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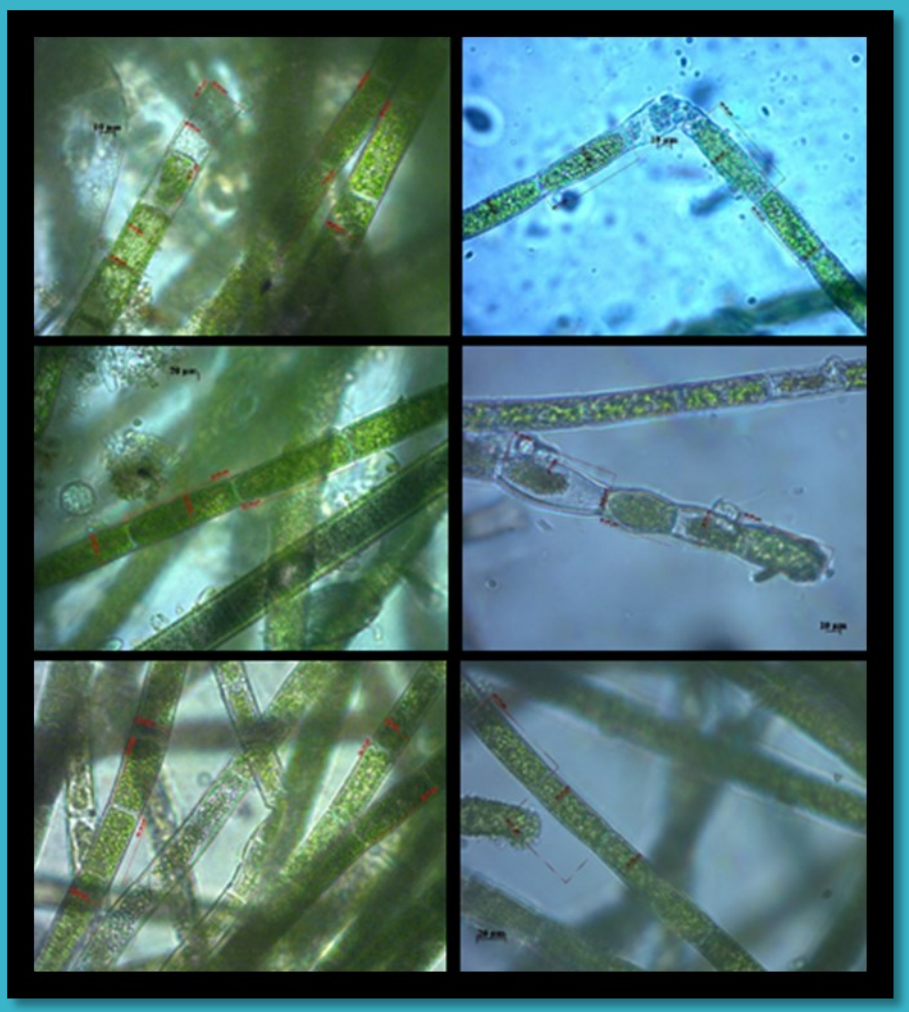

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Keterangan foto cover depan (*Notes of cover picture*): Pertumbuhan *Oedogonium* sp. pada perlakuan cahaya yang berbeda. *Oedogonium* sp. Pada kultur Outdoor tampak lebih padat daripada kultur indoor, sesuai dengan halaman 309 (*Growth of Oedogonium sp. at different light treatments. Oedogonium* sp *in outdoor culture appeared denser than in indoor culture, as in page 309)*

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ANTIFUNGAL ACTIVITY OF CRUDE EXTRACT FROM *Nocardia* sp. ATS-4.1 AGAINST *Candida albicans* InaCC-Y116

[Aktivitas Antifungi Ekstrak Isolat *Nocardia* sp. ATS-4.1 Terhadap Jamur *Candida albicans* InaCC-Y116]

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ABSTRACT

Candida albicans is a microorganism that knows as caused of candidiasis. *Nocardia* is known to have the ability to produce antifungal bioactive compounds to overcome cases of fungal infections. This research aims to determine the presence of antifungal activity and the good concentration from crude extract of *Nocardia* sp. ATS-4.1 to inhibit *C. albicans* InaCC-Y116 and. Antifungal activity test of isolate *Nocardia* sp. ATS-4.1 was performed using a well diffusion method on Mueller Hinton Agar (MHA) medium with a concentration of 92%, 94%, 96%, 98%, 100% and nystatin (positive control) 0,0125% and DMSO 10% (negative control) incubated at 37 °C for 24–48 hours. The results showed that the extract of *Nocardia* sp. ATS-4.1 can inhibit the growth of *C. albicans*. *Nocardia* sp. ATS-4.1 isolate extract concentration of 96% with a resistance diameter of 13.63 ± 0.53 mm with a strong category against the growth of *C. albicans* InaCC-Y116.

Keywords: antifungal, *Candida albicans*, *Nocardia* sp., inhibitory zone

ABSTRAK

Candida albicans merupakan salah satu mikroorganisme penyebab infeksi penyakit kandidiasis. *Nocardia* diketahui memiliki kemampuan menghasilkan senyawa bioaktif antifungi untuk mengatasi kasus infeksi jamur. Penelitian ini bertujuan untuk mengetahui adanya aktivitas antifungi, konsentrasi yang baik dari ekstrak kasar isolat *Nocardia* sp. ATS-4.1 untuk menghambat jamur *C. albicans* InaCC-Y116. Pengujian aktivitas antifungi ekstrak isolat *Nocardia* sp. ATS-4.1 dilakukan menggunakan metode difusi sumuran pada media *Mueller Hinton Agar* (MHA) dengan konsentrasi 92%, 94%, 96%, 98%, 100% serta nistatin (kontrol positif) 0,0125% dan DMSO 10% yang diinkubasi pada suhu 37°C selama 24–28 jam. Hasil penelitian menunjukkan bahwa ekstrak isolat *Nocardia* sp. ATS-4.1 dapat menghambat pertumbuhan jamur *C. albicans*. Ekstrak isolat *Nocardia* sp. ATS-4.1 konsentrasi 96% menghasilkan zona hambatan sebesar 13,63±0,53 mm dengan kategori kuat terhadap pertumbuhan jamur anggota spesies *C. albican* InaCC-Y116.

Kata kunci: antifungi, *Candida albicans*, *Nocardia* sp., zona hambat

INTRODUCTION

Candida albicans is a fungal known as the cause of candidiasis. It attacks the vagina, mucous membranes of the mouth, and some parts of the digestive tract and inflammation of the lining of the brain, heart, and blood (septicemia) to ultimately cause death (Pelczhar and Chan, 2007). According to research conducted by Kalista *et al.* (2017) at Cipto Mangunkusumo's hospital that the prevalence of mortality of candidiasis vaginalis reached 64.8% of hospitalized patients. Therefore, cases of fungal infections need to get effective treatment.

Topical medicines are used to treat candidiasis that is often such as miconazole, ketoconazole, clotrimazole, fluconazole, and others. However, there are some disadvantages of using antifungal drugs such as side effects on the body, bad penetration in certain tissues and the appearance of resistant fungal (Anindita and Santi, 2006; Central for Disease and Prevention, 2013). The researchers often continue to look for alternative antifungal sources that are safer, are able to kill and prevent fungal infections recently. Some secondary metabolite compounds from the class of *Actinomycetes,* such as the genus of *Nocardia,* is known to be able to produce antifungal bioactive compounds to overcome cases of fungal infections (Solecka *et al.*, 2012).

Nocardia is a microorganism mostly distributed in soils as a decomposer agent that belongs to the class of *Actinomycetes*. Some of these species that have been successfully isolated from soil can produce antifungal compounds (Komatsu *et al.*, 2004). Research by Sharma *et al.* (2016) successfully tested the ability of *Nocardia* sp. PB-52 isolated from rice samples from Pobitora, Assam, India as a source of antifungal compounds in the form of phenols, esters and *phenoland 2,4-di-t-butyl-6-nitrophenol belonging* against fungi as *C. albicans*.

*Kontributor Utama
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Besides, other antifungal compounds that can be produced by *Nocardia* in the form of belonging to the *pradimicins* and *polyenes* (Kerr, 1999). Other studies have shown *Actinomycetes* isolate belonging to the genus of *Nocardia* sp. TP1 and TP5 isolated from the mountain region of Tangkuban Perahu have an antifungal compound activity of 16 mm and 12 mm in inhibiting the growth of *Microsporum gypseum* (Sukandar *et al*., 2015).

Based on the results of isolation and initial identification obtained *Actinomycetes* isolate belonging to the *Nocardia* sp. ATS-4.1 originating from a rice field with a depth of 20 cm in the village of Cempaka, Kapuas Hulu Regency, West Kalimantan (Abdullah *et al*., 2020). According to research conducted by Sharma *et al*. (2016), Gendy *et al*. (2008), Kavitha *et al*. (2009), Mukai *et al*. (2009), Sun *et al*. (2007), the bacteria group of *Nocardia* has the potential to produce antifungal compounds. However, there is no scientific data that explains the ability of isolate *Nocardia* sp. ATS-4.1 in terms of producing antifungal compounds so that the need for further research on *Nocardia* sp. ATS-4.1 isolates as antifungal producers.

MATERIALS AND METHODS

Experimental design

The research used a completely randomized design with seven levels of treatment namely dimethyl sulfoxide (DMSO) 10% (negative control), nystatin (positive control) 0,0125%, extract concentrations of 92%, 94%, 96%, 98%, and 100%, respectively. The level was repeated four times so that 28 units of the experiment were obtained.

Tools sterilization

The tools as Petri dishes, test tubes, 250 ml Erlenmeyer, 500 ml Erlenmeyer, 1000 ml Erlenmeyer, beaker glass, measuring cup and test tube were first wrapped in plastic so that no air is left in it at all. Furthermore, these tools were inserted into the autoclave and regulated at 121 °C with a pressure of 1 atm for 15 minutes (Waluyo, 2008).

Preculture of isolates

The isolate of *Nocardia* sp. ATS-4.1 were pre-cultured by taking one of bacteria colony with a sterile inoculating 1 loop and then transferred to Petri dish containing Starch Casein Agar (SCA) medium and incubated for 8 x 24 hours at 28°C (Sharma *et al*., 2016). The isolate of *Candida albicans* InaCC-Y116 precultured by taking one of fungi colony with a sterile inoculating loop and then transferred to a Petri dish containing Sabouraud Dextrose Agar (SDA) medium. Subsequently incubated at 37 °C for 1 x 24 hours (Nadeem *et al*., 2013; Soleman and Setiawan, 2017).

The growth curve of isolate *Nocardia* sp. ATS-4.1

Preparation of growth curves of *Nocardia* sp. ATS-4.1 was initiated by making a starter culture. Five ml of *Actinomycetes* isolate from pure culture were inoculated into 50 ml of Starch Casein Broth (SCB) medium then incubated in a shaker at 150 rpm at room temperature for five days (Haque *et al*., 2016). Furthermore, as much as 0.5 ml of starter culture was inoculated back into the new SCB medium by 5 ml in 23 test tubes (Pertiwi *et al*., 2015). Culture then incubated in the shaker with 150 rpm agitation at room temperature for 23 days. Observations were made by means of the incubation results filtered using Whatman paper no. 1 then dried using an oven at 50 °C to separate the liquid and cells. The weighing process is carried out to dry weight of cells then recorded the results to obtain a constant weight (Sharma *et al.,* 2016; Rendowaty *et al.,* 2017). The observation process began on day 0–22.

The metabolites extraction of *Nocardia* sp. ATS-4.1

One loop of isolate *Nocardia* sp. ATS-4.1 was inoculated on 50 ml of SCB medium into a 100 ml Erlenmeyer, then incubated in a shaker at 150 rpm for 5th days at room temperature (Alimuddin *et al*., 2016). The results of the 5th-day culture are referred to as starter cultures. As many as 5 ml starter culture of *Nocardia* sp. ATS-4.1 was inoculated into 50 ml SCB in a 100 ml Erlenmeyer and then incubated in a shaker at room temperature for 14 days. The culture was then transferred to a centrifuge tube and centrifuged at 3,000 rpm for 15 minutes. After centrifugation, the supernatant was collected and

used for screening using Whatman no. 1 filter paper. The supernatant layer is taken as a source of secondary metabolites. The process of extracting metabolites from isolates *Nocardia* sp. ATS-4.1 was carried out by means of supernatant that had been successfully separated beforehand soaked in 100% methanol solvent for 24 hours at a v/v ratio (1:1). The extract was concentrated using a rotary evaporator then let stand for 24 hours (Omran and Kadhem, 2016; Mulyadi and Sulistyani, 2012). The extract was dissolved in DMSO 10% prior to assay (Sharma *et al.,* 2016).

Preparation of fungal suspension test

As many as one loop of *Candida albicans* InaCC-Y116 previously put into 2 ml of 0.9% physiological NaCl solution. Then the solution is homogenized with a vortex. The level of turbidity adjusted to Mc Farland 0.5 standard which is equivalent to a cell concentration of 1.5×10^8 CFU / ml (Song *et al.*, 2015).

Antifungal activity test

Antifungal activity test was conducted using wells method as previously described (Magaldi *et al.*, 2004) on Mueller Hinton Agar (MHA) medium surface. A well was made in the medium with a 6 mm diameter. The extract produced by *Nocardia* sp. ATS-4.1 as much as 25 µL 10% DMSO as a negative control, positive control nystatin 0,0125% and supernatant extract dilution to 92% (0.92 g/ml), 94% (0.94 g/ml), 96% (0.96 g/ml), 98% (0.98 g/ml), and 100% (1 g/ml) were incubated for 24–48 hours at 37 °C to measure the diameter of the clear zone formed using calipers (CLSI, 2017; Nadeem *et al.*, 2013). Antifungal produced by *Nocardia* sp. ATS-4.1 was analyzed based on the clear zone (zone of resistance) formed (Mulyadi and Sulistyani, 2012). Based on the calculation of the inhibition zone area observed in the medium: the inhibition zone can be categorized as follows, for diameters >20 mm are categorized as very strong, 11–20 mm are categorized as strong, 6–10 mm are categorized as moderate and <5 mm are categorized as weak (Rios *et al.*, 1988).

Data analysis

Measurement data on inhibition zone diameters were statistically analyzed using an Analysis of Variance (ANOVA) one way with a level of 5% using the SPSS program version 20.0. If there are significantly different, further tests were conducted using the Duncan Multiple Range Test (DMRT) with an error level of 0.05.

RESULTS

The growth curve of *Nocardia* sp. ATS-4.1

The growth curve of *Nocardia* sp. ATS-4.1 was performed by measuring the dry weight of cells formed in the growing medium. Measurement of the dry weight of cells was carried out for 22 days as shown in Figure 1.

Figure 1 shows the growth phase of the *Nocardia* ATS-4.1 for approximately three weeks incubation. On day 0, *Actinomycetes* isolates were in the phase of adjustment to growth medium (*lag phase*). The coming day, on day 1 to day 14 (last log phase) the isolate was in the accelerated growth phase (log phase) which was seen in a curve that experienced a significant increase in the dry weight of cells . Then on the 15th day until the 22th day was in the phase of death (*death phase*) which is shown in a curve that has decreased due to the reduced value of dry weight of cells.

The average inhibitory zone of isolate extract *Nocardia* sp. Ats-4.1 against the growth of *Candida albicans*

Antifungal activity of the supernatant extract of *Nocardia* sp. ATS-4.1 to the growth of *Candida albicans* InaCC-Y116 species can be detected from the average value of the inhibition zone diameter formed in each treatment incubated for 24 hours and 48 hours (Figure 2). Each treatment had a significantly different effects on the growth of *C. albicans* during the 24-hour incubation period ($F_{(4.15)} = 3.071$, $\rho = 0.049$; ANOVA) and 48 hours ($F_{(4.15)} = 3.197$, $\rho = 0.044$; ANOVA). Based on Duncan's further test results at an error level of 0.05 during the incubation period of 24 hours and 48 hours (Table 1).

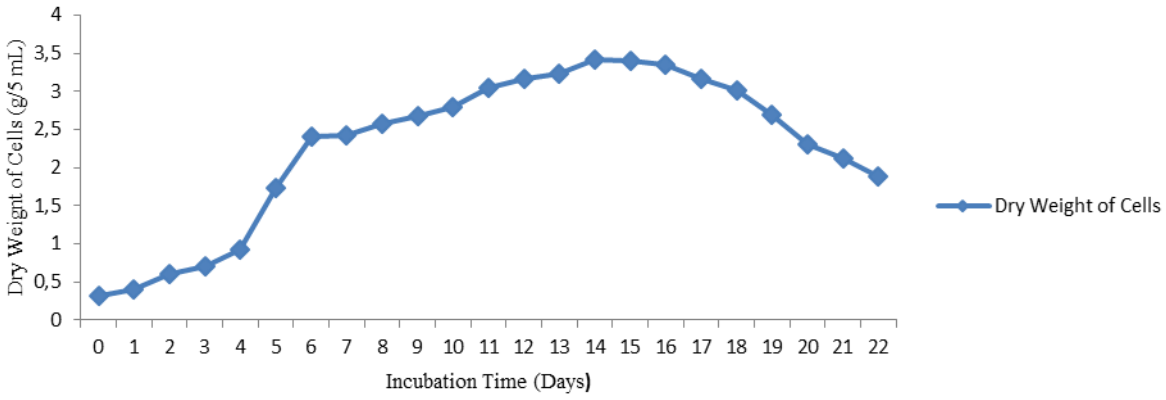

Figure 1. The growth curve of isolate *Nocardia* sp. ATS-4.1 is based on the length of incubation time for the dry weight of cells *(kurva pertumbuhan isolat Nocardia sp. ATS-4.1 berdasarkan lama waktu inkubasi terhadap berat kering sel)*

Table 1. The average diameter of the inhibitory zone of isolate extract *Nocardia* sp. ATS-4.1 against the growth of *Candida albicans* InaCC-Y116 *(Rata-rata diameter zona hambat ekstrak isolat Nocardia sp. ATS-4.1 terhadap pertumbuhan Candida albicans InaCC-Y116)*

Treatments	The average diameter of the inhibitory zone (mm)		The average diameter of the inhibitory zone (mm)			
	24 Hours	48 Hours	48 Hours	24 Hours	24 Hours	48 Hours
Negative control (DMSO 10%)	0±0[a]	0±0[a]	No	No	No	No
Positive control (nystatin)	16.43±0,36[c]	16.44±0,36[c]	Strong	Strong	Strong	Strong
Concentration 92%	0±0[a]	0±0[a]	No	No	No	No
Concentration 94%	13.63±0,53[b]	13.65±0,53[b]	Strong	Strong	Strong	Strong
Concentration 96%	15.38±0,44[bc]	15.41±0,43[bc]	Strong	Strong	Strong	Strong
Concentration 98%	15.69±0,30[bc]	15.73±0,30[bc]	Strong	Strong	Strong	Strong
Concentration 100%	17.45±0,57[c]	17.65±0,59[c]	Strong	Strong	Strong	Strong

Note: Figures followed by the same letters show results that are not significantly different at the0,05 level according to the Duncan test *(Angka yang diikuti huruf yang sama menunjukkan hasil yang tidak berbeda nyata pada taraf 0,05 menurut uji Duncan)*

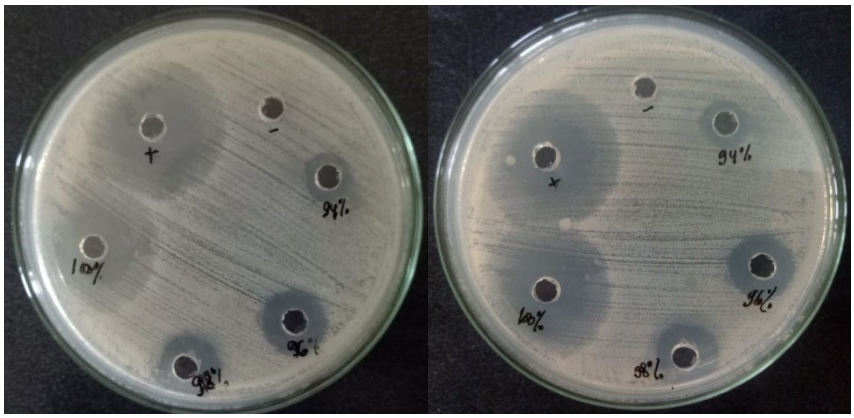

Figure 2. Inhibition zones of isolate extract *Nocardia* sp. ATS-4.1 against *C. albicans* InaCC-Y116
(Zona hambat ekstrak isolat Nocardia sp.ATS-4.1 terhadap jamur C. albicans InaCC-Y116)

DISCUSSION

Based on the research's results, *Nocardia* sp. ATS-4.1 has the ability to produce antifungal compounds against the fungus *Candida albicans* InaCC-Y116. The previous study by Kavitha *et al.* (2009) and Sun *et al.* (2007) showed antifungal compounds produced by *Nocardia* to inhibit *C. albicans*. Based on the results of the growth curve (Figure 1), the extraction time in this research was carried out on day 14th (the last of log phase). A study by Kumari *et al.* (2013) that isolates of *Streptomyces* sp. US7 MTCC 8723 showed an increase in the amount of dry weight of cells is directly correlated with the level of antimicrobial activity that occurred on the 21st day which is the last log phase. This is confirmed by the opinion of Augustine *et al.* (2005) that the production of bioactive compounds occurs in the last log phase and remains constant during the stationary phase. Bioactive compounds are produced by *Nocardia* through genomic pathways in genomic polyketides under conditions that are unfavorable for their growth and development (stressed response) (Cane and Walsh, 1999). However, there's no stationary phase of *Nocardia* sp. ATS-4.1 is based on measurements of dry weight of cells for the incubation time in Figure 1. This is thought to occur because the cell sensitivity of a bacteria group of *Actinomycetes* is relatively higher against the influence of stress both inside and outside the cell which causes the bacteria to be in a stationary growth phase in a relatively shorter period of time (Nolan and Cross, 1988).

Nocardia sp. ATS-4.1 has the effect of inhibiting the growth of *Candida albicans*. This inhibitory ability is detected by the formation of inhibition zone diameters in the antifungal activity tested using the wells method with the basic principle that the antifungal compounds used are diffused to the agar surface (Table 1). The results showed that the concentration of *Nocardia* sp. ATS-4.1 of 94%, 96%, 98%, and 100% have the ability to suppress the growth of *C. albicans* InaCC-Y116 with a strong resistance category. In addition, positive control using nystatin also forms a zone of inhibition against the growth of *C. albicans* InaCC-Y116 with a strong category. The inhibitory zone is formed because there is an antifungal compound(s) can inhibit fungal growth (Brooks *et al.*, 2013). The extract concentration treatment of 92% (Table 1) did not show any inhibitory activity with an inhibition zone formed of 0 mm. The antifungal substances of isolate extract *Nocardia* sp. ATS-4.1 is used to inhibit *C. albicans*' growth still low, so it is not able to react with the cell's component of *C.albicans*. This was confirmed by Cappucino and Sherman (1983) that a type of microorganism requires concentrations of certain antimicrobial compounds so that their growth is inhibited or killed. The isolate culture duration factor also influences the amount of

antifungal substances that can be formed by *Nocardia* sp. ATS-4.1. According to Aliero *et al.* (2018) higher or lower number of bioactive compounds produced by *Actinomycetes* can be influenced by the duration of culture.

Based on the observation's result, it was seen that an increase in the diameter of the zone of inhibition of *Nocardia* sp. ATS-4.1 on *Candida albicans albicans* InaCC-Y116's growth in the whole extract concentration treatment with incubation time for 24–48 hours (Table 1). This shows that the extract of isolate *Nocardia* sp. ATS-4.1 has the properties of killing *C. albicans* InaCC-Y116 or known as a fungicide. According to Brooks *et al.* (2013) fungicide is a mechanism of killing a type of fungus due to the side effects of a compound that reacts enzymatically so that it causes loss of function of fungal cells to grow and develop.

Based on the test's results Duncan Multiple Range Test, the average inhibition zone yield of *Nocardia* sp. ATS-4.1 on the growth of *Candida albicans* InaCC-Y116 with extract concentration of 96% had a significant effect on the concentration of the extract of the isolate *Nocardia* sp. ATS-4.1 namely 92%, 94%, 98%, 100%, and positive control with inhibition zone diameter of 15.38 ± 0.44 mm. This means that the extract concentration of 96% will have the same effect if treatment of the extract concentration is given namely 94%, 98%, 100%, and the treatment of antifungal substances (positive control).

Nystatin was used as a positive control in this research. Nystatin works by binding to one component of the fungal cell membrane in the form of ergosterol forming a pore that causes leakage of molecules from inside to exit the fungal cell (Baron, 1996). The results showed that a positive control of nystatin 0.0125% produced an inhibitory zone against *Candida albicans* InaCC-Y116 of 16.43 ± 0.36 mm. According to the Clinical and Laboratory Standard Institute (2009), nystatin is said to inhibit if it produces a zone of inhibition zone against *C. albicans* between 11–14 mm and is said to be sensitive to a diameter of resistance ≥15 mm. If it compares to the positive control treatment, the extract concentration of *Nocardia* sp. ATS.-4.1 as 94%, 96%, 98%, and 100% belong to the sensitive category for inhibiting *C. albicans* InaCC-Y116. Based on the intervals concentration tested, the extract concentration 96% can be categorized as an effective concentration to suppress the growth of *C. albicans* InaCC-Y116 with inhibition zone is 13,63±0,53 mm. An antifungal compound can be said to be optimal if in low concentrations but has a high ability to inhibit the growth of pathogenic fungi (Riesselman *et al.*, 2000). However, the treatment of 100% extract is better because the diameter of the inhibition zone is greater (17.65±0.59 mm compared to Nystatin as positive control.).

Based on the research's result that isolate *Nocardia* sp. ATS-4.1 is able to inhibit the growth of *Candida albicans* InaCC-Y116 due to the content of the bioactive compounds which has antifungal activity from the isolate extract *Nocardia* sp. ATS-4.1. Previous study by Sharma *et al.* (2016) mentioned that extracts of *Nocardia* PB-52, originating from the Brahmaputra river plain soils, able to produce approximately 20 types of active compounds. They are belong to phenol, quinone, hydrocarbons, esters and several acid compounds which have been known as an antifungal agent. Research by Gendy *et al.* (2008) succeeded in detecting the presence of phenol compounds from *Nocardia* sp. ALAA2000 originating from the sea. Phenol compounds are known to be used as anti-agents for *Candida* consisting of several derivatives of compounds such as *p-hydroxibenzoic* (Teodoro *et al.*, 2015), *p-coumaric* and caffeic acid (Maskovic *et al.*, 2011).

The Bacteria from genus of *Nocardia* are able to produce bioactive compounds in the form of antifungals. Most of these compounds are formed, one of which is through the flow of the polyketides genome (Herisse *et al.*, 2019). Polyketides are a group of natural compounds arranged in a structured manner which is formed by the *acyl-polimaloate* biosynthetic pathway (Money, 1973). Research by Ishikawa *et al.* (2004) managed to reveal the existence of 7 polyketide genes from the complete genome sequence from *N. farcinica* IFM 10152.

CONCLUSIONS

Based on the results, it can be concluded that there is an antifungal activity of crude extract from *Nocardia* sp. ATS-4.1 against *Candida albicans* InaCC-Y116, Concentration of extract of bacterial isolates from *Nocardia* sp. ATS-4.1 which is good for inhibiting the growth of *C. albicans* InaCC-Y116 is 96% with a relatively strong antifungal activity level based on the inhibition zone diameter of 13,63±0,53 mm.

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan atau baru, agar dapat segera diketahui oleh umum. Hasil dan pembahasan dapat digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul ditulis dalam huruf tegak kecuali untuk nama ilmiah yang menggunakan bahasa latin, Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*). Jika penulis lebih dari satu orang bagi pejabat fungsional penelitian, pengembangan agar menentukan status sebagai kontributor utama melalui penandaan simbol dan keterangan sebagai kontributor utama dicatatan kaki di halaman pertama artikel.
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam Bahasa Inggris merupakan terjemahan dari Bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
7. **Pembahasan**
   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, implikasi dari hasil penelitian dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review*. Apabila harus menyitir dari "laporan" atau "komunikasi personal" dituliskan '*unpublished*' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers* dan penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak spasi tunggal. Batas kiri-kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
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7. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
8. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.

8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al*. Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al*., 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995). Jika sitasi beruntun maka dimulai dari tahun yang paling tua, jika tahun sama maka dari nama penulis sesuai urutan abjad. Contoh: (Anderson, 2000; Agusta *et al*., 2005; Danar, 2005). Penulisan daftar pustaka, sebagai berikut:

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   b. **Buku**
      Anderson, R.C. 2000. *Nematode Parasites of Vertebrates, Their Development and Tramsmission*. 2nd ed. CABI Publishing. New York. pp. 650.
   c. **Prosiding atau hasil Simposium/Seminar/Lokakarya.**
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      Sausan, D., 2014. Keanekaragaman Jamur di Hutan Kabungolor, Tau Lumbis Kabupaten Nunukan, Kalimanan Utara. Dalam: Irham, M. & Dewi, K. eds. *Keanekaraman Hayati di Beranda Negeri*. pp. 47–58. PT. Eaststar Adhi Citra. Jakarta.
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      Himman, L.M., 2002. A Moral Change: Business Ethics After Enron. San Diego University Publication. *http:ethics.sandiego.edu/LMH/oped/Enron/index.asp*. (accessed 27 Januari 2008) bila naskah ditulis dalam bahasa inggris atau (diakses 27 Januari 2008) bila naskah ditulis dalam bahasa indonesia

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Setiap penulis yang mengajukan naskahnya ke redaksi Berita Biologi akan diminta untuk menandatangani lembar persetujuan yang berisi hak alih terbit naskah termasuk hak untuk memperbanyak artikel dalam berbagai bentuk kepada penerbit Berita Biologi. Sedangkan penulis tetap berhak untuk menyebarkan edisi cetak dan elektronik untuk kepentingan penelitian dan pendidikan. Formulir itu juga berisi pernyataan keaslian naskah yang menyebutkan bahwa naskah adalah hasil penelitian asli, belum pernah dan tidak sedang diterbitkan di tempat lain serta bebas dari konflik kepentingan.

Penelitian yang melibatkan hewan dan manusia
Setiap naskah yang penelitiannya melibatkan hewan (terutama mamalia) dan manusia sebagai obyek percobaan/penelitian, wajib menyertakan *'ethical clearance approval*' yang dikeluarkan oleh badan atau pihak berwenang.

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