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Antibacterial Potency of *Pleurotus ostreatus* Extract from Fruiting Body and Its Solid Substrate on *Staphyllococcus aureus*

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ABSTRACT

The objective of this research was to evaluate the antibacterial potency of ethanol extract from fruiting body *of Pleurotus ostreatus* and Its solid substrate made from coffee husk and sawdust on the growth of *Staphylococcus aureus ATCC 25922*. The extract of *Ganoderma lucidum* fruiting body and Tetracycline antibiotic paper disk 30µg/disk were used as control. The samples were extracted by using maceration method in 30% ethanol solution. The extracts were diluted with sterile distilled water to concentration 500, 1000, and 5000ppm. The result showed that the ethanol extracts from fruiting body *of Pleurotus ostreatus* and *Ganoderma lucidum* and the extracts of coffee husk and sawdust substrate fermented by *Pleurotus ostreatus* could inhibit the growth of bacteria for all the concentration. There was a significant difference in diameter of cleared zones between Tetracycline antibiotic disc 30µg and the ethanol extracts of the samples ($p<0.01$). The diameter of cleared zones among the sample extracts and each dilution concentrations were not a significant difference ($p>0.05$). Tetracycline was sensitive to *S.aureus* ATCC25922, while all the extracts were resistant. This study confirmed that there were the antibacterial potency of mushroom extracts from the fruiting body and also its solid substrates.

Key words : antibacterial, extract, *Pleurotus ostreatus*, fruiting body, solid substrate.

INTRODUCTION

Mastitis is a major problem of the dairy animals. It is an inflammation of mammary gland parenchyme which is caused by bacteria and its toxins (Sharma *et al*., 2011). Mastitis influence the quantity and the quality of milk, be a food safety problem and an economic losses. The change of mammary tissues due to mastitis leading the progressive change to the secretory apparatus and resulting in the loss of milk production. The closed association between milk production and somatic cell count (SCC) has been increasing used to estimate production loss (Barlett *et al*., 1990). Mastitis changed milk composition and milk quality. The mastitis or elevated SCC is associated with a decrease in lactose, α-lactalbumin, and fat in milk because of reduced synthetic activity of mammary tissue (Harmon, 1994). Milk from mastitis udder or with high SCC causes arise in whey protein and a decrease in casein, resulting in a considerable lower cheese yields. Shorter shelf life and adverse milk flavor are other consequences of high SCC (Sharif and Muhammad, 2008).

Several pathogens can cause mastitis but *Staphylococcus aureus* is the most frequently contagious pathogen. *S. aureus* secretes several toxins contributing to the pathogenesis of mastitis and also plays a role in foodborne disease, even with pasteurized milk because of the thermostable enterotoxins (Contreras *et al.*, 2007). Because of that it is important to get the way to erradicate *S. aureus* in dairy animals.

The antibiotic usage is one of the way that often used to treat mastitis. But due to the growing concern of antibiotic resistance and residual effect to animals and humans, the use of antibiotic have already been banned or been minimized. A potential alternative to antibiotic is natural or traditional medicine and mushroom have been exploited for the treatment of many diseases.

Mushrooms have been used as food supplement from times immemorial not only for their flavour, aroma and nutritive values but also for their medicinal properties as evident from ancient literature (Patel *et al*., 2012). The mushroom medicinal properties possess many typical pharmacological features like, to act as metabolic activators, prevent/control intoxication and microbial/viral infections, help in immune-balancing and immunomodulation, as antioxidants with rejuvenating and energy boosting properties (Wasser, 2002)

Mushrooms need antibacterial and antifungal compounds to survive in their natural environments. This antimicrobial compunds could be isolated from many mushrooms and that coul be of benefit for human (Lindequist *et al*., 2005). In the world of medicinal mushrooms, ganoderma is number one and has been considered as king of medicinal mushrooms followed by *Lentinula* and *Pleurotus*. The phytochemical or bioactive subtances have been documented such as saponin, alkaloid, flavonoid, triterpenoid and so on (Ijeh *et al*., 2009). The extract of the mushrooms were able to inhibit some pathogens including *Staphylococcus aureus* (Karaman *et al*., 2010).

The medicinal substances of mushroom can be found in fruiting body and mycelia (Stadtler & Sterner, 1998; Martin *et al*., 2011). The mycellium can be grown in the solid state substrates in which generally come from lignocellulose wastes such as agro-industry byproduct including coffee husk and sawdust. Since *Pleurotus ostreatus* has the biopotentialities to bioconversion of lignocellulosic wastes and production or improved animal feed, research concerning the use of solid fermentation substrate to produce bioactive compounds also interested to be explored. The use of agro-industry residues through solid state fermentation provides an important way to overcome environmental problems caused by their disposal, and being an economic way for production of value added compounds. The aim of this research work was to explore potentiality of *Pleurotus ostreatus* extract from fruiting body and its solid substrate on the growth of *Staphylococcus aureus in vitro*.

MATERIALS AND METHODS

Preparation of Extraction

The four samples consisted of fruiting body *from Pleurotus ostreatus* and *Ganoderma lucidum*, full mycellium of solid substrate from coffee husk and sawdust fermented by *Pleurotus ostreatus* were dried and pulverized into fine powder. The composition of solid substrate from coffee husk dan sawdust listed in Table 1. The ethanol extracts were prepared using the maceration. The powdered samples were macerated in the 30% ethanol solution with the ratio samples to solution = 1:3. The suspensions were stirred and precipitated overnight then be refined. The supernatants were retained while two further similar extractions were carried out. The three supernatants were combined and the residue was discarded. The supernatants were dried in vacuum rotavapor at 50^{o}C to obtain the concentrate extracts. The extracts were diluted in sterile distilled water to obtain the concentration 500ppm, 1000ppm and 5000ppm for antibacterial test.

Preparation of Bacterial Culture

The bacterial culture was prepared according to the method of Tilton *et al.* (1989) with a slight modification. The stock culture of bacterial colony used were *Staphylococcus aureus* (ATCC 25922). The stock culture of bacterial on growing nutrient agar media were taken and suspended into 5 ml sterile saline solution. When the turbidity of about 9 x 10^8 bacterial cells/ml (equal to Mc. Farland no.III standard) was attained, the cell suspensions were diluted in sterile saline solution to obtain a concentration of 9 x 10^6 bacterial cells/ml for further antibacterial test.

Antibacterial Test

Antibacterial testing was done by using agar diffusion paper disk method as described by Cowan and Steel (1989). As much as 20 ml sterile Mueller Hinton agar was poured into a petri dish. As much as 1 ml bacterial suspensions 9 x 10^6 cells/ml was spread over the surface of agar plates, and the dishes were incubated at temperature room for 15 minutes.

As much as three sterile blank papers disk were immersed into the extract solution that had been diluted (500ppm, 1000ppm dan 5000ppm) for five minutes. The papers disk were then be placed on the surface of agar media. The tetracycline paper disk 30μg/disk were used as a positive control. All experiments were performed in triplicate. After incubation at 37^{o}C for 18-24 hours, the diameter of cleared zones was measured in milimeter (mm). The antimicrobial activity was evaluated by measuring the diameter of cleared zone. The cleared zone was a tranparent zone formed around the paper disk showed bacteriostatic activity.

Phytochemical Properties of the Extracts

The obtained extracts were examined for the presence of alkaloids, flavonoids, phenol hidroquinon, steroids, triterpenoids, tannin, and saponin as described by Harborne (1989).

Table 1. The composition of coffe husk solid substrate and sawdust solid substrate

Solid substrate	Coffee husk (%)	Saw dust (%)	Rice bran (%)	Gips (%)	$CaCO_3$ (%)
Coffee husk	82.5	-	15	1.5	1.0
Sawdust	-	82.5	15	1.5	1.0

Source : Badarina *et al.* (2013)

Statistical Analysis

A completely randomized design with four extract samples and three dilution concentrations was used. The trials were in triplicate. Data were subjected to one-way analyses of variance (Steel and Torrie, 2003).

RESULTS AND DISCUSSION

The diameter of cleared zones from ethanol extract of *P.ostreatus* and *Ganoderma lucidum* fruiting body and full mycellium of solid substrate from coffee husk and sawdust fermented by *Pleurotus ostreatus* were presented in Table 2. The result showed that there was a tranparent zone or cleared zone surroundings the paper disk on the concentration 500, 1000 and 5000ppm respectively. This condition indicated that there was antibacterial activity from ethanol extract of the samples on *Staphylococcus aureus* (ATCC 25922).

This research showed that the diameter of cleared zones of ethanol extracts in all concentrations formed by mushrooms were smaller than tetracyclin 30 μg/disk ($p<0.01$). There was no significant difference ($P>0.05$) among the samples of ethanol extracts and the concentration of the extracts in the diameter of cleared zones. This condition implied that the mushroom ethanol extracts from each sources had the same ability in antibacterial activity.

Tetracyclin included the one of broad spectrum antibiotics. The diameter of cleared zones or inhibiting zone according to NCCLS (National Committee for Clinical Laboratory Standard) with <14 mm was resistance, 15-18mm was intermediate and >19mm was sensitive (Cowan and Steel, 1989).

Table 2. The diameter of cleared zones on *Staphylococcus aureus* ATCC 25922 from ethanol extract of *P.ostreatus* and *Ganoderma lucidum* fruiting body and full mycellium of solid substrate from coffee husk and sawdust fermented by *Pleurotus ostreatus*

Samples	The concentration of ethanol extracts		
	500ppm	1000ppm	5000ppm
Tetracyclin	30.98±0.86	31.13±0.47	31.73±0.24
P. ostreatus	9.17±0.49	10.05±3.15	15.07±5.92
Ganoderma lucidum	8.12±0.88	8.23±2.08	10.45±0.95
Coffee husk	7.88±3.043	8.95±1.51	8.70±0.16
Sawdust	8.43±1.15	8.38±1.21	8.02±1.11

This research showed that the tetracyclin antibiotic was sensitive to *Staphylococcus aureus* ATCC 25922 while the ethanol extracts from mushrooms in this research had weak antibacterial activites. The diameter of transparent zones in this research were in 7-

15mm. This condition was possibility because the samples extracts were still in crude extract. Kalyoncu *et al*. (2010) reported that the spectrum of biological activities of mushroom is very broad. The maximum antibacterial effect to *S. aureus* in tested macrofungi was shown by *P.ostreastus* as 24 mm. The least active was shown by species were *Morchella elata*, *Morchella esculenta*, *P.eryngii* and *Paxillus involutus* as 8 mm. They recommended to obtain the wider antibacterial effect, further works could be done on the isolation and purification of biological active component from crude extract.

Antibacterial effect exhibited by fungal extracts could be related to its pharmacologically bioactive substances. The phytochemical screening from the sample extracts are presented in Tabel 3.

Table 3. Phytochemical properties of fruiting body extracts of *P.ostreatus, G.lucidum* and the extracts of coffee husk and sawdust substrates fermented by *P.ostreatus*

Phytochemical properties	*Ganoderma lucidum*	*Pleurotus ostreatus*	Coffee Husk	Sawdust
Alkaloid	++	++	+++	-
Flavonoid	+	+	++	+++
Phenolhidroquinon	+	-	+	+
Steroid	-	-	-	-
Triterpenoid	+	+	+	+
Tanin	++	-	++	-
Saponin	+++	-	+++	++

The pharmacologically bioactive substances that have great therapeutic significance produced by macrofungi were the secondary metabolites (Table 2). Flavonoid and triterpenoid were found in all samples extracts. While steroid was not detected at all. Saponin were found in the extracts of *Ganoderma lucidum* fruiting body, coffee husk and sawdust fermented substrate. Alkaloid wasnot found in sawdust fermented extract. Fermented coffee husk extract were rich in alkaloid. Tannin were found in *Ganoderma lucidum* and coffe husk fermented substrate. From the phytochemical properties screening, the extracts of *Ganoderma lucidum* and coffee husk fermented by *P.ostreatus* contained the bioactive substances in higher contrentration than others.

The production of certain secondary metabolites may depend on the characteristics of the strain or culture condition. The precise composition of examined extracts of fungi is unknown and can only be assumed that the effect of crude extracts, which are concentration dependent, is a consequence of complex interactions between cells and mixtures of compounds in the extracts (Karaman *et al*., 2009). Coffee husk subtrate fermented by *P.ostreatus* showed a great potency in phytochemical contents. Coffee husks were rich in phenolic compound (tannin, saponin, flavonoid) and Alkaloid (caffeine) (Bressani, 1979; Fan *et al.,* 2000; Badarina *et al*., 2013).

These compunds have the antibacterial activity. Cowan (1999) said that the presence of phenol hydroxyl groups has the correlation to antimicrobial activity. Antimicrobial properties of phenolics are explained by the presence of phenol hydroxyl groups, which number is in correlation with their toxicity toward microorganisms. Caffeine is an alkaloid named trimethylxanthine that often used for its medicinal properties (Nolan 2001: Hosseinzadeh *et al*. 2006). Charles and Rawal (1977) said that caffeine could inhibit the growth of *Staphylococcus* by inhibiting *Staphylococcus* penicillinase enzyme.

The result of this study confirmed that there were the antibacterial potency of mushroom extracts from fruiting body and also its solid subtrates. Further study with more focus on the isolation and purification of biological active component from crude extract was therefore suggested.

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