Berita Biologi
LIPI
Jurnal Ilmu-ilmu Hayati

10.14203/beritabiologi.v20i1.3991
P-ISSN 0126-1754
E-ISSN 2337-8751

# BIOPROSPECTION OF ENGGANO MACROSCOPIC FUNGI AS ANTIBACTERIAL AND ANTIOXIDANT AGENTS

## [Bioprospeksi Jamur Makroskopis Enggano Sebagai Antibakteri dan Antioksidan]

Evana[1*], Kartika Dyah Palupi[1], Listiana Oktavia[1], Ahmad Fathoni[1*]✉

[1]Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kawasan PUSPIPTEK, Muncul, Tangerang Selatan 15314, Indonesia
email: fathoni.chem@gmail.com

**ABSTRACT**

Macrofungi in Indonesia have not been widely studied for their pharmacological activity, especially as a source of antibacterial and antioxidant properties, even though Indonesia as a tropical country has quite a high diversity of macrofungi. This study aims to reveal the potential of macrofungi from the Enggano forest as a source of antibacterial and antioxidant compounds. Four types of macrofungi were collected and their metabolites were extracted using four types of organic solvents. Antibacterial and antioxidant activity assay of the extract was carried out using the TLC Bioautography method. From the sixteen macrofungal extracts, there is one extract that has the strongest antibacterial activity compared to the others, namely n-hexane *Coriolopsis polyzona*. It showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with MIC values of 256–128 µg/mL, respectively. Meanwhile, the antioxidant activity of the macrofungal extracts showed weak activity with $IC_{50}$ values of 3080–7370 µg/mL (AAI values of 0.033–0.079).

**Key words**: Macrofungi, Enggano, antibacterial, antioxidant, *Coriolopsis polyzona*

**ABSTRAK**

Jamur makro di Indonesia masih belum banyak diteliti aktivitas farmakologinya terutama sebagai sumber antibakteri dan antioksidan, padahal sebagai negara tropis keragaman jamur makro di Indonesia cukup tinggi. Penelitian ini bertujuan mengungkap potensi jamur makro dari hutan Enggano sebagai sumber senyawa antibakteri dan antioksidan. Empat jenis jamur berhasil dikoleksi dan diekstrak senyawa metabolitnya menggunakan empat jenis pelarut organik. Pengujian aktivitas antibakteri dan antioksidan dilakukan terhadap ekstrak dengan metode KLT bioautografi. Dari ekstrak jamur tersebut terdapat satu ekstrak jamur memiliki aktivitas antibakteri yang paling kuat dibanding lainnya yaitu fraksi n-heksana Coriolopsis polyzona. Ekstrak tersebut menunjukkan aktivitas antibakteri yang moderat melawan Staphylococcus aureus dan Escherichia coli dengan nilai KHM masing-masing adalah 256–128 µg/mL. Sementara aktivitas antioksidan ekstrak jamur makro memperlihatkan aktivitas yang lemah yaitu dengan $IC_{50}$ 3080–7370 µg/mL, dengan nilai IAA 0,033– 0,079.

**Kata kunci:** Jamur makro, Enggano, antibakteri, antioksidan, *Coriolopsis polyzona*

## INTRODUCTION

The increasing number of antibiotic-resistant bacteria drives the development of more effective antibiotics. In the presence of antibiotics, bacteria gradually become immune and these resistant bacteria begin to replicate (Dandawate *et al*., 2019). As a result, novel anti biotic agents derived from various biological sources are constantly searched. Furthermore, the discovery of antioxidant agents is also important for protecting against chronic degenerative diseases. Fungi have received a lot of attention because they are recognized to possess bioactive compounds that are effective against microbial organisms.

The fungi have long been known to thrive in humid conditions that encourage the development of pathogenic microorganisms. They have developed certain defensives mechanisms to survive in their environment by producing antibacterial and antifungal compounds (Lindequist *et al*., 2005). Fungi are medicinal foods that are widely used in the treatment of diet-related diseases, the prevention of chronic diseases, and to slow down the aging process (Khatun *et al*., 2012). The medicinal properties of fungi are due to the presence of a diverse variety of secondary metabolites of high therapeutic potential (Ogidi and Oyetayo, 2015). Polysaccharides and glycoproteins derived from the fungi, such as lentinan, krestin, and schizophyllan, have been used as a stimulant of the body in the treatment of cancer in Asia (Wasser, 2002).

*Main Contributor (*Kontributor Utama*)
**Diterima: 4 Mei 2021 - Diperbaiki: 28 Juli 2021 - Disetujui: 29 Juli 2021*

Macrofungi are a fascinating fungi group because of their potential. Most macrofungi belong to *Basidiomycota* and *Ascomycota* (Hibbett *et al*., 2007). Metabolites from macrofungi have antibacterial, antioxidant, antifungal, anti-tumor, antiviral, anti-inflammatory, antiatherogenic, hypoglycemic, hepatoprotective, immunomodula tory, anti-Alzheimer's, antidiabetic, antimalarial, and hypocholesterolemic activity (Suay *et al*., 2000; Lindequist *et al*., 2005; Reis *et al*., 2011; Ren *et al*., 2012). The fungi can be a potential source of medicinal raw materials because of their abundance in nature, with a variety of potential activities. Fungi are the second-largest organism in existence, behind terrestrial plants, with about 3–5 million organisms (Blackwell, 2011). Indonesia, which is rich in animal and plant diversity, also has a high diversity of fungi. This is due to the humidity and tropical temperatures in Indonesia, which promote the growth of fungi, and it is estimated that Indo nesia has 200,000 species of fungi (Dewi *et al*., 2019). Nevertheless, only a limited percentage of the overall number of fungi species found in the wild has been described.

Enggano Island is one of the outermost islands in Indonesia, located in Bengkulu Province. The information about Enggano Island's biodiversity is still limited. Thirtyone species of macrofungi were identified from the village in Enggano Island. Those species belong to Basidiomycota and Ascomycota. The nine new species were identified as new record named as *Phellinus gilvus*, *Fomitella supina*, *Flaviporus liebmannii*, *Coriolopsis polyzona*, *Flabellophora sp*., *Trichaptum byssogenum*, *Stecherrinum sp.*, *Stereum cf. pergameneum*, and *Trametes cf. villosa*. On the other hand, two species, namely *Cookeina cremeirosea* and *Fomitella supina* are known to be a new record for macrofungi in Indonesia. Fungi *Phellinus gilvus* was reported as a harmful parasite for rubber plantation, while edible mushrooms were described as *Pleurotus ostreatus, Volvariella volvacea, Schizophyllum commune, Auricularia auricula- judae, Lentinus sajor-caju, and Panus neostrigosus*. According to previous use, two species *Favolus grammocephalus* and *Panus neostrigosus* are known as therapeutic agents (Susan and Retnowati, 2017).

The bioactivities of macrofungal extracts as antimicrobials and antioxidants need to be investigated because antibacterial and antioxidant compounds play an important role in protecting and improving the quality of human existence. Therefore, this research aims to evaluate the antibacterial and antioxidant activity of macrofungal extracts from Enggano Island.

## MATERIALS AND METHODS

### Sample collection

Macrofungi were collected from Enggano Island, Bengkulu. Identification was carried out at the Herbarium Bogoriense, Research Center for Biology-Indonesian Institute of Sciences, Cibinong, Bogor.

### Sample extraction

As much as 10 g of macrofungi fruiting bodies were extracted with 100 mL ethanol for 3 x 24 hours by changing ethanol every 24 hours, then successively partitioned with 100 mL of each solvent (n-hexane, dichloromethane, ethyl acetate, and methanol) respectively for 3 x 24 hours. To obtain a crude extract, the filtrates of each solvent were gathered and dried using a rotary evaporator at 35 °C.

### Thin Layer Chromatography (TLC) analysis of chemical compounds

TLC was used to analyze the chemical compounds in macrofungal extracts. The dried extract was created at a concentration of 10 mg/mL. The macrofungal extract was transferred (10 µL) to TLC silica plates (GF 254, Merck) at the prepared concentration. n-hexane extract was eluted with an eluent system of hexane:ethyl acetate (3:1), dichloromethane, and ethyl acetate extracts were eluted with an eluent system of dichloromethane: methanol (10:1), and methanol extract was eluted with an eluent system of dichloromethane:methanol (4:1). Under UV light at 254 and 366 nm, eluted chemical compounds were visualized before being sprayed with coloring reagent (1% vanillin-sulphuric acid and 1% cerium (IV) sulfate) (Praptiwi *et al*., 2018).

### Antibacterial activity assay by TLC-bioautography

The antibacterial activity of the macrofungal extract was evaluated qualitatively using TLC-bioautography-guided screening (Dewanjee *et al*., 2015) against *Staphylococcus aureus* InaCC B4 and *Escherichia coli* InaCC B5. 10 µL of extract (10 mg/mL) were transferred to a TLC plate and dipped in Mueller-Hinton Broth (MHB) containing $10^6$–$10^8$ CFU/mL of bacterial inoculants. The plates were incubated at 37 °C for 18 hours. The incubated plates were sprayed with p-iodonitrotetrazolium 4 mg/mL (Sigma-Aldrich) solution to observe bacterial growth. As a positive control, chloramphenicol solution (Sigma-Aldrich) was used. Bacterial growth inhibition is implied by the existence of a white zone around the extract on a purple background (Das *et al*., 2010).

**Determination of Minimum Inhibitory Concentration (MIC) value**

The MIC of both extracts and the reference antibiotic, chloramphenicol, were determined by serial microdilution using a 96-well microplate. The extracts of the macrofungi and the reference antibiotic were prepared in dimethylsulfoxide (DMSO, Merck) at a concentration of 20480 µg/mL. The Mueller-Hinton broth (MHB, Merck) and extracts were applied to the 96-well microplate, and serial dilutions were conducted to achieve final concentrations of 1024, 512, 256, 128, 64, 32, 16, and 8 µg/mL in that order, and then 100 µL of bacterial suspension (*S. aureus* and *E. coli*) ($10^5$ CFU/mL) was added to each well. After incubating the microwell plate at 37 $^oC$ for 18 hours, 10 µL of 4 mg/mL INT was applied to each well. The MIC value is the lowest concentration of extract needed to inhibit bacterial growth, as determined by the well that does not change color after the addition of INT (Pessini *et al*., 2003).

**Antioxidant activity assay by TLC-bioautography**

The TLC-bioautography-guided screening was used to assess the antioxidant efficacy of macrofungal extract and positive control, (+)-catechin against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Wang *et al*., 2012). 10 µL of extract (10 mg/mL) and (+)-catechin were transferred to a TLC plate and sprayed with a solution of 1 mM DPPH (Sigma-Aldrich) in methanol. After spraying with DPPH reagent, the yellow spot against the purple background revealed antioxidant activity.

**Antioxidant Activity Index (AAI) and $IC_{50}$ value determination**

Determination of the $IC_{50}$ value of the extract was carried out by the serial microdilution method using a 96-microwell plate (Takao *et al*., 2015). The first wells were prepared with a 100 µL methanol solution, while the remaining wells were prepared with 50 µL methanol. 100 µL of extracts (10000 mg/L) were added to the first wells and thoroughly mixed. Serial dilutions were performed to obtain final concentrations of 10000–78.125 mg/ L. After diluting, 80 µL DPPH (1mM) was applied to each well.

The microplates were incubated for 90 minutes at room temperature in dark conditions. The absorbance of the extracts was measured at 517 nm using a microplate reader (Varioscan flash, Thermo scientific). The $IC_{50}$ value was calculated using the extract concentration and the IC values from the linear regression equation. The Antioxidant Activity Index (AAI), which is formulated as follows, was used to classify antioxidant activity (Scherer and Godoy, 2009; Praptiwi *et al*., 2018):

$$\text{AAI} = \frac{\text{The final concentration of DPPH in the reaction } (\mu g/ml)}{IC_{50}\,(\mu g/ml)}$$

## RESULTS

**TLC analysis of the chemical compounds of the extracts**

TLC chromatogram profiles of macrofungal extracts revealed a distinct chromatogram band (Figure 1). According to the data, the macrofungal extract from Enggano Island contains various chemical compounds, as evidenced by the different retention factors (Rf) of each stain created. The less polar compound eluted first, whereas a more polar compound interacted stronger with the polar stationary phase (silica), resulting in a lower Rf value.

**Antibacterial activity**

The TLC-bioautography approach was used to qualitatively assess the antibacterial activity of macro fungal extracts against *S. aureus* and *E. coli.* The results revealed that extract numbers 3,5,6,9, and 14 were able to inhibit the growth of *S. aureus*, while extract numbers 3, 5, 6, 7, 8, 9, and 11 were able to inhibit the growth of *E. coli*, with various diameters of inhibition zones, characterized by the presence of a white zone around the extract (Figure 2).

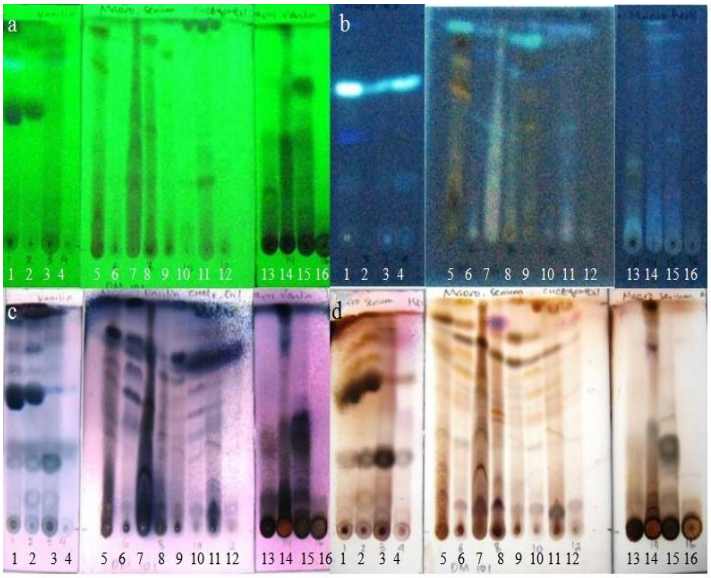


**Figure 1**. Chromatograms of macrofungal extracts from Enggano developed with an eluent system of n-hexane:ethyl acetate (3:1) (no.1–4), dichloromethane:methanol (10:1) (no.5–12), and dichloromethane:methanol (4:1) (no.13–16). The chromatogram was observed under the following conditions: (a) UV 254 nm, (b) UV 366 nm, (c) sprayed with 1% cerium (IV) sulfate, and (d) 1% vanillin-sulphuric acid. (*Kromatogram ekstrak jamur makro dari Enggano dielusi dengan sistem pelarut n-heksana:etil asetat (3:1) (no.1-4), diklorometana:metanol (10:1) (no.5-12), dan diklorometana:metanol (4:1) (no.13-16). Kromatogram diamati pada kondisi: (a) UV 254 nm, (b) UV 366 nm, (d) disemprot dengan 1% serium (IV) sulfat, dan (d) 1% vanillin-sulfat*).

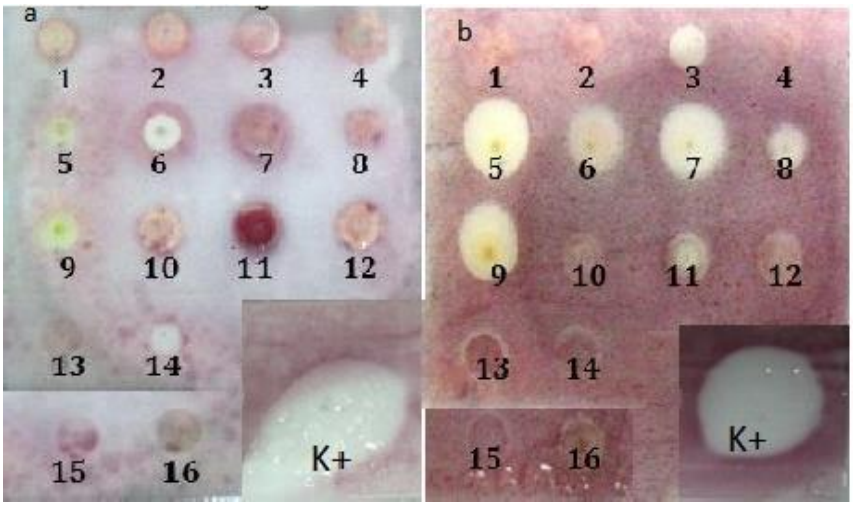


**Figure 2**. Bioautogram of antibacterial activity of macrofungi extracts from Enggano against (a) *S. aureus* and (b) *E. coli*. Extracts no. 1–16 refer to Table 1. K+: chloramphenicol. (*Bioautogram aktivitas antibakteri ekstrak jamur makro dari Enggano terhadap (a) S. aureus dan (b) E. coli. Ekstrak no. 1-16 sesuai dengan Tabel 1. K+: kloramfenikol*).

The dehydrogenase enzyme in living microorganisms converts INT into purple formazan, resulting in the development of a purple stain on the TLC plate after being sprayed with INT solution (Silva *et al*., 2005). The extract of macrofungi that inhibited bacterial growth was then quantitatively tested.The microdilution approach was used to assess the MIC value of each extract in a quantitative study of macrofungal extract from Enggano Island (Table 1).

**Table 1**. MIC values of macrofungal extract from Enggano (*Nilai KHM ekstrak jamur makro dari Enggano*)

| No. | Macrofungal extracts *Ekstrak jamur makro* | MIC value *Nilai KHM* (µg/mL) | |
|---|---|---|---|
| | | ***S. aureus*** | ***E. coli*** |
| 1 | n-hexane *Pycnoporus sanguineus* | NT | NT |
| 2 | n-hexane *Lentinus sajor-caju* | NT | NT |
| 3 | n-hexane *Coriolopsis polyzona* | 256 | 128 |
| 4 | n-hexane *Earliella scabrosa* | NT | NT |
| 5 | Dichloromethane *Pycnoporus sanguineus* | 512 | > 512 |
| 6 | Dichloromethane *Lentinus sajor-caju* | 512 | 512 |
| 7 | Dichloromethane *Coriolopsis polyzona* | NT | > 512 |
| 8 | Dichloromethane *Earliella scabrosa* | NT | > 512 |
| 9 | Ethyl acetate *Pycnoporus sanguineus* | 512 | 512 |
| 10 | Ethyl acetate *Lentinus sajor-caju* | NT | NT |
| 11 | Ethyl acetate *Coriolopsis polyzona* | NT | 512 |
| 12 | Ethyl acetate *Earliella scabrosa* | NT | NT |
| 13 | Methanol *Pycnoporus sanguineus* | NT | NT |
| 14 | Methanol *Lentinus sajor-caju* | 512 | NT |
| 15 | Methanol *Coriolopsis polyzona* | NT | NT |
| 16 | Methanol *Earliella scabrosa* | NT | NT |
| 17 | Chloramphenicol | 4 | 4 |

*NT: not tested, a qualitative assay revealed poor antibacterial activity. (*TD: tidak diuji, pengujian kualitatif menunjukkan aktivitas antibakteri yang lemah*).

The antibacterial activity of the extract is classified as follows: MIC values of < 100 µg/mL (strong), 100 < MIC ≤ 625 µg/mL (moderate), and MIC values > 625 µg/mL (weak) (Kuete and Efferth, 2010). Based on the classification of antibacterial activity, it can be concluded that extract no. 3,6,9, and 11 have moderate activity in inhibiting the growth of *S. aureus* and *E. coli*, while extract no. 5 was only moderately effective in inhibiting *S. aureus* growth. The antibacterial activity of n-hexane extract of *Coriolopsis polyzona*, is the highest among the macrofungal extracts, both against *S. aureus* and *E. coli*.

**Antioxidant activity**

The antioxidant activity of macrofungal extracts against the free radical DPPH was evaluated qualitatively using the TLC-bioautography method. The formation of a yellow zone around the extract in the qualitative assay indicated that only twelve extracts have the potential to scavenge DPPH radicals (Figure 3).

The formation of yellow color on the plate after being sprayed with DPPH is caused by the antioxidants in the extract donate electrons or hydrogen atoms to the DPPH, causing DPPH to be reduced and the color change from purple to yellow. The strength of the yellow color showed the

antioxidant potential. Because DPPH is a stable free radical, it is the most widely used to assess antioxidant activity. Furthermore, twelve extracts were quantitatively evaluated using a 96-microwell plate to determine the $IC_{50}$ and AAI values (Table 2). The $IC_{50}$ value is defined as the extract concentration needed to reduce DPPH free radicals by 50% (Molyneux, 2004).

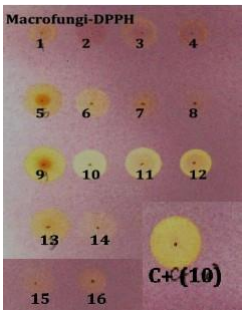


**Figure 3**. Bioautogram of antioxidant activity of macrofungal extracts from Enggano. Extracts no. 1–16 refer to Table 1. C+: (+)-catechin. (*Bioautogram aktivitas antioksidan ekstrak jamur makro dari Enggano. Ekstrak no. 1-16 sesuai dengan Tabel 1. C+: (+)-katekin*).

The classification of antioxidant activity for extracts is described as follows: AAI < 0.5 (weak), AAI 0.5–1.0 (moderate), AAI 1.0–2.0 (strong), and AAI > 2 (very strong) (Scherer and Godoy, 2009). The results in table 2 revealed that twelve extracts were classified as having weak antioxidants, while (+)-catechin as a positive control exhibited very strong antioxidant properties.

**Table 2**. $IC_{50}$ and AAI values of macrofungi extract from Enggano (*Nilai $IC_{50}$ dan IAA ekstrak jamur makro dari Enggano*)

| No. | Macrofungi extracts (*Ekstrak jamur makro*) | $IC_{50}$ value *Nilai $IC_{50}$* (µg/mL) | AAI value (*Nilai IAA*) |
|---|---|---|---|
| 1 | n-hexane *Pycnoporus sanguineus* | NT | NT |
| 2 | n-hexane *Lentinus sajor-caju* | NT | NT |
| 3 | n-hexane *Coriolopsis polyzona* | 4670 | 0.052 |
| 4 | n-hexane *Earliella scabrosa* | NT | NT |
| 5 | Dichloromethane *Pycnoporus sanguineus* | 3390 | 0.072 |
| 6 | Dichloromethane *Lentinus sajor-caju* | 7370 | 0.033 |
| 7 | Dichloromethane *Coriolopsis polyzona* | 7070 | 0.034 |
| 8 | Diklorometana *Earliella scabrosa* | NT | NT |
| 9 | Ethyl acetate *Pycnoporus sanguineus* | 5060 | 0.048 |
| 10 | Ethyl acetate *Lentinus sajor-caju* | 7360 | 0.033 |
| 11 | Ethyl acetate *Coriolopsis polyzona* | 3080 | 0.079 |
| 12 | Ethyl acetate *Earliella scabrosa* | 3810 | 0.064 |
| 13 | Methanol *Pycnoporus sanguineus* | 4520 | 0.054 |
| 14 | Methanol *Lentinus sajor-caju* | 3340 | 0.073 |
| 15 | Methanol *Coriolopsis polyzona* | 5410 | 0.045 |
| 16 | Methanol *Earliella scabrosa* | 3770 | 0.064 |
| 17 | (+)-Catechin | 2.03 | 15.18 |

*NT: not tested, a qualitative assay revealed poor antioxidant activity. (*TD: tidak diuji, engujian kualitatif menunjukkan aktivitas antioksidan yang lemah*).

## DISCUSSION

Based on morphological characteristics, four types of macrofungi collected from Enggano Island in Bengkulu were identified as *Pycnoporus sanguineus, Lentinus sajor-caju, Coriolopsis polyzona, dan Earliella scabrosa.* The four types of these macrofungi belong to Basidiomycota. *Pycnoporus sanguineus* is white-rot basidiomycetes widely studied because of its ability to produce bioactive compounds and valuable enzymes (Rohr *et al*., 2013). This macrofungus is widely used for wound and skin lesion therapy, as well as antiviral, antibacterial, pigment-producing, lignin cellulose-producing enzymes, antioxidants, and antifungals (Smânia *et al*., 2003; Hwang *et al*., 2004; Lomascolo *et al*., 2011). Based on the research of Achenbach and Blum (1991), this macrofungi produced red pigment, which contains alkaloids such as phenoxazine-3-one, which acts as an active compound. Smania *et al*., (1995) reported that fraction B of *P. sanguineus* had antibacterial activity against several bacteria with MIC values of 190–1250 µg/mL. Cinnabarin, a bioactive compound isolated from *P. sanguineus* had antibacterial activity against several bacteria with MIC values of 62.5–4000 µg/mL (Smania *et al*., 1998). Antibacterial activity of low-molecular-weight secondary metabolite subfractions from cultures of *P. saguineus* grown at 30 °C against *S. aureus* with MIC value of 120 µg/mL, meanwhile the $IC_{50}$ value for DPPH assay was 51.46 µg/mL (Jaszek *et al*., 2015).

Meanwhile, *Earliella scabrosa* is a polypore fungus that can be found in rainforests. This fungus has been used as an antioxidant and free radical scavenger (Zmitrovich *et al*., 2017). In other studies, it was stated that water and methanol extracts of *E. scabrosa and P. sanguineus* were active against several wood-degrading fungi with MIC values of 100–5000 µg/mL (Teoh *et al*., 2011; Peng and Don, 2013). *E. scabrosa* contains triacetin, furanone derivative, and pyran derivative, while *P. sanguineus* contains 4H-pyranone derivatives that have been shown to inhibit the growth of several wood-degrading fungi (Teoh *et al*., 2011; Peng and Don, 2013). The bioactive compound such as isocoumarin is successfully used in cancer therapy (Zmitrovich *et al*., 2017).

While other macrofungi, *L. sajor-caju* or *Pleurotus sajor-caju* is a type of macrofungi that is most commonly found in Indonesia (Susan and Retnowati, 2017). It has antioxidant, antibacterial, and nutritive properties (Singdevsachan *et al*., 2013). The aqueous and organic solvent extracts of *L. sajor-caju* or *P. sajor-caju* had antibacterial activity against several pathogenic bacteria with inhibition growth zone diameter of 12–22 mm (Tambekar *et al*., 2006). Based on the research of Mossebo *et al*., (2020), the MIC value of crude extract of L. sajor-caju was 6.25 mg/mL for *S. aureus* and 12.5 mg/mL for *E. coli.*

Macrofungi *Coriolopsis polyzona* has been known as lignin modifying enzyme, and decolorization of olive oil mill wastewaters (Jaouani *et al*., 2006), and the methanolic extract had antibacterial against several positive and negative-Gram bacteria, and antifungal activity against some fungi with inhibition zone diameter of 10–25 mm (Al-Fatimi *et al*., 2012).

This study revealed that n-hexane extract of *C. polyzona* had the highest activity in inhibiting the growth of *S. aureus and E. coli*. n-hexane has a lower polarity compared to dichloromethane, ethyl acetate, and methanol. The bioactive compounds in n-hexane extract contributed to antibacterial have a lower polarity. The previous study stated that secondary metabolites related to the polarity properties might be attributed to the antimicrobial activity of *C. polyzona* extract. The bioactive compounds as an antimicrobial activity have the following functional groups: hydroxyl (OH), alkyl (C-H), carbonyl (C=O), an aromatic ring (Ogidi and Oyetayo (2015). The previous study stated this macrofungus is an antifungal, organic polymer degrading, antioxidant, and anticancer agent with secondary metabolites containing saponins, flavonoids, tannins, alkaloids, and steroids in *C. polyzona* extracts (Oyetayo *et al*., 2013; Ogidi and Oyetayo, 2015). According to another study, these phytochemicals have already been identified to have an antifungal capacity (De Silva *et al*., 2013).

Moreover, n-hexane *C. polyzona* extract also demonstrated greater antibacterial activity against *E. coli* (a Gram-negative bacteria) compared to *S. aureus* (a Gram-positive bacteria). This is probably related to the capability of antibiotic resistance of *S. aureus* bacteria. These bacteria have been reported to overgrow and succeed because they produce the enzyme coagulase associated with pathogenicity in these bacteria (Elfirta and Saskiawan, 2020). Pathogenicity is affected by coagulase action, which can coagulate and accumulate fibrin across the bacteria, making it difficult for antibiotics to reach the bacterial cell wall (Kumar *et al*., 2017).

*P. sanguineus* produces poliporin that has an antibacterial activity (Bose, 1946). *P. sanguineus* also synthesizes an orange pigment called cinnabarin that is more active against gram-positive bacteria (Smânia *et al*., 1998). A study by Rosa *et al*., (2003) displayed that *P. sanguineus* CCB277 extract could inhibit the growth of *C. krusei*, *L. monocytogenes*, and *S. aureus*. In addition, another study also observed a severe disruption of bacterial cells which were treated with low-molecular-weight secondary metabolite subfractions from cultures of *Pycnoporus sanguineus* grown at 30 °C (Jaszek *et al*., 2015). However, the mechanism of action of these antimicrobial compounds from *P. sanguineus* is still not clear and described in the available reports.

Ngai and Ng (2004) reported that *L. sajor-caju* produces a ribonuclease that is active against *S. aureus* and *P. aeruginosa*. This RNase has an N-terminal sequence that resembles the C-terminal sequence of bacteriocin peptide and also has a similarity to a part of RNA polymerase and an RNA-specific sequence. Hence, this resemblance may play a role in the antibacterial and RNase activities of this *L. sajor-caju*. *C. polyzona* has been reported to have antibacterial and antifungal properties (Oyetayo and Ogidi, 2011; Ogidi and Oyetayo, 2015;). However, the underlying mechanisms, as well as the compounds that are responsible for its bioactivities, have not been reported. Liew *et al*., (2015) reported that crude ethanolic extract of *E. scabrosa*, which contained saponin was most active against *Pseudomonas aeruginosa* (MIC = 28.13 µg/mL) and less active against *S. pyogenes, Clostridium difficile, S. aureus*, and *E. coli*. However, further studies are needed as the mode of action as well as the responsible compound for this antibacterial activity are still unclear.

## CONCLUSION

Several macrofungal extracts from Enggano Island, including n-hexane extracts (*C. polyzona*), dichloromethane extract (*L. sajor-caju*), ethyl acetate extracts (*P. sanguineus and C. polyzona*), have moderate antibacterial activity in inhibiting the growth of *S. aureus and E. coli.* Among all tested extracts, n-hexane extract *Coriolopsis polyzona* showed moderate antibacterial activity against *Staphylococcus aureus and Escherichia coli* with MIC values of 256 and 128 µg/mL, respectively. Meanwhile, the antioxidant activity of all macrofungal extracts displayed weak activity with IC50 values of 3080-7370 µg/mL (AAI values of 0.033-0.079). Therefore, according to this study, macrofungal extracts from Enggano Island may contain valuable bioactive compounds with antibacterial activity. However, further investigation studies need to be carried out to isolate and characterize the bioactive compounds of the extract.

## ACKNOWLEDGMENT

We gratefully acknowledge financial assistance for this work provided by the DIPA Research Center for Biology, Indonesian Institute of Sciences, and IFS under contract F-4613-2. We would like to express our gratitude to Dr. Atik Retnowati. Dewi Susan, M.Si, and Dewi Rosalina for assisting in identifying the varieties of fungi, and Fakhri Ihsan for his assistance in the laboratory.

## REFERENCES

Achenbach, H. and Blümm, E. 1991. Investigation of the Pigments of *Pycnoporus sanguineus*-Pycnosanguin and New Phenoxazin-3-ones. *Archiv der Pharmazie, 324*, pp. 3–6.

Al-Fatimi M, Schröder, G., Kreisel, H. and Lindequist, U., 2013. Biological activities of selected basidiomycetes from Yemen. *Pharmazie*, *68*, pp. 221–226.

Blackwell, M., 2011. The Fungi: 1, 2, 3 ... 5.1 Million Species?. *American Journal of Botany*, *98*(3), pp. 426–438.

Bose, S. R., 1946. Antibiotics in a Polyporus (*Polystictus sanguineus*). *Nature*, *158*, pp. 292–296.

Dandawate, P., Padhye, S., Schobert, R. and Biersack, B., 2019. Discovery of natural products with metal-binding properties as promising antibacterial agents. *Expert Opinion on Drug Discovery*, *14*(6), pp. 563–576.

Das, K., Tiwari, R. K. S., and Shrivastava, D. K., 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, *4*(2), pp. 104–111.

De Silva, D. D., Rapior, S., Sudarman, E., Stadler, M., Xu, J., Aisyah Alias, S., and Hyde, K. D., 2013. Bioactive metabolites from macrofungi: Ethnopharmacology, biological activities, and chemistry. *Fungal Diversity*, *62*(1), pp. 1–40.

Dewanjee, S., Gangopadhyay, M., Bhattacharya, N., Khanra, R. and Dua, T. K., 2015. Bioautography and its scope in the field of natural product chemistry. *Journal of Pharmaceutical Analysis*, *5*(2), pp. 75–84.

Dewi, M., Aryantha, I. N. P. and Kandar, M., 2019. The Diversity of Basidiomycota Fungi that Have the Potential as a Source of Nutraceutical to be Developed in the Concept of Integrated Forest Management. *International Journal of Recent Technology and Engineering*, *8*(2S), pp. 81–85.

Elfirta, R. R. and Saskiawan, I., 2020. The Functional Character Of *Auricularia auricula* Crude Polysaccharides: Antioxidant And antibacterial Activity. *Berita Biologi*, *19*(3), pp. 433–440.

Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., … Zhang, N., 2007. A higher-level phylogenetic classification of the Fungi. *Mycological Research*, *111*(5), pp. 509–547.

Hwang, H. J., Kim, S. W., Xu, C. P., Choi, J. W. and Yun, J. W., 2004. Morphological and rheological properties of the three different species of basidiomycetes Phellinus in submerged cultures. *Journal of Applied Microbiology*, *96*, pp. 1296–1305.

Jaouani, A., Tabka, M.G., Penninckx, M.J. 2006. Lignin modifying enzymes of *Coriolopsis polyzona* and their role in olive oil mill wastewaters decolourisation. *Chemosphere*, *62*(9), pp. 1421–1430.

Khatun, S., Islam, A., Cakilcioglu, U., and Chatterjee, N. C., 2012. Research on Mushroom as a Potential Source of Nutraceuticals: A Review on Indian Perspective. *American Journal of Experimental Agriculture*, *2*(1), pp. 47–73.

Kuete, V. and Efferth, T., 2010. Cameroonian medicinal plants: pharmacology and derived natural products. *Frontiers in Pharmalogy*, *1*, pp. 1–19.

Kumar, S. S., Shankar, S. and Mohan, S. C., 2017. In vitro Antioxidant and Antimicrobial Activity of Polysaccharides Extracted from Edible Mushrooms *Pleurotus florida* and *Agrocybe cylindracea*. *Singapore Journal of Chemical Biology*, *6*(1), pp. 17–22.

Jaszek, M., Jaroszuk, M., Sulej, J., Matuszewska, A., Stefaniuk, D., Maciag, K., Polak, J., Matuszewski, L. and Grzywnowicz, K., 2015. Stimulation of the Antioxidative and Antimicrobial Potential of the Blood Red Bracket Mushroom *Pycnoporus sanguineus* (Higher Basidiomycetes). *International Journal of Medicinal Mushrooms*, *17* (8), pp. 701–712.

Koch, A.L., 2003. Bacterial wall as target for attack: past, present, and future research. *Clin Microbiol Rev.*, *16*, pp. 673–687.

Lade, H. and Kim, J.S., 2021. Bacterial Targets of Antibiotics in Methicillin-Resistant *Staphylo coccus aureus*. *Antibiotics, 10*, pp. 398.

Liew, G. M., khong, H.Y., Kutoi, C.J. and Sayok, A.K., 2015. Phytochemical Screening, Antimicrobial and Antioxidant Activities of Selected Fungi from Mount Singai, Sarawak, Malaysia. *International Journal of Research Studies in Biosciences*, *3*(1), pp. 191–197.

Lindequist, U., Niedermeyer, T. H. J., and Jülich, W. D., 2005. The Pharmacological PotentiaMushrooms. *Evidence-Based Complemen- tary and Alternative Medicine*, *2*(3), pp. 285– 299.

Lomascolo, A., Uzan-Boukhris, E., Herpoël-Gimbert, I., Sigoillot, J. C. and Lesage-Meessen, L., 2011. Peculiarities of *Pycnoporus* species for applications in *Biotechnology. Applied Microbiology and Biotechnology*, *92*, pp. 1129–1149.

Molyneux, P., 2004. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklana- karin J. Sci. Technol.*, *26*(2), pp. 211–219.

Mossebo, D. C., Metsebing, B. P., Oba, R., Tsigaing Tsigaing, F., Ryvarden, L., Fonkui, T. Y., Mungoh Tata, C. and Ndinteh, D. T., 2020. Comparative evaluation of antifungal and antibacterial activities of crude extracts of *Pleurotus sajor-caju*, *Pleurotus tuber-regium* and *Lentinus squarrosulus* (Basidiomycota, Pleurotaceae, Lentinaceae) from Cameroon. *European Journal of Biology and Biotechnology*, *1* (5), pp. 1–7.

Ngai, P.H.K. and Ng, T.B., 2004. A ribonuclease with antimicrobial, antimitogenic, and antiproliferative activities from the edible mushroom *Pleurotus sajor-caju*. *Peptides*, *25* (1), pp. 11–17.

Ogidi, C. O. and Oyetayo, V. O., 2015. Antifungal effect of *Coriolopsis polyzona* (Pers) on fungi isolated from remnant foods and wastewater from restaurants in Akure metropolis, Nigeria. *African Journal of Microbiology Research*, *9*(8), pp. 527–533.

Oyetayo, O. V., Nieto-Camacho, A., Ramırez- Apana, T. M., Baldomero, R. E. and Jimenez, M., 2013. Total Phenol, Antioxidant and Cytotoxic Properties of Wild Macrofungi Collected from Akure Southwest Nigeria. *Jordan Journal of Biological Sciences*, *6*(2), pp. 105–110.

Oyetayo, V.O., and Ogidi, C.O., 2011. Phytochemical Screening and Antibacterial Properties of a Wild macrofungus *Coriolosis polyzona* against Microbial Isolates from wastewater and leftover foods. *Asian Journal of Pharmaceutical and Biological Research, 1*(4) 486–492.

Peng, T.Y. and Don, M.M., 2013. Antifungal Activity of In-vitro Grown *Earliella scabrosa*, a Malaysian Fungus on Selected Wood-degrading Fungi of Rubberwood. *Journal of Physical Science*, *24*(2), pp. 21– 33.

Pessini, G.L., Filho, B.P.D., Nakamura, C.V. and Cortez, D. A. G., 2003. Antibacterial Activity of Extracts and Neolignans from *Piper regnellii* (Miq.) C. DC. var. pallescens (C.DC.) Yunck. *Mem Inst Oswaldo Cruz, 98* (8), pp. 1115–1120.

Praptiwi, Raunsai, M., Wulansari, D., Fathoni, A. and Agusta, A., 2018. Antibacterial and Antioxidant Activities of endophytic Fungi Extracts of medicinal plants from Central Sulawesi. *Journal of Applied Pharmaceutical Science*, *8*(08), pp. 69–74.

Reis, F. S., Pereira, E., Barros, L., Sousa, M. J., Martins, A. and Ferreira, I. C. F. R., 2011. Biomolecule profiles in inedible wild mushrooms with antioxidant value. *Molecules*, *16*, pp. 4328–4338.

Ren, L., Perera, C., and Hemar, Y., 2012. Antitumor activity of mushroom polysaccharides: A review. *Food and Function*, *3*, pp. 1118–1130.

Rohr, C.O., Levin, L.N., Mentaberry, A.N., Wirth, S.A. 2013. A First Insight into *Pycnoporus sanguineus* BAFC 2126 Transcriptome. *PLoS ONE*, *8*(12): pp. 1–14.

Rosa, L. H., Machado, K. M. G., Jacob,C. C., Capelari, M., Rosa, C. A., Zani, C. L., 2003. Screening of Brazilian basidiomycetes for antimicrobial activity. *Memórias do Instituto Oswaldo Cruz*, *98* (7), pp. 967–974.

Scherer, R., and Godoy, H. T., 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, *112*, pp. 654–658.

Silva, M. T. G., Simas, S. M., Batista, T. G. F. M., Cardarelli, P., and Tomassini, T. C. B., 2005. Studies on antimicrobial activity, in vitro, of *Physalis angulata* L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination. *Mem Inst Oswaldo Cruz*, *100*(7), pp. 779–782.

Singdevsachan, S. K., Patra, J. K., and Thatoi, H., 2013. Nutritional and Bioactive Potential of Two Wild Edible Mushrooms (*Lentinus sajor -caju* and *Lentinus torulosus*) from Similipal Biosphere Reserve, India. *Food Science and Biotechnology*, *22*(1), pp. 137–145.

Smânia. A., Monache F.D., Smânia, E.F.A., Gil, M.L., Benchetrit, L.C., and Cruz, F.S., 1995. Antibacterial activity of a substance produced by the fungus *Pycnoporus san- guineus* (Fr.) Murr. *J Ethnopharmacol* 45, pp. 177–181.

Smânia, E.F.A., Smânia Júnior, A., and Loguercio- Leite, C., 1998. Cinnabarin synthesis by *Pycnoporus sanguineus* strains and antimicrobial activity against bacteria from food products. *Revista de Microbiologia*, *29* (4), pp. 317–320.

Smânia, A., Marques, C.J.S., Smânia, E.F.A., Zanetti, C. R., Carobrez, S. G., Tramonte, R. and Loguercio-Leite, C., 2003. Toxicity and Antiviral Activity of Cinnabarin Obtained from *Pycnoporus sanguineus* (Fr.) Murr. *Phytotherapy Research*, *17*, pp. 1069–1072.

Suay, I., Arenal, F., Asensio, F. J., Basilio, A., Cabello, M. A., Díez, M. T., … Vicente, M. F., 2000. Screening of basidiomycetes for antimicrobial activities. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *78*, pp. 129–139.

Susan, D., and Retnowati, A., 2017. Catatan Beberapa Jamur Makro Dari Pulau Enggano: Diversitas Dan Potensinya. *Berita Biologi*, *16* (3), pp. 243–256.

Takao, L. K., Imatomi, M. and Gualtieri, S. C. J., 2015. Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from Cerrado *(Brazilian Savanna)*. *Braz. J. Biol.*, *75*(4), pp. 948–952.

Tambekar, D.H., Sonar, T.P., Khodke, M.V., and Khante, B.S., 2006. The novel antibacterials from two edible mushrooms: *Agaricus bisporus* and *Pleurotus sajor caju*. *Int J Pharmacol*, *2*, pp. 584–587.

Teoh Y.P, Don M.M, and Ujang S., 2011. Media Selection for Mycelia Growth, Antifungal Activity Against Wood-Degrading Fungi, and GC-MS Study by *Pycnoporus sanguineus. BioResources*, *6*(3), pp. 2719–2731.

Wang, J., Yue, Y.-D., Tang, F., and Sun, J., 2012. TLC Screening for Antioxidant Activity of Extracts from Fifteen Bamboo Species and Identification of Antioxidant Flavone Glycosides from Leaves of *Bambusa. textilis* McClure. *Molecules*, *17*, pp. 12297–12311.

Wasser, S. P., 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, *60*, pp. 258–274.

Zmitrovich, I.V., Ezhov, O.N., Ranadive, K.R., Wasser, S.P. 2017. Profiles of Little-Known Medicinal Polypores: *Earliella scabrosa* (Agaricomycetes). *International Journal of Medicinal Mushrooms*, *19*(11), pp. 1023–1027.