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Antimicrobial Activity and Identification The Compounds of Methanol Extract from The *Pleurotus Ostreatus* Fruiting Body

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Abstract

Pleurotus ostreatus is an edible mushroom that also has potential as medicinal values. In this study, fruiting body of *P. ostreatus* was tested for its ability to inhibit the growth of fungi and bacteria. The fruiting body powder of *P. ostreatus* was extracted using methanol by maceration method. Analysis of this compound was done by using anisaldehyd sulfuric acid, Dragendorff reagent, and $FeCl_3$. Using the agar well diffusion technique, the extracts were tested for their antimicrobial activity against *Staphylococcus aureus* (Gram positive), *Enterobacter aerogenes* (Gram negative) and *Candida albican* (yeast). The spot results on TLC using crude extract of *P. ostreatus* is terpenoids. Zone of inhibition for the various extracts varied between 10.9 - 23.2 mm. Ten miligrams extract exhibit maximum antimicrobial activity against most of the tested pathogens

1. Introduction

Infectious disease is an health problems in most of the developing countries, including in Indonesia. Although the chemical antimicrobial agent have been synthesized, but a careless use could led to the development of antibiotics resistance to be more dangerous (Raghunath, 2008). The resistant pathogens to antibiotic are becoming one of the most serious threats

to microbial diseases treatment. A novel antimicrobial agent from different natural biological source is needed (Cordell, 2000). Research on drugs derived from fungal sources has also a considered (Rosa et al., 2003).

Oyster mushroom (*Pleurotus ostreatus*) is a mushroom that used not only as a food because of their flavor, aroma, and nutrition values but also for their medicinal properties.

According to Jose & Janardhanan (2000), oyster mushroom has antibacterial, anticancer, and antitumor potential. It has been reported that *P. ostreatus* is able to inhibit the proliferation of breast cancer cells and colon cancer. According to Jedinak & Sliva (2008), oyster mushrooms have a therapeutic potential in breast cancer and colon. Water solvent extract from *P. ostreatus* produce the most significant cytotoxicity on prostate cancer cells (PC-3) [Gu & Sivam, 2006].

Research on antimicrobial methanol extracts of fruiting bodies of *P. ostreatus* cultivated in Indonesia have not been reported, so it is necessary to investigate the antimicrobial extract *P. streatus* against fungi and bacteria.

2. Materials and methods

a) Collection of mushrooms

Pleurotus ostreatus which used in this study was obtained from Persatuan Petani Jamur Indonesia Asrifarm, located at Rembang, Central Java, Indonesia. The fruiting bodies were washed thoroughly to remove any debris or dust particles; thereafter, they were allowed to dry in an oven at 40°C.

b) Methanol Extract of The *P. ostreatus* Fruiting Body

Maceration is done by grinding the dried fruit body of *P. ostreatus*, then soaked in methanol (CH₃OH) for three days at room temperature and protected from light, liquid extract will go into the cells pass through the cell wall. The contents of the cells will dissolve as the concentration difference between the solution inside the cell and outside the cell. During the process of maceration stirring and methanol replacement is done every day. Replacement of methanol done until the solution becomes clear with the assumption that there is no active compound contained in the dry powder. Maceration solution is dried until all the solvent evaporates and the results obtained a viscous extract. The extract obtained was weighed, and put in a flacon and

stored in a refrigerator. The final result of the maceration process is a pulp and methanol extract.

c) Chromatography Extracts of *P. ostreatus*

Bioactive methanol extract screening is done by using Thin Layer Chromatography (TLC). The stationary phase such as silica gel 60 F₂₅₄ with mobile phase for Terpenoid compound is hexane: ethyl acetate = 93: 7. Alkaloids compound using toluene: ethyl acetate: diethylamine = 7: 2: 1. Phenolic compounds using ethyl acetate: formic acid: toluene: water = 6: 1.5: 3: 0.5. Activation TLC plate is done by using a hair dryer for 10 minutes. Extract spotted using microhematocrit at the bottom of the TLC plate with a diameter of 2-3 mm, distance between spot is 1 cm, then dried and spotted back. TLC plates which had been spotted and then inserted into a vessel, wait until the phase up to the mark (0.5 cm from the top), take TLC and dried. The sulfuric acid anisaldehyd, Dragendorff reagent, and FeCl₃ sprayed and inserted in the oven at 100° C for 1 minute. Chromatogram profile was visualized using visible light, UV light with $\lambda = 254$ nm and 366 nm.

d) Microbial Test Organisms

The antimicrobial activity from methanol extract of *P. ostreatus* fruiting body was evaluated using agar well diffusion assay (Perez *et al.*, 1990). In this method, 100 μ l of 24 h culture of each test organism which is equivalent to a 0.5 McFarland standard was inoculated on the Muller Hilton Agar (MHA) for bacteria and Sabouraud Glucose Agar (SGA) for yeast and the thickness is 4 mm. Then it is spread on to the surface of the agar using a sterilized glass spreader. After 30 minutes of inoculation, the wells were prepared using sterilized steel cork borer (5 mm diameter). Four wells were made in each plate, out of which four wells were loaded with 100 μ l of different test *P. ostreatus* extracts. Standard antibiotics (Amphicillin, Chloramphenicol and Nistatin) were inoculated in each separate

plate to be used as a positive control and to be compared to the mushroom extracts. All the plates were then incubated aerobically at 37°C for 24 – 48 h. Antimicrobial activity of the extracts was determined by measuring the diameter of inhibition zone and compared with the standard antibiotics inhibition results obtained from the inhibition diameter zone that measured at a cross angle and three measurements taken three times and was taken as mean of three independent measurements. Antibacterial activity was recorded when the zone inhibition greater than 6 mm (Nehra et al, 2012).

3. Result

a. Identification of the Compounds

Extraction is the process to take an active compound that is in the cells using a solvent that can dissolve the active compound wish extracted. Type of the extracted compounds is

determined by the type of solvent used (Tiwari et al., 2011). Methanol is semipolar, that are expected able to extract polar and non-polar compounds. Methanol is used because it is more volatile, resulting in the maceration process to extract bioactive compounds done faster. Results of the extraction with methanol *P. ostreatus* from 19.8 gram powder (about 2.1 gram extract) shows that the yields is 10.6% with a brownish paste extract in physical properties. This shows *P. ostreatus* contains active compounds that can dissolve by methanol. Methanol extract bioactive compounds identified using Thin Layer Chromatography (TLC) with the stationary phase in the form of silica gel 60 F₂₅₄ then tested by sulfuric acid anisaldehyd, Dragendorff and FeCl₃ to determine the class of compounds. The results of TLC shown in Figure 1 and Table 1

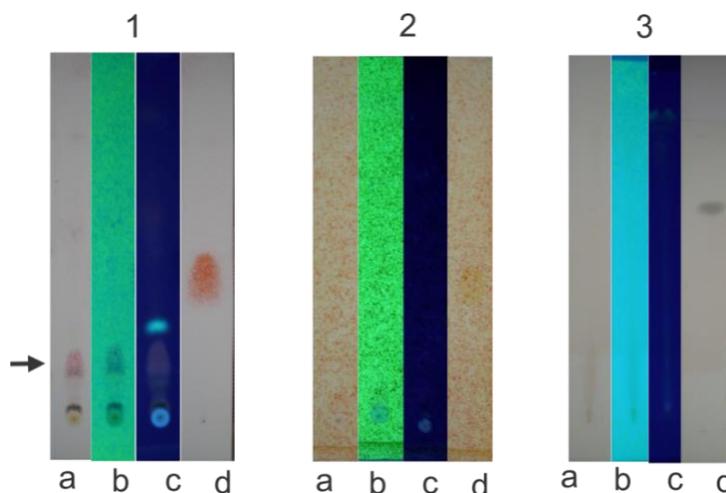


Figure 1. Results of spotting reagents on TLC: anisaldehyd sulfuric acid (1); Dragendorff reagent (2); FeCl₃ (3). a. Visible light; b. UV 254 nm; c. UV 366 nm; d. comparator (tymol 10 mg / 1 ml ethanol) (quinine 10 mg / 1 ml ethanol) gallic acid 10 mg / 1 ml ethanol in visible light. The arrow indicates the positive spots.

Table 1. Positive Test Reagent Spray against Spotting Results TLC

No	Spotting reagents	Cmpounds	Color positive test	Test result
1	Anisaldehyd sulfuric acid	Terpenoid	Orange spot	+
2	Dragendorff reagent	Alkaloid	Orange spot	-
3	FeCl ₃	Fenolik	Dark spot	-

Based on the results of spotting the reaction, TLC shows the results of this class of compounds included in terpenoids. These results are consistent with research Jayakumar et al. (2011) that the oyster mushroom produces secondary metabolites such as terpenoids.

b. Antimicrobial extract assay

Test results of the *P.ostreatus* antimicrobial extract against *Staphylococcus aureus*, *Enterobacter aerogene*, and *Candida albicans* is characterized by the formation of inhibition zone. Data observation of inhibition zone from methanol extracts of *P. ostreatus* shown in Table 2.

Result showed zone of inhibition for the various extracts varied between 10.9-23.2 mm. Ten mg extract exhibiting maximum antimicrobial activity against most of the tested pathogens. Among four the number of the methanol extract of *P. ostreatus* highest antimicrobial activity is indicated by a concentration of 10 mg against *S. aureus* with a diameter of 23.2 mm, and in *E. aerogenes* 21.0 mm and *C. albicans* 21.8 mm. Amongst the microbial tested, *E. aerogenes* (Gram negative) and *C. albicans* (yeast) were found to be more resistant to the extracts as they formed a zone of inhibition against lesser number of extracts, as compared to the *S. aureus* (Gram positive).

Extracts showed a higher response than the response exhibited by the antibiotics against a few microbial. All the extracts 10 mg

the showed a higher response (23.2 mm zone of inhibition) than ampicillin (20.2 mm zone of inhibition) against *S. aureus*; 21.0 mm zone of inhibition that chloramphenicol (19.2 mm zone of inhibition) against *E. aerogenes*; 21.8 mm zone of inhibition that nistatin (20.5 zone of inhibition) against *C. albicans*.

4. Discussion

Antibiotics are the main basis for the microbial infections therapy. However, the high genetic variability of microbial enables them to result a strain that more resistant to antibiotics. Thus, a new potential antibiotics is needed. More recently, mushrooms have attracted a lot of attention as it is being a potential source of several compounds that have an antimicrobial activity (Lindequist, et al., 2005). The present study has revealed the antimicrobial potential of *P. ostreatus*. Methanol extracts solvents of the fruiting body showed antibacterial activity.

The quantity of secondary metabolite influenced by external and internal factor. According to Dewick (2002), external factors affecting among others are the induction of pathogen, moisture, salinity and temperature. External factors are controlled (by taking a sample in a homogeneous environment, from Persatuan Petani Jamur Indonesia Asrifarm) so that only internal factors that differentiate the results of this study.

Table 2. The average of inhibition zone (mm) from methanol extract of *Pleurotus ostreatus* against *Staphylococcus aureus*, *Enterobacter aerogene*, and *Candida albicans*

Microbe	<i>P. ostreatus</i> methanol extract				Standard antibiotics (diameter of inhibition zone)
	2.5 mg	5 mg	7.5 mg	10 mg	
<i>Staphylococcus aureus</i>	18.0	21.8	22.4	23.2	ampicillin 10 µg (20.2 mm)
<i>Enterobacter aerogenes</i>	10.9	16.0	18.2	21.0	chloramphenicol (19.2 mm)
<i>Candida albicans</i>	12.3	13.7	17.0	21.8	nistatin 1% (20.5 mm)

The results showed that the extraction using methanol in *P. ostreatus* belongs to a class of compounds terpenoids. Terpenoids compounds are secondary metabolites, the most abundant with varied structure.

Precursor terpenoids are isoprene [$(C_5)_n$] form dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) derived from pyruvic acid as the reaction product of glycolysis. Sesquiterpenes and triterpenes

synthesized in the cytosol or endoplasmic reticulum, monoterpenes, diterpene, and tetraterpenoid (including plastoquinon and tocopherol) is synthesized in plastids and ubiquinone synthesized in mitochondria and Golgi apparatus (McGarvey and Croteau, 1995). This research result showed an antimicrobial activity (zone of inhibition in the range of 10.9 - 23.2 mm) against *S. aureus*, *E. aerogenes*, and *C. albicans*. Similar results have been reported in earlier studies (Nehra et al., 2012).

The mechanism of antimicrobial activity terpenoid compound in the TLC results still unknown but according Bontjura et al. (2015) terpenoids compounds can inhibit the growth of bacteria by damaging the cytoplasmic membrane and cell wall of bacteria. This creates an imbalance of macromolecules and ions in the cell, so the cell become lyses. Terpenoids also would disrupt vital ion transport processes in yeast cells, which can cause impaired permeability of fungal cell membranes. So that the process of diffusion of materials or substances needed by the fungus also affected (Sugianitri, 2011).

5. Conclusion

The spots result on TLC using crude extract of *P. ostreatus* was terpenoid. Zone of inhibition for the various extracts varied between 10.9-23.2 mm. Ten mg extract of *P. ostreatus* were the most effective against the tested pathogens.

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