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An Alternative Antimicrobial Approach to Fight Bacterial Pathogens from *Phellinus linteus*

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***Abstract*—**The objective of this research was focused on investigating *in vitro* antimicrobial activity of *Phellinus linteus* fruiting body extracts on *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*. *Phellinus linteus* fruiting body was extracted with ethanol and ethyl acetate and was vaporized. The disc diffusion assay was used to assess antimicrobial activity against tested bacterial strains. Primary screening of chemical profile of crude extract was determined by using thin layer chromatography. The positive control and the negative control were used as erythromycin and dimethyl sulfoxide, respectively. Initial screening of *Phellinus linteus* crude extract with the disc diffusion assay demonstrated that only ethanol had greater antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*. The MIC assay showed that the lower MIC was observed with 0.5 mg/ml of *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus* and 0.25 mg/ml. of *Escherichia coli* and *Staphylococcus aureus*, respectively. TLC chemical profile of extract was represented at $R_f \approx 0.71$-0.76.

Keywords*—Staphylococcus aureus, Phellinus linteus*, methicillin-resistant *Staphylococcus aureus*, antimicrobial activity, *Escherichia coli.*

I. Introduction

IN recent years, the development of new antibiotic drugs is a key issue for antimicrobial because microorganism resistant to current drugs, especially opportunistic pathogens, can cause mortality [1]. Antibiotic resistance is seen in a range of pathogenic and non-pathogenic bacteria; however, Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most commonly reported. There are very few drugs on the horizon. This problem is not just confined to the hospital or health care environment. Nowadays, many antibiotic-resistant microorganisms are seen in the community and also among animals. This has resulted in a number of changes in practice and intenseness of the data made available to those able to prescribe antibiotics and natural agent. Hence, there has been an increasing focus on developing novel research into natural antibiotics and more targeted treatment strategies, by study clinical trial to assay the efficacy of secondary metabolites; however, there is still a need for in vivo animal studies to appropriately evaluate the antibiotics for the treatment of multidrug resistant bacteria, especially screening the active compounds with good pharmacologic properties and drug safety, with a return to traditional remedies medicines.

The traditional folk herbal medicine as an alternative antimicrobial therapy has attracted substantial attention due to its low toxicity and costs as well as wisdom knowledge. *Phellinus linteus* (Fig. 1), a basidiomycete, is a species of medical mushroom whose fruiting body is commonly called Krathin Phiman in Thailand and *sangwhang* in Taiwan. This mushroom has been widely used in ancient herbal medicines and alternative herbal therapy in oriental countries including China, Japan, Korea, and Thailand [3]. *Phellinus linteus* was previously described as immunodulatory, antitumor [2], [4], [5], antioxidant, anti-inflammatory [6], and antimicrobial activity [7]. Therefore, the antimicrobial activity of *P. linteus* with Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were evaluated, together with their chemical profile.

Fig. 1 The fruiting of *P. lineus* [8]

II. Materials and Methods

A. Fungal Cultivation

The fungal mycelial was cultivated on potato dextrose agar (PDA) by placing agar blocks of actively growing pure fungal culture (Fig. 2) in 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated at 30 °C for four weeks with periodical shaking at 100 rpm.

B. Analysis of Radial Growth

The fungal strain *Phellinus linteus* kept in tested tube containing PDA slant culture tubes in refrigerator was inoculated in Petri dishes with PDA at least for 14 days. After incubation, mycelial fragment (0.8 cm diameter) of the strains under study was transferred to Petri-dishes using 20 ml of PDA and incubated at 30 °C. Fungal growth was determined

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by daily measuring the diameter of the colony. The experiment was measured in triplicates.

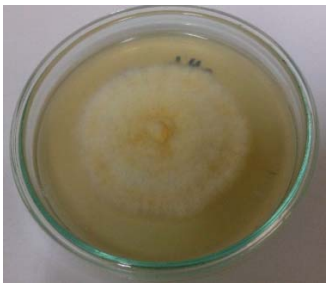

Fig. 2 The mycelium of *Phellinus linteus* on PDA plate

C. Preparation and Extraction of Mushroom

Fruiting bodies of Thai *Phellinus linteus* were obtained from a folk medicine company in Thailand. The mushroom was extracted with 95% ethanol and ethyl acetate. After extraction, all solvents were further evaporated by a vacuum rota-evaporator. The crude extract was stored at -20 °C for further determination.

D. Evaluation of the Antimicrobial of P. linteus Extract

The crude extract was dissolved in 10% dimethyl sulfoxide (DMSO) solution at initial concentration of 100 mg/ml. and submitted to antimicrobial assay. The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by disc diffusion method [9].

E. Anti-Bactericidal Activity

The tested bacterial strains as Methicillin-resistant *Staphylococcus aureus*, *S. aureus*, *P. aeruginosa* and *E. coli* were obtained from the national institute of health of Thailand. The antibacterial activity was performed according to a previously described methodology. The MBC was determined by serial two-flow dilution. The lowest concentration that shows inhibition zone was read as the MBC. Standard drug, erythromycin was used as positive controls, and 10% DMSO was used as negative control.

F. Thin Layer Chromatography of the Crude Extract

TLC was carried out using aluminum silica gel 60GF254, 0.2 mm thick, (Germany). Standard chromatograms of *P. linteus* extracts were prepared by applying 20 µl extract solution to a silica gel TLC plate and developed with chloroform/methanol (1:1; v/v) under saturated conditions. Chromatograms were detected by UV-light (254 nm and 365 nm), and by the color reaction with 5% sulfuric acid-ethanol spraying solution after heating at 100 °C.

G. Statistical Analysis

Data obtained were expressed as mean±sd for groups (n=3).

III. Results and Discussion

A. Fungal Cultivation

Table I and Fig. 3 show the mean of the radius with their standards deviations for *Phellinus linteus* culturing times when cultured on PDA. On the other hand, some scientists reported that *Phellinus* spp. was favorable in Malt Extract Agar (MEA), Malt Yeast Agar (ZMYA), and Soybean Powder Malt Sucrose (SMS medium) [10].

TABLE I
THE RADIAL OF MYCELIAL GROWTH OF *PHELLINUS LINTEUS* DURING 7 DAYS

Days	Mycelial growth of *P. linteus* (mm)			
	Plate 1	Plate 2	Plate 3	Average ±sd
1	0	0	0	0
2	8	8	7	7.67±0.58
3	14	15	16	15.00±1.00
4	20	23	20	22.00±1.73
5	30	30	30	30.00±0.00
6	33	35	33	33.67±1.15
7	38	38	40	38.67±1.15

B. Antibacterial Activities

The antibacterial activities of *P. linteus* fruiting bodies extracts are shown in Table II. The ethanol extract displayed the antimicrobial activity against *P. aeruginosa*, *S. aureus*, Methicillin-resistant *Staphylococcus aureus*, and *E. coli* at the zone of inhibition of 16.33±1.53, 14.33±0.58, 11.67±1.53, and 14.00±1.73, respectively while the ethyl acetate extract did not show any activities. In contrast, a previous report [11] showed that crude extracts of mushroom *P. linteus* also did not inhibit the growth of food borne pathogens as *E. coli* ATCC25922, *Salmonella typhi* GMST5784, *Shigella flexneri* DMST4423, *Shigella dysenteriae* DMST1511, and *Vibrio chloerae* ATCC14035 but displayed strongest antioxidant activity and anti-malarial activity [12]. Similar to a previous report [13], *Phellinus baumii* had effect on Methicillin-resistant *Staphylococcus aureus.*

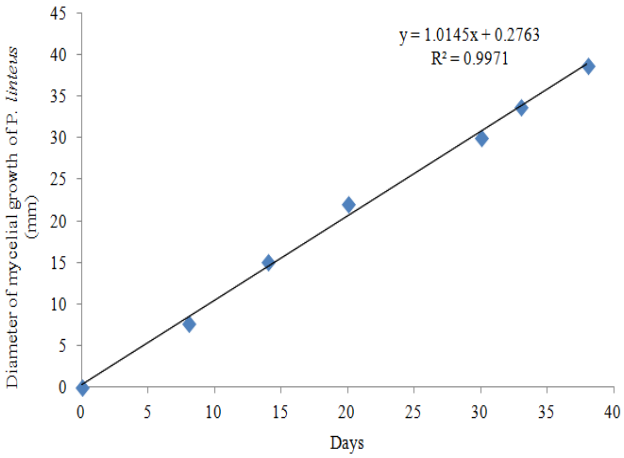

Fig. 3 The diameter of fungal colony *P. linteus*

C. Bacteriostatic and Bactericidal Effect

For MIC assay, a more sensitive measure of antimicrobial activity [14] showed that growth of all four bacterial pathogens. A lower MIC was observed in Table III. The MICs of the *P. linteus* extracts were 0.5 mg/ml with all bacterial strains, while *P. linteus* extract showed MBC at the

concentration of 1.0 mg/ml. except *P. aeruginosa* of 0.5 mg/ml (Fig. 4).

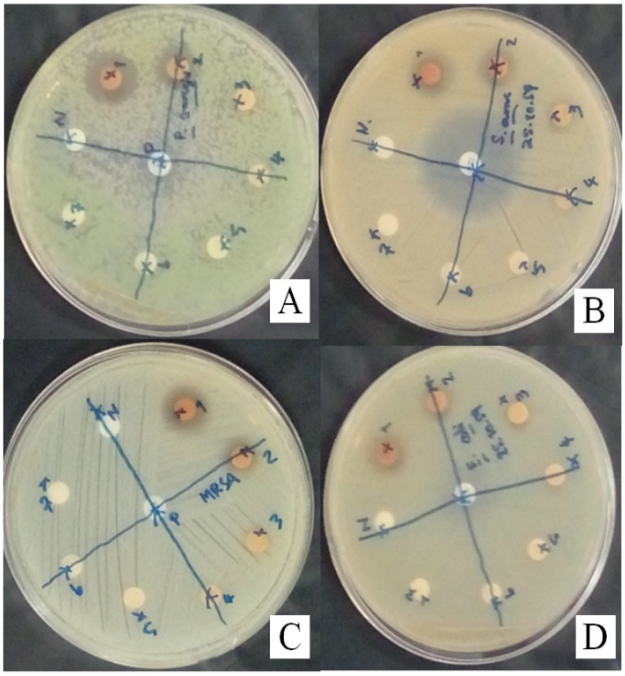

Fig. 4 The MIC of *Phellinus linteus* extract on *Pseudomonas aeruginiosa* (A), *Staphylococcus aureus* (B), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, (D). Note: 1 = 1.0 mg/ml, 2= 0. 5 mg/ml, 3 = 0.25 mg/ml, 4= 0.125 mg/ml, 5=0.025 mg/ml, 6= 0.03125 mg/ml, N= negative control (10% DMSO), P= erythromycin (10 µg/ml)

TABLE II
THE ANTIBACTERIAL ACTIVITIES OF THE CRUDE EXTRACT OF *PHELLINUS LINTEUS*

Bacterial strains	Inhibition (mm.)	
	Ethanol extracted	Ethyl acetate extracted
P. aeruginosa	16.33±1.53	nz
S. aureus	14.33±0.58	nz
S. aureus (MRSA)	11.67±1.53	nz
E. coli	14.00±1.73	nz

nz: no inhibition zone

TABLE III
THE MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF *PHELLINUS LINTEUS* TO INHIBIT 100% OF THE MICROBIAL GROWTH (N=3)

Bacterial strains	*P. linteus* extract	
	MIC (mg/ml)	MBC (mg/ml)
P. aeruginosa	0.5	0.5
S. aureus	0.5	1.0
S. aureus (MRSA)	0.5	1.0
E. coli	0.5	1.0

MIC = minimum inhibitory concentration
MBC = minimum bactericidal concentration

D.TLC of Crude P. linteus Extract

Developed TLC plates with visualized spots are presented in Fig. 5. There was intensive blue with spot of extracts under 365 nm and 254 nm UV light ($R_f \approx 0.71$-0.76). After colorized by 5% sulfuric acid-ethanol solution, polar spots appeared at the same of UV light position.

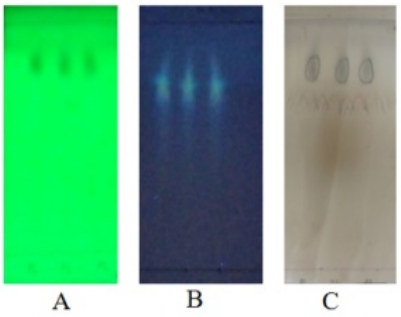

Fig. 5 TLC separation of crude extracts of selected *Phellinus linteus*. (A) exposed with 254 nm. UV light, (B) exposed with 365 nm. UV light, (C) spry with 5% sulfuric acid-ethanol solution

IV. CONCLUSION

From the results contained in this report, we conclude that, due to high antimicrobial activity, ethanol extract of *P. linteus* fruiting bodies may warrant further investigation into its use as a possible alternative therapy for health care application.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with this study.

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