The potential of white-oyster mushroom (*Pleurotus ostreatus*) as antimicrobial and natural antioxidant

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Abstract. Egra S, Kusuma IW, Arung ET, Kuspradini H, 2018. The potential of white-oyster mushroom (*Pleurotus ostreatus*) as antimicrobial and natural antioxidant. Biofarmasi J Nat Prod Biochem 16: 17-23. White-oyster mushroom (*Pleurotus ostreatus*) is a favorite meal in Indonesia. Previously this fungus was known as a useless plant, but after the nutrition is known, everything changes. People tried to cultivate it because the nutrients contents are very good for body health. Therefore, to support this added value in the field of health, especially antimicrobials and antioxidants, this research needs to be done. This research used successive extraction with hexane solvent, acetate ethyl, ethanol, water, and crude ethanol by antimicrobial assay, antioxidants assay (DPPH), total antioxidant content, total phenolic content. The highest results on barrier antimicrobial test which occurred against Candida albicans bacteria was 47.60 % with 100 ppm concentration. While, on antimicrobial assay using Propionibacterium acnes bacteria, there was no significant inhibition. Regarding the antioxidant test against DPPH, the result showed the occurrence of free radical by 25 % on water extraction at the concentration of 100 ppm. Continuously, the total antioxidant content assay showed the ethyl acetate had the highest value of 368.708 mg gae/g. The results of the total content phenolic assay showed the solvent hexane had the value of 78.495 mg gae/g. These findings indicated that mushroom has an active phenolic compound with no contribution to impede its working on *Candida albicans* assay.

Keywords: Antimicrobial, total antioxidant, total phenol, white-oyster mushroom

INTRODUCTION

Mushrooms become more popular in Indonesia when people realize their benefits. Over the years, people have consumed several varieties of mushrooms, such as: white-pink oyster, shiitake, black jelly, and straw mushrooms because of their benefits. At this time, mushrooms have been processed and consumed in various forms as vegetables, crackers, and herbs for health purposes. Most Indonesian people start cultivating oyster mushrooms because of their efficacy and high economical value that they provide high income for the cultivators. Nasrul (2004) stated that historically, it has been used as food since 3000 years ago which was exclusively presented to the King of Egypt while Chinese has used it as herbs since 2000 years ago. Mushrooms are one of highly nutritious and cholesterol-free foods (Nasrul 2004). Sumarmi (2006) states that 100 grams of oyster mushrooms contain protein (19-35 %) which consists of 9 amino acids; fat (1.7-2.2 %) in which 72 % are unsaturated fatty acids, carbohydrates, vitamins B (thiamin, riboflavin, and niacin), vitamin D and C, minerals (K, Na, Ca, Mg, Zn, Fe, Mn, Co, and Pb), and the levels of metal-microelements are very low so it is safe to be consumed every day.

According to Chang and Buswell (1996), mushrooms are delicious food and some of mushrooms have been known to have biological activities as anti-cancer, anti-diabetes, overcoming hyperlipidemia and increasing the immune system. According to Bobek et al (1998), oyster mushrooms are good for cardiovascular patients; it also can control cholesterol levels. Limited knowledge about the benefits of mushrooms is owned by the community, so they consume it only as additional dishes. This research was conducted to determine the potential of white-oyster mushrooms (*Pleurotus ostreatus*) as antimicrobial and natural antioxidant

MATERIALS AND METHODS

Plant materials and chemicals

This research was carried out at Chemistry Laboratory, Department of Forest Products Technology, Faculty of Forestry, Mulawarman University, Samarinda, Indonesia. The research material (fresh oyster mushrooms) was obtained from the Faculty of Forestry, Mulawarman University, Samarinda, Indonesia. The chemical used are n-hexane, ethyl acetate, ethanol, acetone, isolates of *Propionibacterium acnes* and *Candida albicans* provided by Forest Products Chemistry Laboratory in Mulawarman University, nutrients agar (Difco, USA), chloramphenicol, and glucose (Merck, Germany). The equipment used in this research was Shimadzu UV-VIS 1240 spectrophotometer (Shimadzu, Japan), evaporator (Eyela, Japan) and autoclave provided by all American Model 25X-2.
**Extraction**

White-oyster mushroom (*Pleurotus ostreatus*) (500 grams) was cut into small pieces, dried in the oven at 39°C for 48 hours. The dried mushroom was ground into coarse powder by blender. The powder was extracted successively with n-hexane, ethyl acetate, and ethanol. The powder was extracted separately with ethanol to obtain crude ethanold. The extraction was conducted at room temperature with continuous shaking on a shaker (7400 Tübingen; EdmundBuchler, Germany) for 48h., followed by filtration of suspension with Whatman filter paper No. 2 (Maidstone, UK). The crude alcohol extracts were evaporated at 40°C and put in a vacuum oven to near-dried extracts to yield the mushroom extracts.

**Total phenolic content**

Total phenolic content was determined using the method by Slinkard and Singleton (1977). Sample (5 mg) was dissolved in 1000 µL ethanol. 200 µL of the sample solution was put into a test tube and added with 1.25 mL of distilled water and 75 µL of 5% NaNO₂ and incubated at room temperature for 5 minutes. 150 µL was added from 10% AlCl₃ and incubated at room temperature for 6 minutes. Then it was added with 500 µL of 1 M NaOH and 275 µL of distilled water, was incubated for 20 minutes for a reaction. Measurements were carried out with a spectrophotometer with an absorbance of 510 nm with the use of distilled water as a blank.

**RESULTS AND DISCUSSION**

**Extraction**

The extraction method used was successive maceration at room temperature which three different solvents (n-hexane, ethyl acetate, and ethanol). The ideal solvent for extracting process must have some conditions, namely: (i) it must be able to dissolve the extractive substances, (ii) it must have similar boiling level with the substance, (iii) it must be inert (it does not react with substances that will be extracted, (iv) it must have low boiling point for easy evaporation, but not too low that it can cause the loss of some solvents as a result of the evaporation (Guenther 1987). The extraction is started by macerating the sample for 24 hours with n-hexane. The filtrate was filtered with filter paper and evaporated to obtain concentrated hexane extract. The powder of mushroom which has been extracted with hexane was re-extracted with ethyl acetate followed by ethanol.

The extract yield can be used as a reference to find out the amount of simplicial needed to make a certain number of thick extracts. According to Lo et al. (1983), the extraction method is one of the factors that will affect the yield of an extract. Extraction using solvents consists of several methods, including maceration, percolation and heat methods, reflux, Soxhletation, infusion, decadence, and digestion. Besides, the amount of extract is also influenced by the polarity index in the solvent; the lowest to highest polarity index in this study is, respectively, hexane, ethyl acetate, and ethanol.

Table 1 showed that the highest yield of extract was with the ethanol extract (7.88%), while with the hexane extract it was less than 1% (0.51%). The yield on extracting mushroom with acetate ethyl was 6.02%. The n-hexane solvent has 0.51% yield.

**Antimicrobial activity**

Antimicrobial assay on white-oyster mushroom (*P. ostreatus*) was done against *P. acnes* and *C. albicans* with agar diffused method. Chloramphenicol was used as a positive control because it has broad-spectrum as antibacterial, while acetone was used as a negative control. Microbial resistance to antibiotics is a major problem today. Many biological active components released from plant species are commonly used as drugs, because they...
can offer a new source of antimicrobial activity. The search for antimicrobial bioactivity from natural materials gives the result of antimicrobial activity from white oyster mushroom (*Pleurotus ostreatus*) which is shown in Table 2.

Table 1. Yield of oyster mushroom extract in several solvents

| Extract         | Sample weight (g) | Extract yield (g) | Extract (%) |
|------------------|-------------------|-------------------|-------------|
| n-Hexane         | 40                | 0.18              | 0.51        |
| Ethyl acetate    | 40                | 2.14              | 6.02        |
| Ethanol          | 40                | 2.80              | 7.88        |
| Crude ethanol    | 23                | 0.08              | 0.39        |

Note: The percentage of dried-mushroom extract based on the weight.

Chloramphenicol with the zone barriers 24.5 mm has presented the results of the test activity of bacteria *P. acnes*. This study showed the extract has no resistance to *P. acnes*. This indicates that there is no active anti-bacterial compound towards *P. acnes*. The absence of active compounds in the extract is thought to be due to very little concentration, perhaps if the concentration is raised it will increase inhibition. Antimicrobial activity of oyster mushroom extract has also conducted against one of the fungi, *C. albicans*. The results of the assay displayed that oyster mushroom extract indicated the formation of barriers on some level zone concentration of extract. Mushroom extract on the concentration of 25 µg showed the lowest activities of anti-candida with inhibition of 8.53 mm width. Increasing inhibition activity was shown on the concentration of 50 µg with inhibition of 10.5 mm width. The best activities were indicated by mushroom extract on the concentration of 100 µg with inhibition of 10.8 mm width. Davis and Stout (1971) reported the inhibiting diameter regions on 5 mm or less then inhibiting activities is categorized as weak; 5-10 mm is categorized as medium; 10-19 mm is categorized as strong, whilst 20 mm or more is categorized as very strong.

Table 2 showed the inhibition as a whole in medium category as it is around 8-10 mm. Of all solvent, the highest barriers were produced on the rough extract ethanol with 10.8 mm. It was identified on the concentration of 100 µg. Oyetayo (2009) reported that fungus extracts are able to inhibit the growth of all kinds of organisms on the concentration between 12.5 mg/mL until 100 mg/mL. This research used less concentration, 25 µg, 50 µg, 100 µg, and these concentrations have been able to inhibit the growth of the mushroom *C. albicans*.

Nwachukwu and Uzoeto (2010) found that the hot water extract of the *R. vesca* mushroom is ably inhibiting the growth of *E. coli, S. typhi, P. mirabilis*, and *C. albicans*. The ethanol extraction of *A. auricular* showed a broad spectrum of microbial effect against microorganisms experiments with the exception of *S. tophi* and *P. aeruginosa*. *P. squarrosulus* displaying the microbial activity against *K. pneumonia* (6.14 mm), *S. pneumoniae* (5.12 mm), and *C. albicans* (4.10 mm). *P. aeruginosa* has swapped off almost all extract from four species of fungus except hot water extraction from *P. squarrosulus* which shows the inhibition zone (3.41 mm). *V. vulvae* showed the microbial activity against *S. typhi* (4.60 mm). However, Vamanu (2012) reported, the *P. ostreatus* is able to inhibit *Candida albicans* with MIC 12.5, 12.5, 25, 25 mg/mL, continuously on a different nitrogen source, corn, malt, and yeast extraction, and peptone used in the culture media.

Table 2. Antimicrobial activity of oyster mushroom against *Candida albicans*

| Sample extracts | Inhibition (mm) | Inhibition (%) |
|-----------------|-----------------|---------------|
|                 | 25 µg | 50 µg | 100 µg | (+) | 25 µg | 50 µg | 100 µg | (+) |
| n-Hexane        | 9.86  | 9.46  | 8.63   | 23.76 | 41.5  | 39.8  | 36.3  | 100 |
| Ethyl acetate   | 9.76  | 9.96  | 9.7    | 21.86 | 44.65 | 45.56 | 44.4  | 100 |
| Ethanol         | 8.53  | 9.16  | 9.06   | 23.96 | 35.6  | 38.2  | 47.6  | 100 |
| Crude ethanol   | 9.3   | 10.5  | 10.8   | 22.7  | 40.96 | 46.3  | 37.8  | 100 |

Note: (+) is positive control (Chloramphenicol)
Compounds must continue to be done, because it is very important in finding solutions to diseases caused by free radicals, in testing oxidative reactions in food, and in protecting against DNA damage and carcinogenesis. In the future, this substance will function in many ways such as pharmacological activity; anti-inflammatory, anti-bacterial, and anti-fungal.

**Total antioxidant capacity**

Total antioxidant content assay has been done to find out how many active compounds are able to scavenge free radicals on the mushroom. Surekha et al. (2011) concluded that mushroom is healthy food, moreover, its rich protein and antioxidant compounds which are essential compound are able to fight disease. Total antioxidant content assay results from several extraction methods on mushroom are presented in Table 4.

Table 4 showed that the oyster mushroom extractions have good antioxidant activity. The ethyl acetate has the highest antioxidant content values which are followed by hexane extract, ethanol, and crude ethanol extract. Table 4 displayed the different colors on the assay solvent indicating the existing active compounds. Polyphenol is one of contributed antioxidant activity on the fruit, vegetables, and fungus (Ferreira et al. 2007).

**Table 3. Antioxidant activity of oyster mushroom extract against DPPH**

| Sample          | Inhibition (%) |
|-----------------|----------------|
|                 | 25 ppm | 50 ppm | 100 ppm |
| Vitamin C (positive control) | 96.84  | 96.84  | 96.85   |
| n-hexane        | 3.92   | 2.41   | 0.30    |
| Ethyl acetate   | 2.72   | 2.72   | 6.34    |
| Ethanol         | 2.25   | 8.20   | 13.88   |
| Crude ethanol   | 17.85  | 17.85  | 21.43   |

**Table 4. The total antioxidant capacity of oyster mushroom**

| Samples          | Absorbance | Antioksidan capacity (mg GAE/g) |
|------------------|------------|---------------------------------|
|                  | Rep. 1     | Rep. 2     | Rep. 3     |                |
| n-hexane         | 1.283      | 1.238      | 1.352      | 313.625        |
| Ethyl acetate    | 1.485      | 1.523      | 1.526      | 368.702        |
| Etanol           | 0.548      | 0.563      | 0.544      | 128.792        |
| Crude Etanol     | 0.479      | 0.502      | 0.505      | 114.708        |

**Note:** Rep. = Repetition

**Table 5. The total phenolic content of oyster mushroom**

| Samples          | Absorbance | Antioksidan capacity (mg GAE/g) |
|------------------|------------|---------------------------------|
|                  | Rep. 1     | Rep. 2     | Rep. 3     |                |
| n-hexane         | 0.28       | 0.3        | 0.28       | 78.495         |
The high antioxidant content on oyster mushroom extracts allegedly because the polyphenol is found on the oyster mushroom. Hapsari et al. (2012) reported that oyster mushroom extraction has antioxidant and tyrosine activities with the result that the ethanol extraction has higher activity than water extraction. It indicated that solvent ethanol is more dissolving antioxidants active compounds than water which is polar. The Culinary-medicinal mushroom research which found a good antioxidant content and potentially antihypertensive can be seen from the occurrence of inhibition of oyster mushroom to ACE (angiotensin I-converting enzyme).

### Total phenolic content

Total phenolic content assay has been done to find out how much the content of active phenol compounds on the oyster mushroom. Phenol components, flavonoid, anthocyanin, and carotenoids develop the main components of the natural antioxidant which scavenges free radicals due to its ability to divide the hydrogen atoms or electron and the balance of radical compound (Shahidi and Wanasundra 1992). Table 5 shows the results in total phenolic content from oyster mushroom extract on some of the solvents.

| Solvent          | Total Phenolic Content (mg GAE/g) |
|------------------|-----------------------------------|
| n-Hexane         | 0.23                              |
| Ethyl acetate    | 0.2                               |
| Ethanol          | 0.1                               |
| Crude Ethanol    | 0.09                              |

Table 5 presented that the highest rate of phenol content is from the extract by hexane followed by ethyl acetate, ethanol and crude ethanol which indicates that oyster mushroom has active phenolic compounds. Alvarez (2007) reported that mushroom can be used as the source of low calories and fat on food with the high polyphenol and antioxidants activity level. Phenol compound is the component with the highest antioxidant activity on the white-oyster mushroom. The activities are not only mainly caused by the capability in reducing hydrogen and singlet oxygen quencher but the component also has the potential metal chelation effect (Polite 2010).

Phenolic acid plays the main role in phenolic components in the mushroom (Ferreira 2009). According to Puttaraju (2006), galic, tannin, protocatechuic, and gentisic acids are some main phenolic content detected in the water extraction from some Indian mushroom traditional food. Abdullah et al. (2012) stated that the total phenolic from some varieties of mushroom extraction assay started at 6.19 to 63.51 mg GAE/g with G lucidum having the highest phenolic content (63.51 ± 1.11 mg GAE/g). Iwalokun (2007) reported the phenolic content and the antioxidant
content on acetone extract of *P. ostreatus* is equal to petroleum ether extract of *P. ostreatus*.

![Figure 4. The result of total flavonoid content](image)

| Samples          | Absorbance | Antioksidan (mg GAE/g) |
|------------------|------------|------------------------|
|                  | Rep. 1     | Rep. 2     | Rep. 3    |
| n-hexane         | 1.758      | 1.905      | 1.461     | 653.5    |
| Ethyl acetate    | 0.357      | 0.394      | 0.231     | 122.474  |
| Ethanol          | 0          | 0          | 0         | 0        |
| Crude Ethanol    | 0          | 0          | 0         | 0        |

**Table 6. The total flavonoid content of white oyster mushroom**

The highest flavonoid content in hexane is probably due to the density that occurs because of the reaction of the reagent solvent as shown in Figure 5. It is caused by hexane which has non-polar properties, so it has no ability to attract or dissolve active flavonoid compounds in oyster mushrooms. Hapsari et al. (2012) reported that from the chemical composition of the oyster mushroom simplicia, the presence of alkaloids, saponins, phenols, and tannins has been detected, but the presence of flavonoids was undetected. This was confirmed by Kim's research (2009) reporting that carotenoids such as lutein, lycopene, β-carotene, zeaxanthin staining were not detected in oyster mushroom. The absence of flavonoids in oyster mushrooms probably is a biological factor and a factor in the ecology of oyster mushrooms because the bioactive components inhibit enzyme activity (tyrosinase) for the development process and growth of oyster mushroom pigmentation (Xie et al. 2003).

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