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

SAAT EGRA[1,♥], IRAWAN WIJAYA KUSUMA[2,♥♥], ENOS TANGKE ARUNG[2,♥♥♥], HARLINDA KUSPRADINI[2,♥♥♥♥]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Borneo Tarakan. Jl. Amal Lama No. 1, Tarakan 77123, North Kalimantan, Indonesia. ♥email: saat.egra.shaumi@gmail.com

[2]Faculty of Forestry, Universitas Mulawarman. Jl. Penajam, Samarinda 75123, East Kalimantan, Indonesia. Tel.: +62-541-749068, Fax.: +62-541-735379, ♥♥email: kusuma_iw@yahoo.com, ♥♥♥tangkearung@yahoo.com, ♥♥♥♥alinkuspra@gmail.com

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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, shitake, 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, P, Na, Ca, Mg, Zinc, 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 39ºC 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 ethanol. The extraction was conducted at room temperature with continuous shaking on a shaker (7400 Tübingen; EdmunBuchler, Germany) for 48h., followed by filtration of suspension with Whatman filter paper No. 2 (Maidstone, UK). The crude alcohol extracts were evaporated at 40ºC and put in a vacuum oven to near-dried extracts to yield the mushroom extracts.

Antimicrobial assay

Antimicrobial assays were conducted using the agar well disk diffusion method of Kuspradini (2012). *Propionibacterium acnes* and *Candida albicans* were used in all experiment. Nutrient agar (Difco) was used as the main media to culture bacteria. 20 mL aliquots of sterile media were transferred to Petri dishes and allowed to solidify. The media were inoculated with 20 µL of microbial suspension spread uniformly on the surface of the plates. After that, the media agar plates were cut using a sterile cork borer with size seven-mm well and 20 µL acetone solution containing 25-100 µg extracts were added to the well. Chloramphenicol was used as a positive control at the concentration of 10 µg/20 µL in each well. The plates were incubated in the dark at 32ºC for 24 h. Microbial activity was measured (mm) by the existence of inhibiting zone around agar diffusion well-containing extract. Microbial activity was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug. (Kuspradini 2012).

Total antioxidant capacity

Total antioxidant capacity was evaluated according to the method described by Prieto et al. (1999). 5 mg sample was dissolved in 1000 µL ethanol. 100 µL sample solution was added by 1 mL Reagent (0.6 M sulfuric acid + 28 mM sodium phosphate + 4 mM ammonium molybdates). Then, sample was incubated in water bath shaker at 95ºC for 90 minutes and cooled to room temperature. Measurement was made using spectrophotometer with absorbance wavelength of 695 nm. The gallic acid was used as a standard on 0.02 in 0.1 mg/mL and ethanol as a blank.

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. 20 µL sample solution was added to the tube with 100 µL (2 N Reagan Folin-Ciocalteu) and 300 µL from 0.2 mg/mL solution of sodium carbonate and 1180 µL aquadest and the volume growth 1600 µL in the reactive tube. Incubation was done at temperature 37ºC for 2 hours. The measurement was conducted with spectrophotometer with wavelength of absorbance 760 nm. The gallic acid was used as standard.

Total flavonoid content

Total flavonoid content was determined using the method of Choi et al. (2006). 5 mg sample was dissolved in 1000 µL ethanol. 250 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% $NaNO_2$ and incubated at room temperature for 5 minutes. 150 µL was added from 10% $AlCl_3$ 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 24.5mm 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.

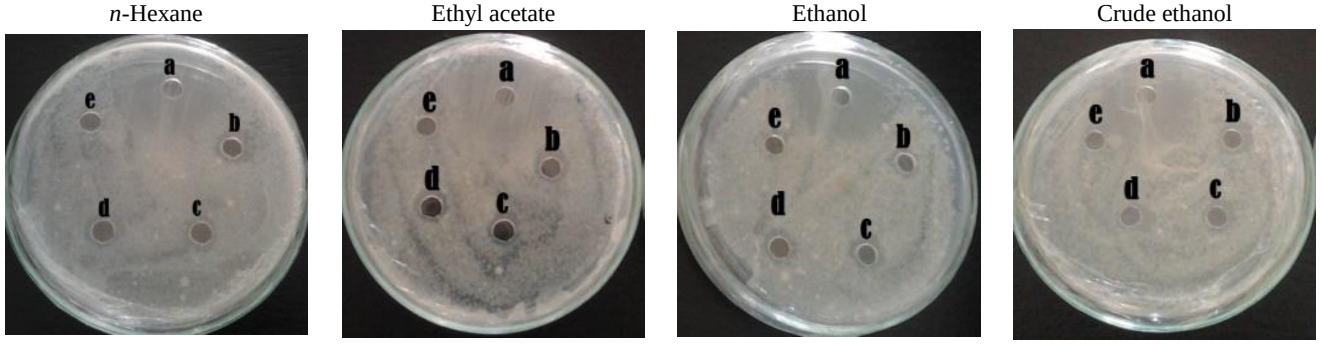
Table 2 showed the inhibition as a whole in medium category as it is around 8-10 mm. Of all solvent, the highest barriers were produced on the rough extract ethanol with 10.8 mm. It was identified on the concentration of 100 µg. Oyetayo (2009) reported that fungus extracts are able to inhibit the growth 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)



Picture 1. Inhibition of oyster mushroom against *Candida albicans*. (a) control (-); (b) control (+); (c) concentration 100 µg; (d) concentration 50 µg and (e) concentration 25 µg

Hapsari et al. (2012) reported that the chemical composition at oyster mushroom is alkaloid, saponin, phenolic, and tannin. Tannin plays important role in degradation of microbial cell wall. Ajizah (2004) reported, tannin also has antibacterial capability by precipitating proteins. The effect of antibacterial tannin is through the reaction with the membrane cell, inactivation enzymes, and function of genetic material. This condition makes some fungi used extensively as food for its nutrition in traditional medicine (Stamets 2000; Lindequist et al. 2005). The strong activity of plant extracts against *C. albicans* shows the potential for use as a drug for candidemia, nosocomial infections, and diseases caused by other *Candida infections*

DPPH antioxidant activity

Antioxidant assay was done by scavenging free radicals which are measured at spectrophotometer with DPPH free radicals agent. The positive control used vitamin C because it is known as the best natural antioxidant. The number of free radical scavenging activity is presented as the percentage of the inhibition with an indication of color changes of DPPH. The result of the antioxidant activity mushroom extract oysters is presented in Table 3.

Table 3 displayed that the antioxidants activity by scavenging free radical DPPH is influenced by the type of extraction and concentration levels. The crude ethanol provided the highest inhibition by 21.43% on the concentration of 100 ppm. The results indicated that the use of higher concentrations is potential to improve antioxidant activity. These active compounds were soluble on ethanol extraction.

Antioxidant activity assay aimed to find out how big a plant will be able to scavenge free radicals. Mechanism of scavenging free radicals is by inhibiting lipid oxidation that can be used to calculate the activity of antioxidant (Pornariya 2009). The crude ethanol extract showed relatively better inhibition with 21.43 % on 0.1 mg/mL even though it was still lowest than vitamin C which was used as a positive control. Mau et al. (2001) and Lo (2005) found that free radical activity in oysters mushroom towards DPPH was 81.8 % on 6.4 mg/mL and 68.4% on 5 mg/mL. It specified that extracting concentration on antioxidant activity assay plays an important role to produce high antioxidant activity.

White Oyster mushroom (*Pleurotus ostreatus*) is able to be an antioxidant because it provides phenolic, L-ergotine, selenium compound and vitamin C (Jayakumara et al. 2006). Phenolic compounds are capable to inhibit the oxidation reaction and reduce hydroxyl radical, superoxide, and peroxide. Phenolic also has an effect on the process of synthesis transcription of endogenous antioxidant, namely glutathione (Khotimah 2008). 250 mg/kg BW white oyster mushroom extraction has a great antioxidant effect because it contains antioxidant substances, phenol, ergotine, vitamin C, selenium, and beta-carotene. Phenol compound is the component with the highest antioxidant activity on white oysters mushroom. The research to find antioxidant 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

Ethyl acetate	0.23	0.2	0.21	57.733	Crude Etanol	0.09	0.08	0.09	21.352
Ethanol	0.07	0.09	0.1	22.495					

Note: Rep.= Repetition

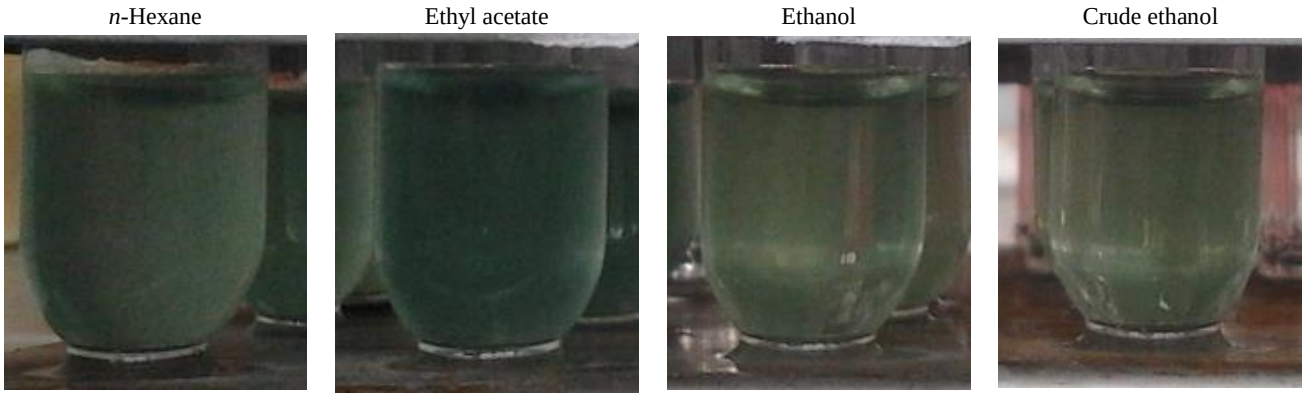


Figure 2. The result of total antioxidant capacity assay

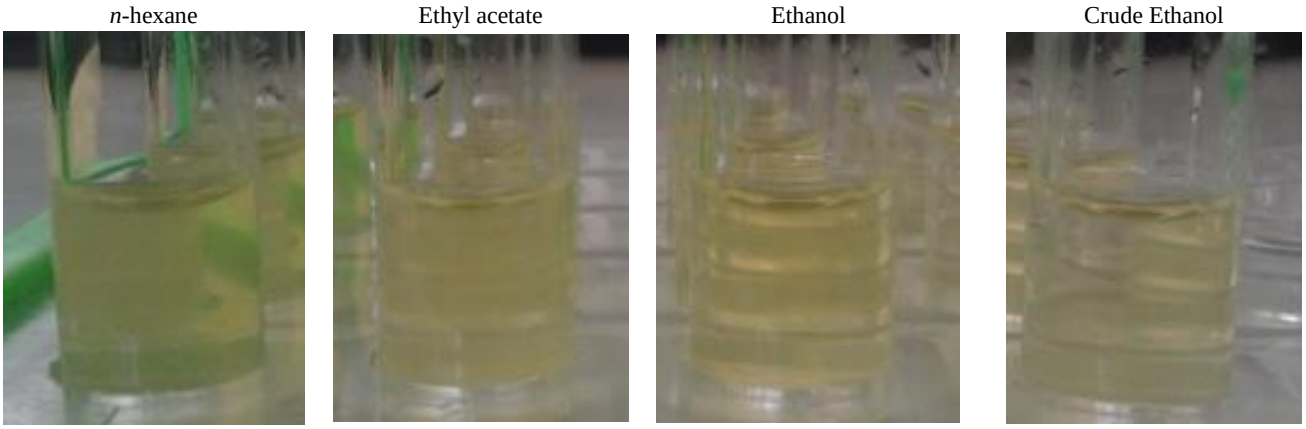


Figure 3. The result of total antioxidant content

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.

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*.

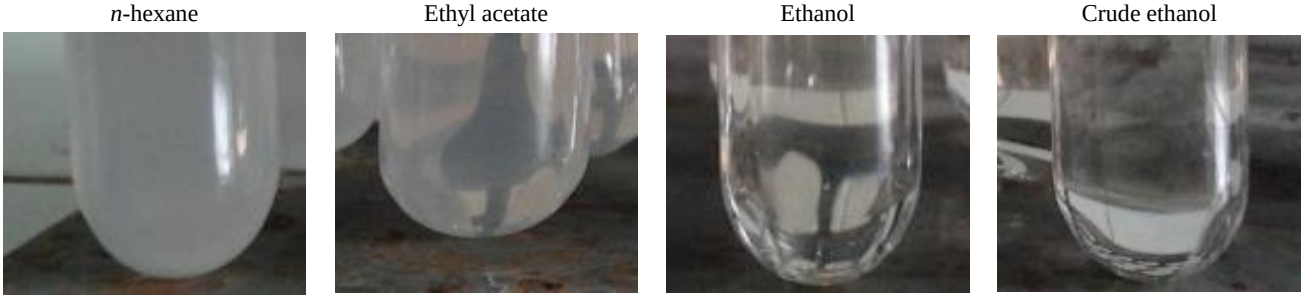


Figure 4. The result of total flavonoid content

Table 6. The total flavonoid content of white oyster mushroom

Samples	Absorbance			Antioksidan capacity (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

Total flavonoid content

Total flavonoid content assay has been done to find out how much the active compound of flavonoids in oyster mushrooms. Flavonoids are usually glycosylated and can be classified as anthocyanidins, flavanols (catechins), Flavone, flavanone, and flavonol, which are commonly found in fruits and vegetables in orange, red, and blue. Generally, they are found in light-colored fruit, vegetables or foods that are known to be healthy for the human body (Lin and Tang 2007). Table 6 shows that oyster mushroom extract has flavonoid levels. The highest level of flavonoid is in hexane solvent and is followed by ethyl acetate.

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 is probably 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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