{50} value is 347.5 $\mu g \cdot mL^{-1}$.

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