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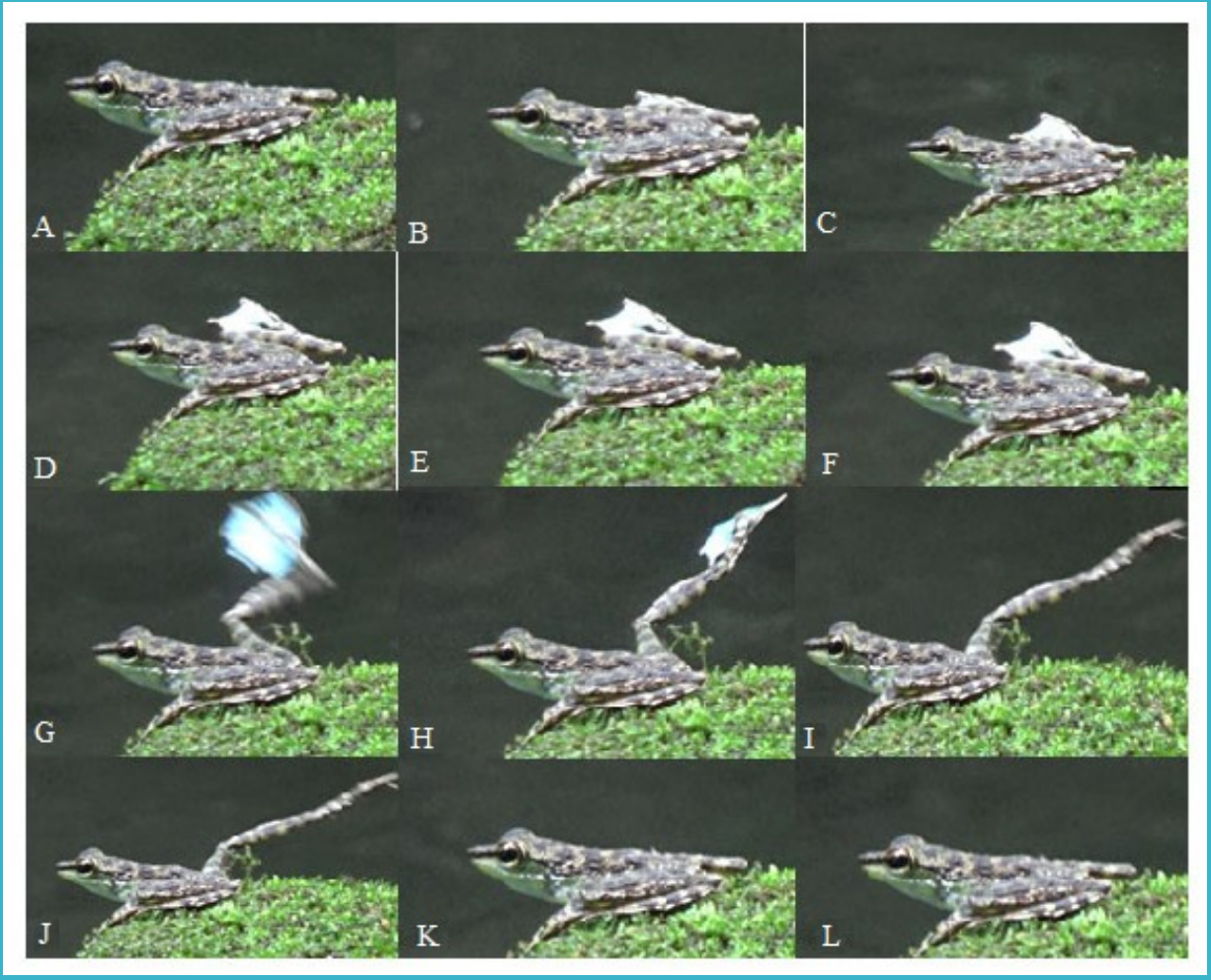

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Keterangan foto cover depan: *Sequence* gerakan yang ditunjukan selama *foot-flagging* pada katak jantan (*S. gutattus*); (A) saat istirahat; (B) angkat kaki; (C-F) ekstensi kaki parsial; (G-J) ekstensi kaki penuh; (K-L) istirahat, sesuai dengan halaman 385

(*Notes of cover picture*): (*Sequence of movements shown during foot-flagging in male frogs (S. gutattus); (A) at rest; (B) leg lift; (C-F) partial leg extension; (G-J) full leg extension; (K-L) rest*), *as in page 385)*

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THE FUNCTIONAL CHARACTER OF *Auricularia auricula* CRUDE POLYSACCHARIDES: ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

[Karakter Fungsional dari Ekstrak Kasar Polisakarida *Auricularia auricula*: Aktivitas Antioksidan dan Antibakteri]

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ABSTRACT

The food stuffs can be clasified as functional food since the foods can improve the human health. One of them are the food stuffs which have function as antioxidant and antibacterial. These activities were studied on crude polysaccharides from *Auricularia auricula*. The sample was subsequently isolated using hot water and 1 M NaOH to obtain water and alkali soluble of crude polysaccharides. The antioxidant activity was evaluated using β-caroten-linoleat assay. The results showed that the alkali soluble of crude polysaccharides had the highest antioxidant activity (85.82%) at 350 µg/ml. The water and alkali soluble of crude polysaccharides from *A.auricula* were evaluated for their antibacterial activity using disc diffusion method. The alkali soluble of crude polysaccharides was found to have the highest antibacterial activity at 100 mg/ml against *Staphylococcus aureus* InaCC B4 and *Escherichia coli* InaCC B5 with clear zone values of 3.18 mm and 5.10 mm, respectively. The findings indicated that the alkali soluble of crude polysaccharides from *A. auricula* could potentially be used in part of well-balanced diets and could be considered as a potential source of natural antioxidant and antibacterial products.

Keywords: antioxidant, antibacterial, *Auricularia auricula*, crude extract of polysaccharides

ABSTRAK

Bahan pangan dapat diklasifikasikan menjadi pangan fungional karena memiliki peran dalam meningkatkan kesehatan manusia. Salah satunya adalah bahan pangan yang memiliki fungsi sebagai antioksidan dan antibakteri. Aktivitas antibakteri dari ekstrak kasar polisakarida dari *A. auricula* telah dipelajari. Sampel diisolasi secara bertingkat menggunakan air panas dan NaOH 1M untuk mendaptakan ekstrak kasar polisakarida larut air dan larut alkali. Aktivitas antioksidan di ukur menggunakan metode β-karoten-linoleat. Hasil penelitian menunjukkan bahwa ekstrak kasar polisakarida larut alkali menghasilkan aktivitas antioksidan tertinggi, yaitu 85,82% pada konsentrasi 350 µg/mL. Ekstrak kasar polisakarida larut air dan larut alkali dari *A. auricula* diuji aktivitasnya terhadap bakteri *Staphylococcus aureus* InaCC B4 dan *Escherichia coli* InaCC B5 pada konsentrasi 100 mg/ml menghasilkan diameter zona hambat berturut-turut sebesar 3,18 mm dan 5,10 mm. Hasil penelitian ini menunjukkan bahwa ekstrak kasar larut alkali *A. auricula* berpotensi digunakan sebagai asupan harian dan berpotensi sebagai sumber alami dari produk antioksidan dan antibakteri.

Kata Kunci : antioksidan, antibakteri, *Auricularia auricula*, ekstrak kasar polisakarida

INTRODUCTION

There are two types of diseases, degenerative and infectious. Degenerative disease mostly caused by the free radicals like reactive oxygen species (ROS) (Araújo *et al*., 2016). They are produced continuously in all cells as part of normal cell metabolism or from environmental sources such as cigarette smoke, pollution, radiation and medications. The quantity of ROS produced by the human body and from environmental sources sometimes higher than the natural antioxidant-superoxide dismutase (SOD). Overproduction of ROS in the body can generate oxidative stress. When the concentration of free radicals increases, damage to amino acids, proteins, lipids, and DNA can occur, affecting homeostasis and cellular function. Furthermore the ROS attacks the cell and initiation of deleterious process that plays a major part in the development of degenerative diseases, such as arteriosclerosis, diabetes, cancer initiation, and has also been implicated in the aging process (Dewi *et al.*, 2012; Montes, Hernandez, dan Fenton 2019; Sylvie *et al*., 2014). The mechanism of antioxidants to prevent oxidative stress is by the neutralize of ROS molecules (Singh *et al.,* 2018). Therefore, antioxidant therapy represents a promising treatment for the degenerative disease.

Infectious diseases remain one of the major threats to human health. One common of infectious diseases is caused by the bacterial activity at human body that is cannot be performed by immune system. The infectious diseases can be treated by the compounds from outside the body which can inhibit or kill the bacteria called as antibacterial (Zaman *et al*., 2017). The discovery of antibacterial agents both natural and synthetic always be a interesting issue as

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long as the resistance of the infectious bacteria also grow up along the time. In particular, infections with methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus* (VRSA) strains have emerged as health problems. Antibiotic resistance was developed when a drug loses its ability to kill or inhibit bacterial growth effectively. Bacteria become resistant and continue to multiply in the presence of antibiotics (Dandawate *et al*., 2019). Therefore, novel antibiotic agents from different biological sources are continuously sought. The natural products inside the mushroom may have high benefit to human as long as the discovery of the products can be stored to the drug library as a drug candicate on the futhur investigation, such as antibacterial drug.

Both additional antioxidant and antibacterial are importantly needed by our body to prevent degenerative and infectious diseases. They may be taken from fruits, vegetables or other foods such as mushroom. Mushrooms have been used for centuries in China, Japan and Korea as food and medicine. In general, cultivated mushrooms contain little fat (1.7 %) and digestible carbohydrates (66.1%), but have higher protein contents than most vegetables (12.5%). They are also rich in vitamins such as vitamin B1 (thiamine) B2 (riboflavin), vitamin B3 (niacin), B7 (biotin), vitamin C (ascorbic acid) , and vitamin E. Making them suitable for low calorie diets (Kadnikova *et al*., 2015; Kim *et al*., 2008). The polysaccharides from mushrooms have also been reported have antioxidant properties (Luo *et al.,* 2011). Several cultivated edible mushrooms such as *Lentinus edodes* have significant antioxidant, higher SOD activity, decrease malondialdehyde (MDA) content, inhibit lipid peroxidation, and effectively protect cells from oxidative damage as well as repair damaged cells, delaying of skin aging and free radical scavenging activities (Zi *et al*., 2018). Mushrooms also need antibacterial and antifungal compounds to survive in their natural environment. Although the research on antioxidant and antibacterial activity of mushroom such as *L. edodes* has been widely carried out, the research on antioxidant and antibacterial activity of crude polysaccharides of *A.auricula* is still not completely done. Therefore, antioxidant and antibacterial compounds isolated from *A. auricula* could bring some benefits for humans. This study aims to observe the antioxidant and antibacterial activity of *A. auricula,* one of famous mushroom. In additional, this study is purposed to prove that *A. auricula* is potential as functional food.

MATERIALS AND METHODS

The fruiting body of *A.auricula* were obtained from working culture isolate at mushroom cultivation in Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences-LIPI. The samples were then extracted using hot water and NaOH 1 M to obtained water and alkali soluble of crude polysaccharides. The alkali soluble of crude polysaccharides was neutralized using CH_3COOH to reach pH 7. The Gram negative *E. coli* InaCC B5 and Gram positive *S. aureus* InaCC B4 were used as a tested microorganism. Other experimental materials included linoleic acid, β-caroten, tween 80, chloroform, Nutrient agar (NA), nurient broth (NB), alcohol 70%. All materials were purchased from Sigma as analytical grade unless otherwise stated.

Preparation of samples

The fresh fruiting body of *A.auricula* is washed using distilled water and mashed using a blender. The fruiting body of sample was frozen and lyophilized at 50^o C. The powder was then sifted until the particles measured less than 20 mesh (Bach *et al.,* 2019).

Isolation of crude polysaccharides

The water and alkali soluble of crude polysaccharides were isolated from the fruiting body of *A.auricula.* A total of 20 g simplicia powder was extracted with hot water for 4 hours at 90 ^{o}C. The suspension was centrifuged at 8,000 g for 30 minutes. The supernatant was collected as water soluble of crude polysaccharides while the pellet was re-extracted using NaOH 1M (alkali solvent). Extraction was done in a cold state for 4 hours. The suspension of *A. auricula* was then centrifuged at 8,000 g for 30 minutes. The supernatant was collected and adjust with acetic acid solution to obtain pH 7. Supernatant of crude polysaccharides

was evaporated using rotary vacum evaporator at 50 ^{0}C and dried using a freeze-dryer (Synytsya *et al*., 2009).

β-Carotene- linoleate model assay

Antioxidant activity of water and alkali soluble of crude polysaccharides were determined using the β-carotene-linoleate model assay according to Jayaprakahsa *et al.* (2001) method. Briefly, a solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml chloroform. One ml of β-carotene solution was transferred into Erlenmeyer flask containing 10 mg linoleic acid and 100 mg Tween-80. Chloroform was removed by placing the β-carotene-linoleic reagent in the fume hood for 30 minutes. 25 mL of distilled water was added in to the residue, slowly with vigorous agitation, to form an emulsion. 2.4 mL aliquots of the emulsion were transferred into different test tubes containing 0.1 mL of samples. The tubes were shaken and incubated at 50° C. Absorbance readings were then recorded between 30 min and 120 min. The absorbance was measured at 470 nm using spectrophotometer BioSpect-1601. Ascorbic acid was used as standard for this assay (Dewi *et al*., 2012).

Antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = \left(1-\frac{As0-Ase}{Ac0-Ace}\right) \text{X } 100\ \%$$

where : As0 was absorbance of the sample at 0 min
Ase was absorbance of the sample at 120 min
Ac0 was absorbance of the control at 0 min
Ace was absorbance of the control at 120 min

Determination of antibacterial activity using disc diffusion method

The antibacterial assay were carried out according to the method of Febriyani *et al.,* (2018) with some modification. The bacterial isolates were cultured in the sterilized medium (both Nutrient Broth and Nutrient Agar). All glassware and medium are sterilized using an autoclave. The *E. coli* InaCC B5 and *S. aureus* InaCC B4 bacteria were cultured in sterilized Nutrient Broth (NB) Sigma medium for 24 h. After 24 h incubation, 0.02 mL bacterial suspension of *S. aureus* and 0.04 mL bacterial suspension of *E.coli* added in to 20 mL Nutrient Agar (NA) 50% (1% NA powder in distilled water). A total of 10 mL Nutrient Agar (NA) were poured into a sterile petri dish. The bacterial suspension (5 mL) was dropped over the media. Paper disc, previously exposed to 30 µL sample with different concentration was planted in the media. Distilled water was used as the negative control while Choramphenicol 100 ppm was used as the positive control. Antibacterial activity was determined by measuring a clear zone of inhibition around the paper disc (Febriyani *et al*., 2018).

RESULTS

Isolation of crude polysaccharides

The crude polysaccharides was successfully obtained by isolation process using hot water and NaOH 1 M. A total of 20 g simplicia powder from *A.auricula* results 1.71 g water soluble of crude polysaccharides and 2.23 g alkali soluble of crude polysaccharides.

Antioxidant activity using β-Carotene- linoleate model assay

Antioxidant activity of water and alkali soluble of crude polysaccharides from *A. auricula* were determined at 100 µg/ml to 350 µg/ml (Table 1). Alkali soluble of crude polysaccharides *A. auricula* demonstrated the highest antioxidant activity at 350 µg/ml (85.82±0.008%) while water soluble of crude polysaccharides (66.75±0.012%) and ascorbic acid as positive control (6.91±0.056%) (Figure 1).

Antibacterial activity

The antibacterial activity of the crude polysaccharides from *A. auricula* as measured using disc diffusion method showed in Table 2. The results showed that the water soluble of crude polysaccharides and alkali soluble of crude polysaccharides were able to inhibit the growth of *S. aureus* and *E. coli* as indicated by the presence of a clear zone around the disc paper (Figure 2). The

Table 1. Antioxidant activity of water and alkali soluble of crude polysaccharides from *A.auricula* (*Aktivitas antioksidan ekstrak kasar polisakarida larut air dan alkali dari A. auricular)*

Concentration of crude polysaccharides (µg/ml)	Antioxidant activity of water soluble of crude polysaccharides (%)	Antioxidant activity of alkali soluble of crude polysaccharides (%)	Antioxidant activity of ascorbic acid (%)
100	9.32±0.005	16.73±0.001	
150	19.36±0.003	42.34±0.007	
200	19.52±0.027	64.24±0.026	
250	21.85±0.075	82.37±0.058	
300	30.84±0.015	85.56±0.003	
350	66.75±0.012	85.82±0.008	6.91±0.056

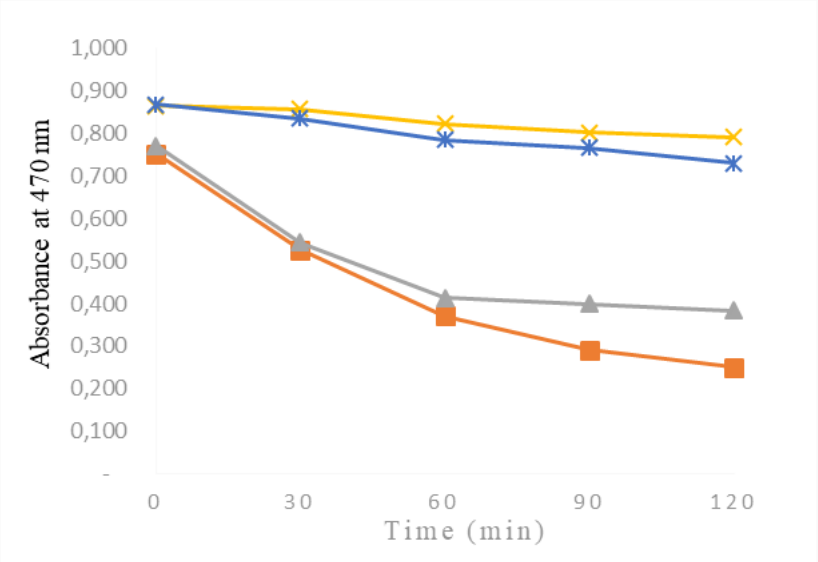

Figure 1. Antioxidant activity of crude polysaccharides from *A.auricula* and ascorbic acid at 350 µg/ml in β-caroten linoleat model system. Water soluble crude polysaccharides ( ), alkali soluble crude polysaccharides ( ), ascorbic acid ( ) and distilled water ( ). Data are presented as the mean ± S.D. of triplicate measurements [*aktivitas antioksidan dari ekstrak kasar polisakarida A.auricula dan asam askorbat pada 350 µg/ml pada sistem β-karoten linoleat. Ekstrak kasar polisakarida larut air ( ), ekstrak kasar polisakarida larut alkali ( ), asam askorbat ( ) and akuades ( ). Data menunjukkan ± S.D. dengan tiga kali pengulangan]*

strongest antibacterial activity was showed by the alkali soluble of crude polysaccharides from *A. auricula* compared to water soluble of crude polysaccharides. The alkali soluble of crude polysaccharides demonstrated the highest antibacterial activity at 100 mg/ml to inhibit the growth of *S. aureus* and *E. coli* with clear zone diameters 3.18±0.333 mm and 5.10±0.665 mm respectively.

Table 2. Clear zone diameters of water and alkali soluble of crude polysaccharides from *A. auricula* on bacteria *S. aureus* InaCC B4 and *E. coli* InaCC B5 (*diameter zona bening dari ekstrak kadar polisakarida larut air dan larut alkali A. auricula terhadap bakteri S. aureus dan E. coli*)

Concentration of crude polysaccharides (mg/ml)	Water soluble of crude polysaccharides		Alkali soluble of crude polysaccharides	
	***S. aureus* (mm)**	***E. coli* (mm)**	***S. aureus* (mm)**	***E. coli* (mm)**
100	1.07±0.134	0.93±0.395	3.18±0.333	5.10±0.665
50	1.03±0.126	1.30±0.367	1.75±263	3.18±0.171
25	0.90±0.384	0.88±0.685	0.93±0.030	1.38±-0.146
Choramphenicol (+ control)	25.49	16.21	16.21	25.49
Distilled water (-control)	0	0	0	0

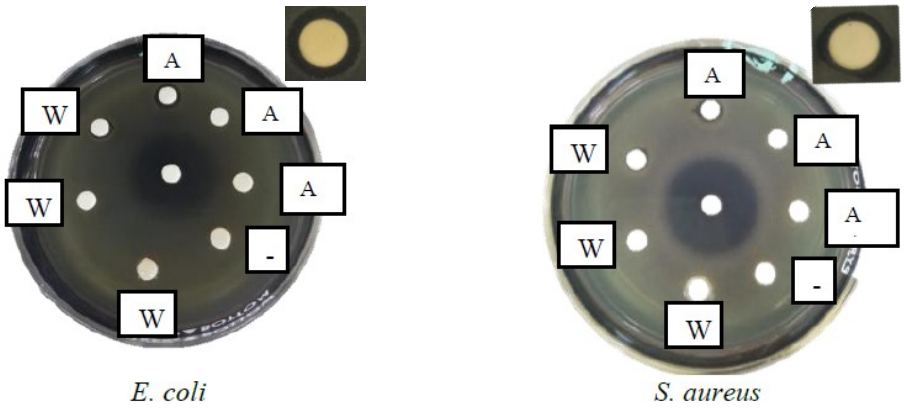

Figure 2. Antibacterial activity of crude polysaccharides on bacteria *E. coli* InaCC B5 and *S. aureus* InaCC B4. Crude polysaccharides: W: water soluble of crude polysaccharides; A: alkali soluble of crude polysaccharides. (*aktivitas antibakteri dari ekstrak kasar polisakarida terhadap bakteri E. coli dan S. aureus. Ekstrak kasar polisakarida : W: ekstrak kasar polisakarida larut air; A: ekstrak kasar polisakarida larut alkali)*

DISCUSSION

The extraction methodologies are based on the solubility of polysaccharide in hot water and in alkaline solutions (Zhu *et al.,* 2015). Synytsya *et al.,* (2009) reported, the analysis of solid residues of water extract confirms that water-insoluble fractions still contained a high amount of curde polysaccharide, which can be isolated using alkali extraction. That is why an alkali extraction step was included into the isolation procedure of curde polysaccharide (Synytsya *et al.,* 2009).

Antioxidant activities of all the samples obtained were measured using β-carotene-linoleate model assay at range concentrations 100-350 µg/ml. For each concentration, the antioxidant activity was calculated and presented in Table 1. The system containing 350 µg/ml of samples water soluble of crude polysaccharides and alkali soluble of crude polysaccharides retained 66.74±0.012% and 85.82±0.008 % of the initial β-carotene after 120 min of the assay whereas ascorbic acid as standard retained only 6.91±0.056%. The presence of antioxidant activity is characterized by the bleaching of β-carotene-linoleic solution from bright yellow to opaque white due to the addition of water soluble or alkali soluble of crude polysaccharides from *A. auricula.* It has been known that carotenoids undergo ‘‘bleaching’’ i. e.,

lose their color, when its exposed to free radicals or to oxidizing species such as ROS (Mueller and Boehm, 2011).

The mechanism of bleaching of β-carotene is a free-radicals-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. When linoleic acid is oxidized, it produces hydroperoxide-derived free radicals which attacks the unsaturated β-carotene models and bleach the yellow colour of β-carotene. Table 1 shows at the concetration 300 and 350 µg/ml, antioxidant activity in water soluble of crude polysaccharides increase twice while the alkali soluble of crude polysaccharides did not show a significant increase. Alkali soluble of crude polysaccharides at 350 µg/ ml may reach maximum antioxidant activity so it can not neutralize free radicals. The presence of antioxidants in water and alkali soluble of crude polysaccharides can hinder the extent of β-carotene -bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system differently (Dewi *et al*., 2012).

However, the absorbance decreased rapidly in negative control (distilled water) or the sample without antioxidant. In the sample with the presence of an antioxidant, the colour could be retained for a longer time. In this assay, β-carotene, a biologically oxidizable substrate, gives direct information on the ability of an extract to prevent oxidation. Accordingly, the β-carotene-linoleate model assay quantifies the ability of the extract to prevent, impede or reduce the formation of free radicals and thus the antioxidant activity of the extract is directly measured by the extent to which the bleaching of β-carotene can be prevented. This ability of water and alkali soluble of crude polysaccharides from *A.auricula* is a product of the presence of different antioxidants which can neutralize the linoleate-free radical and other free radicals formed in the system (Olugbami *et al.,* 2014).

A.auricula is known to have antioksidan called *A.auricula* polysaccharide (AAP). Yang *et al*., (2011) reported that the scavenging ability of AAP on hydrogen peroxide at 2.0 mg/mL were 27.75%. The presence of AAP in *A.auricula* can protect human helth from hydroxyl radical. The hydroxyl radical is considered to be a highly potent oxidant that can easily cross cell membranes and readily react with most biomolecules, including carbohydrates, proteins, lipids and DNA in cells, which thereby causes tissue damage or cell death (Yang *et al.,* 2011). Moreover, other studies also reported that phenolic compounds obtained from mushroom extracts have shown antioxidant activities (Finimundy *et al.,* 2013). Packialakshmi *et al*., (2015) reported that hot water extract of *A. auricula* has total phenolic and flavonoid content about 8.94 mg CE/g, 3.49 mg RE/g respectively (Packialakshmi *et al*., 2015).

Antibacterial activities of water and alkali soluble of crude polysaccharides *A.auricula* mushroom were tested against 2 bacteria i.e *E.coli* (Gram-positive) and *S.aureus* (Gram-negative). The antibacterial activity could be classified into 3 groups: low (clear zone diameter 0-3 mm), moderate (clear zone diameter 3-6 mm) and high (clear zone diameter > 6mm) (Pan *et al.,* 2009). Based on clear zone diameter, the highest antibacterial activity in this experiment was attributed to the alkali soluble of crude polysaccharides. In this study, both crude polysaccharide demonstrated higher antibacterial activity in *E. coli* as gram-negative bacteria compared to *S.aureus* as Gram-positive bacteria. *S.aureus* have a record of developing resistance quickly and successfully to antibiotics. *S. aureus* contains the coagulase enzyme that is associated as a pathogenicity factor for this bacterium. The pathogenicity is caused by the coagulase activity which can coagulate and accumulate fibrin around the bacteria so that the antibiotic agent unable to penetrate the bacterial cell wall easily (Kumar *et al.,* 2016).

The susceptibility of Gram-positive and Gram-negative bacteria to antibacterial agents might be dissimilar due to a different composition of their cell wall structures such as peptidoglycan, lipid, and crosslinking, which could remarkably affect penetration, binding, and activity of the antibaterial agent (Fatisa 2013). Cell wall of Gram positive bacteria contain peptidoglican while cell wall of Gram negative bacteria contain peptidoglican and lipopolysaccharide. *S. aureus* is classified as Gram-

positive bacteria, and their cell walls are composed of polysaccharide and protein with antigen properties and less lipid content (1-4%). Whereas, *E. coli* is classified as Gram-negative bacteria with high lipid content on their cell walls (11- 22%). In addition, their cell walls are composed of three layers: lipoprotein, phospholipid (outer membrane) and lipopolysaccharide (Febriyani *et al.,* 2018). The death of bacteria caused by antibacterial compounds happened because the antibacterial produce chemical components that are able to inhibit the synthesis of cell wall, inhibit function of cell membrane, inhibit the protein synthesis and or inhibit the nucleate acid synthesis (Fitri and Bustam, 2010).

In addition, *A.auricula* is known to have inhibitor of quorum sensing on their fruiting body pigments (Zhu *et al.,* 2011). Quorum sensing is very important for pathogenic bacteria during infection of a host (e.g.humans,) to coordinate their virulence to escape the immune response of the host to be ableto establish a successful infection (Bassler and Losick, 2006). The bioactive constituents from *A.auricula* pigments could interfere with bacterial quorum-sensing system and prevent bacterial pathogenesis (Zhu *et al.,* 2011).

The edible mushroom has a potential to become a functional food which has nutritional value and can give benefits to improve the human health (Siro *et al.,* 2008). The presence of anti-oxidant and antibacterial components in alkali soluble of crude polysaccharides from *A.auricula* makes it a good ingredient that used as functional food for dietary supplements or nutraceuticals.

CONCLUSION

The alkali soluble of crude polysaccharides showed 85.82 % antioxidant activity. The alkali soluble of crude polysaccharides was found to have the highest antibacterial activity against *S. aureus* InaCC B4 and *E. coli* InaCC B5 with clear zone values of 3.18 mm and 5.10 mm, respectively. The clear zone value was indicated that the alkali soluble of crude polysaccharides has moderate anti-bacterial activity. The data obtained in this study showed that the *A. auricula* crude polysaccharides has antioxidant and antibacterial activity. It support that the *A. auricula* can be clasified as functional food. Further deep investigations may be neccessary to support *A. auricula* as a functional food.

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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.
6. Untuk range angka menggunakan en dash (–), contohnya pp.1565–1569, jumlah anakan berkisar 7–8 ekor. Untuk penggabungan kata menggunakan hyphen (-), contohnya: masing-masing.
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:

   a. **Jurnal**
      Nama jurnal ditulis lengkap.
      Agusta, A., Maehara, S., Ohashi, K., Simanjuntak, P. and Shibuya, H., 2005. Stereoselective oxidation at C-4 of flavans by the endophytic fungus *Diaporthe* sp. isolated from a tea plant. *Chemical and Pharmaceutical Bulletin*, *53*(12), pp.1565–1569.
   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.**
      Kurata, H., El-Samad, H., Yi, T.M., Khammash, M. and Doyle, J., 2001. Feedback Regulation of the Heat Shock Response in *Eschericia coli*. *Proceedings of the 40th IEEE Conference on Decision and Control*. Orlando, USA. pp. 837–842.
   d. **Makalah sebagai bagian dari buku**
      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.
   e. **Thesis, skripsi dan disertasi**
      Sundari, S., 2012. Soil Respiration and Dissolved Organic Carbon Efflux in Tropical Peatlands. *Dissertation*. Graduate School of Agriculture. Hokkaido University. Sapporo. Japan.
   f. **Artikel online.**
      Artikel yang diunduh secara online ditulis dengan mengikuti format yang berlaku untuk jurnal, buku ataupun thesis dengan dilengkapi alamat situs dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review misalnya laporan perjalanan maupun artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
      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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Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah yang dipublikasi pada edisi tersebut. Oleh karena itu, setiap naskah yang ada ilustrasinya diharapkan dapat mengirimkan ilustrasi atau foto dengan kualitas gambar yang baik dengan disertai keterangan singkat ilustrasi atau foto dan nama pembuat ilustrasi atau pembuat foto.

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