

Antimicrobial activities of split gill mushroom *Schizophyllum commune* Fr.

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Abstract

Schizophyllum commune or commonly known as split gill mushroom is a widely distributed wood-decaying basidiomycete that has been reported for its health promoting and medical benefits. Hence, the purpose of this study was to evaluate the antimicrobial activity of *S. commune* extracts using well diffusion method. The microorganisms tested were common pathogenic bacteria i.e *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, *Shigella* sp., *Shigella flexneri*, *Plesiomonas shigelloides*, *Salmonella* sp., *Salmonella typhi*, and *Enterobacter faecalis*. The pathogenic fungi tested consisted of *Saccharomyces pombe*, *Candida albicans* and *Candida parapsilosis*. The antimicrobial activities of methanol, ethyl acetate, dichloromethane and water extracts of *S. commune* were qualitatively and quantitatively assessed by the presence and diameter of inhibition zones. Commercially available antibiotic discs were used as positive references. The antibiotic discs were used as positive references. The crude extracts displayed higher antibacterial activity compared to antifungal activity with *S. commune* dichloromethane extract to be the most active with a diameter of inhibition zones of 12 ± 1 mm against *Streptococcus sanguis* at a concentration of 2.0 mg/ml. Both ethyl acetate

and methanol extract had moderate antimicrobial activities. However, the water extract of *S. commune* failed to show good inhibition against all microorganisms tested. This study showed that some extracts of *S. commune* possess antimicrobial properties and therefore, they can be used as potential antimicrobial agents.

Key words: *Schizophyllum commune*, antimicrobial activity

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Introduction

Mushrooms have been shown to have profound health promoting benefits and studies have confirmed their medical efficacy and identified many of the bioactive molecules (Wasser & Weis, 1999b). Studies showed that mushrooms contain a wide variety of bioactive compounds including terpenoids, steroids, phenols, nucleotides and their derivatives glycoproteins and polysaccharides (Borchers *et al.*, 1999). The different bioactive compounds have been extracted from the fruiting body, mycelia and culture medium of various medicinal mushrooms such as *Lentinula edodes*, *Ganoderma lucidum*, *Schizophyllum commune*, *Trametes versicolor*, *Inonotus obliquus* and *Flammulina velutipes* (Wasser & Weis, 1999a). These biologically active substances are claimed to have profound health promoting properties including antimicrobial, antitumor, antiviral, anti-inflammatory, hypoglycaemic, hypocholesterolemic and hypotensive activities useful as therapeutic agents and have nutritional value too (Chang, 1999).

South-East Asian countries have been known to have rich sources of medicinal mushrooms. Some of the medicinal mushrooms are widely used for therapeutic properties and some of them have not been thoroughly studied yet (Wasser, 2002). For this reason, *Schizophyllum commune* was evaluated for its medicinal properties. This split gill mushroom is a popular edible mushroom among the Malay community in Malaysia. In South-East Asia, *S. commune* has been consumed as a nutritional food (Han *et al.*, 2005) and is now being cultivated in Malaysia and Thailand. The production of *S. commune* and other medicinal mushrooms has increased due to the ease of cultivation, increase in popularity and its nutritional value (Chang and Buswell, 1996; Wasser, 2002). Though usually considered as a widely distributed basidiomycetous pathogen (Hobbs, 1995; Rihs *et al.*, 1996; Sigler *et al.*, 1997), *S. commune* has actually been acknowledged for its medical importance (Oso, 1981; Han *et al.*, 2005). Pharmacologically, *S. commune* is extremely important because it produces the polysaccharide schizophyllan which shows considerable medicinal properties (Ooi and Liu, 1999; Wasser, 2002). Thus, the use of *S. commune* metabolites need to be explored as it holds hope as a potential agent for the prevention of variety of human ailments. To our knowledge, this is the first report on the broad spectrum of antimicrobial of *Schizophyllum commune* extracts.

Materials and Methods

Preparation of S. commune extracts

Schizophyllum commune were purchased from local markets. The fruiting bodies (whole mushrooms) were then cut into small pieces and air-dried. Bioactive compounds present in *S. commune* were extracted separately using methanol, ethyl acetate, dichloromethane and water. For the first three

extractions, the air-dried fruiting bodies were soaked in extraction solvents with a ratio of 1:10 for two days at room temperature. The fruiting bodies were then separated from the extraction solvents by filtration using Whatman No.1 filter paper. The solvents were rotary evaporated till complete dryness to yield the crude extracts. Water extract was prepared by boiling the fruiting bodies in distilled water for three hours using a heating mantle. The water extract was then filtered through Whatman No. 1 filter paper and freeze-dried to gain the crude extracts.

Preparation of microorganisms

The test organisms used to screen the antibacterial activity of *S. commune* extracts were Gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Enterobacter faecalis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella sp.*, *S. typhi*, *Shigella sp.*, *S. flexneri*, *Streptococcus mitis*, *S. mutans* and *S. sanguis*). Commercially available antibiotic discs namely streptomycin was used as positive reference. Besides antibacterial activity, antifungal activity of *S. commune* extracts was also evaluated against selected fungi. Among the fungi were *Candida albicans*, *C. parapsilosis* and *Saccharomyces pombe*. The positive control used was nystatin; a common antibiotic used to treat fungal infections. All the microorganisms were obtained from the Mycology Laboratory, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur.

Screening for antimicrobial activity of S. commune extracts

The bacterial and fungal stock cultures were prepared and maintained on selective media. Mueller Hinton broth was used to support the growth of Gram positive and negative bacteria while Brain Heart Infusion broth was used for Gram negative oral bacteria (*S. mitis*, *S. mutans* and *S. sanguis*). Saboraud Dextrose and Yeast-Peptone broth were used for *C. albicans* and *C. parapsilosis* and *S. pombe*

respectively. Bacterial and fungal inoculum were prepared by transferring 100 µl of the stock suspension into 3 ml broth and incubated for 48 hours at 37°C. After two days of incubation, the two-day old bacterial and fungal suspension was evenly streaked on Petri dishes containing the respective nutrient agar media. Antimicrobial tests were then carried out using the well diffusion method. Three 7 mm diameter wells were punched into the inoculated media using a sterile cork borer representing triplicate readings. Each well was filled with 50 µl of 2 mg/ml crude extracts to give 100 µg in each well. The Petri dishes were incubated at 37°C and activities of the extracts were estimated by measuring the diameter of inhibition zones after 24 hours of incubation. At the end of the incubated period, inhibition zones formed on the media were evaluated in mm. The inhibition zones were compared with those of positive reference discs.

Statistical analysis

All values are expressed as the mean standard deviation; significant differences and linear regression analyses were evaluated using the Statgraphic statistical software.

Results and Discussion

The antimicrobial activity of *S. commune* extracts was tested against seven species of Gram-positive bacteria, eight species of Gram-negative bacteria, 2 species of fungi and one species of yeast. As summarized in Table 1, *S. commune* extracts showed mostly partially active (inhibition zones 9 - 12 mm diameter) antibacterial activity against Gram-positive and Gram-negative bacteria at the concentration of 2 mg/ml. Dichloromethane extract of *S. commune* was the most active in antibacterial activity, showing

Table 1: Antibacterial activity of *S. commune* extracts and positive reference standard at 2 mg/ml as evaluated by well diffusion assay (24 hours incubation at 37°C). Inhibition zones were measured (in mm) and the diameter of inhibition zones reported

Microorganisms	<i>S. commune</i> extracts				Positive reference
	Methanol	Ethyl acetate	Dichloromethane	Water	Chloramphenicol
<i>Bacillus cereus</i>	9±1 ^a	8±1 ^a	11±0 ^b	-	26±1
<i>Bacillus subtilis</i>	10±1 ^a	9±1 ^a	11±1 ^b	-	40±1
<i>Enterobacter faecalis</i>	10±1 ^a	10±1 ^a	11±1 ^b	-	30±1
<i>Staphylococcus aureus</i>	10±1 ^a	9±1 ^a	11±1 ^b	-	40±1
<i>Streptococcus mitis</i>	9±1 ^a	9±1 ^a	11±1 ^b	-	25±1
<i>Streptococcus mutans</i>	-	-	-	-	13±1
<i>Streptococcus sanguis</i>	11±1 ^a	11±1 ^a	12±1 ^b	-	18±1
<i>Escherichia coli</i>	9±1 ^a	10±1 ^a	11±1 ^b	-	31±1
<i>Salmonella sp.</i>	9±1 ^a	10±1 ^a	11±1 ^b	-	34±1
<i>Salmonella typhi</i>	9±1 ^a	10±0 ^a	11±1 ^b	-	33±1
<i>Shigella sp.</i>	9±1 ^a	10±1 ^a	11±1 ^b	-	31±1
<i>Shigella flexneri</i>	9±1 ^a	9±1 ^a	11±1 ^b	-	33±1
<i>Plesiomonas shigelloides</i>	9±1 ^a	11±1 ^a	11±1 ^b	-	32±1
<i>Proteus vulgaris</i>	9±1 ^a	10±1 ^a	11±1 ^b	-	42±1
<i>Pseudomonas aeuroginosa</i>	9±0 ^a	9±1 ^a	10±1 ^b	-	34±1

Data expressed as means ± standard deviations of triplicate measurements; diameter of well was 7 mm; inactive (IA) – inhibition zones <9 mm diameter, partially active (PA) - inhibition zones 9-12 mm diameter, active (A) - inhibition zones 13-18 mm diameter, very active (VA) - inhibition zones >18 mm diameter, not active (NA) - no inhibition zone.

a significantly ($P < 0.05$) higher activity than other extracts against the bacteria tested. Hence, the extract is a potential source of antibacterial agent. The highest inhibition zone detected by this extract was against the Gram-positive bacteria, *S. sanguis* with diameter of inhibition zone of 12 ± 1 mm (Table 1)(Plate 1(a)). At the same concentration, methanol and ethyl acetate extracts of *S. commune* exhibited about the same spectrum of antibacterial activity against the bacteria tested. However, water extract of *S. commune* had no inhibitory effect on the growth of all the bacteria at the same concentration evaluated in this study (Table 1). The antibiotic chloramphenicol exhibited either active (inhibition zones 13 - 18 mm diameter) or very active inhibition (inhibition zones > 18 mm diameter) against the bacteria tested. The antibiotic exhibited excellent antibacterial activity especially against the Gram-negative bacteria, *P. vulgaris* with the highest inhibition zone of 42 ± 1 mm (Table 1) (Plate 1(b)).

The antifungal activity of *S. commune* extracts was found to be less pronounced than the antibacterial activity at the same concentration tested (Table 2). In particular, the fungal species were susceptible to dichloromethane and ethyl acetate extracts of *S. commune*. Both extracts gave quite a similar antifungal pattern against the fungi tested with the lowest inhibitory (inactive) recorded against *S. pombe*. Methanol and water extracts, however, failed to show antifungal activity at all. The fungal species were found to be sensitive to the antibiotic nystatin giving very active inhibition ranging from 19 ± 1 mm to 28 ± 1 mm (Table 2).

The present results suggested that *S. commune* extracts displayed higher antibacterial activity (Table 1) when compared to antifungal activity (Table 2). This finding was in the agreement of Suay *et al.* (2000) that the antibacterial activity of polypores and gilled mushrooms was found to be more pronounced than antifungal activity (Suay *et al.*, 2000). As in most cases, it appears that the fungal and yeast strains are more resistant to antimicrobial compounds than bacterial strains (Nishizawa *et al.*, 1990; Papadopoulou *et al.*, 2005). A mushroom extract called *Lentinus adherens* were observed to be

less effective against pathogenic fungi compared to pathogenic bacteria (Lauer *et al.*, 1991). Jonathan and Fasidi (2003) also suggested that the antifungal activities of the mushrooms *Lycoperdon pusilum* and *Lycoperdon giganteum* extracts against pathogenic fungi were very low which supports that antifungal antibiotics are not common among mushrooms.

Table 2: Antifungal activity of *S. commune* extracts and positive reference standard at 2 mg/ml as evaluated by well diffusion assay (24 hours incubation at 37°C). Inhibition zones were measured (in mm) and the diameter of inhibition zones reported

Microorganisms	<i>S. commune</i> extracts				Positive reference
	Methanol	Ethyl acetate	Dichloromethane	Water	Nystatin
<i>Candida albicans</i>	-	10±1	10±0	-	28±1
<i>Candida parapsilosis</i>	-	10±0	10±1	-	12±1
<i>Saccharomyces pombe</i>	-	9±1	8±1	-	29±1

Data expressed as means ± standard deviations of triplicate measurements; diameter of well was 7 mm; inactive (IA) – inhibition zones <9 mm diameter, partially active (PA) - inhibition zones 9-12 mm diameter, active (A) - inhibition zones 13-18 mm diameter, very active (VA) - inhibition zones >18 mm diameter, not active (NA) - no inhibition zone.

The present study showed that water extract of *S. commune* was not inhibitory to all the microorganisms at both the concentrations tested as there was no inhibition zones recorded in this antimicrobial assay (Table 1). This finding confirms the results from previous researchers, which reported that water is not a suitable solvent for extraction of antimicrobial substances compared to other solvent. This observation can be explained by different active compounds were extracted with different solvents and thus resulted in different antimicrobial activity. Jonathan and Fasidi (2003) in their study on edible Nigerian macro-fungi, *Lycoperdon pusilum* and *Lycoperdon giganteum*, found that water was not

a good extracting solvent of antimicrobial compounds compared to the ethanol ($P < 0.05$). The low activity of water extract may also be due to the effect of processing temperature on compound stability. The water extract was reported to be heat-labile (Hirasawa *et al.*, 1999). In the microbial analyses, it was observed that the water extracts of *Lentinula edodes* showed the lowest inhibitory activity against both *Streptococcus mutans* and *Prevotella intermedia* compared to the other extracts. The treatment of extracts at 60°C for 30 minutes reduced the antibacterial activity against the microorganisms by 60% and the activity was completely inactivated by heat treatment at 100°C for five minutes (Hirasawa *et al.*, 1999).



Plate 1(a): The inhibition of *S. sanguis* by 2 mg/ml dichloromethane extract of *S. commune* as measured by well diffusion assay

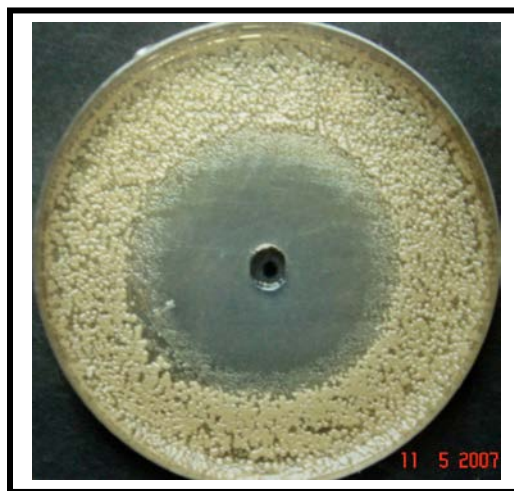


Plate 1(b): The inhibition zone of *P. vulgaris* by 2 mg/ml antibiotic chloramphenicol as measured by well diffusion assay

The present study also described that the *S. commune* extracts were found to exhibit better antibacterial activity against Gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Enterobacter faecalis*, *Staphylococcus aureus*, *Streptococcus mitis*, *S. mutans* and *S. sanguis*) than the Gram-negative bacteria (*Escherichia coli*, *Salmonella sp.*, *S. typhi*, *Shigella sp.*, *S. flexneri*, *Plesiomonas shigelloides*, *Proteus*

vulgaris, and *Pseudomonas aeruginosa*) (Table 1). Gram-positive bacteria are more likely to be more sensitive to antibacterial agents than Gram-negative bacteria probably due to the inhibition of cell wall synthesis; the cells were unable to maintain rigid peptidoglycan component of the wall and therefore become susceptible to weakening and eventual toxic destruction to the cell wall or lysis. It has been proven that the less complex structure of the Gram-positive bacteria cell wall makes it more permeable to the antimicrobial compounds (Papadopoulou *et al.*, 2005). Gram-negative strains are reported to be highly resistant to many known antibiotics due to the thicker peptidoglycan layer of the bacteria cell that protect them from the action of antibiotics (Anke, 1989; Suay, 2000). A previous study by Papadopoulou *et al.* (2005) described that, the diameter of the inhibition zone for *S. aureus* was greater than *E. coli*, indicating that the Gram-positive strain was more sensitive to the active compound found in the extracts than Gram-negative strain.

Conclusion

On the basis of the experimental evidence, this study showed that some extracts of *Schizophyllum commune* had curative properties against bacteria and fungi infections and thus they can be used as potential antimicrobial agents.

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