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Possibility of split mushroom *Schizophyllum commune* by-product extracts as antimicrobial and antioxidant agent for aquaculture

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Abstract *Schizophyllum commune* by product (SCB) extracted was investigated from different organic solvent having the different relative polarity consisting of hexane, ethyl acetate, dichloromethane, 2-butanol, ethanol, methanol and water as antimicrobial and antioxidant agent in order to added value of waste from agriculture. The antibacterial assay to pathogenic bacteria for economic fish culture *Aeromonas hydrophila* and *Streptococcus agalactiae* were tested by disc diffusion method. Antioxidant capacity was measured by DPPH assays. The result found that the inhibitory to pathogenic bacteria was not observed in all SCB extract compared to positive disc of commercial antibiotic (oxytetracyclin and norfloxacin) at the concentration of 20,000 ppm. While, the DPPH radical scavenging activity of each extract was significantly different ($p < 0.05$). SCB extracted by methanol showed the highest activity (IC_{50}=23 μg/mL) with no significant difference to SCB extracted by 2-butanol. The lowest level was observed in SCB extracted by ethanol and ethyl acetate (IC_{50}=35 ug/mL). This study indicated that the different of organic solvent gave the different capacity of antioxidant level and SCB extract might be used as antioxidant agent in aquaculture.

Keywords: Schizophyllum commune, Mushroom by-product, antioxidant, antimicrobial

Introduction

Schizophyllum commune commonly known as split gill mushroom is an edible and medicinal mushroom widely distributed in the world. This fungus is consumed for food in southern part of Asian countries such as Thailand, Taiwan, Malaysia, Vietnam and southern China (Imtiaj *et al.*, 2008). The evident of bioactive compound from fruiting body (cap and stalk) has been

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reported in several studies as well as antioxidant, antimicrobial and immunomodulator in human (Chang, 1999).

Mushrooms usually contain a wide variety of free radical scavenging molecules, such as polysaccharides and polyphenols (Liu *et al.* 1997; Mau *et al.* 2002). The antibacterial activity of split gill mushroom (fruiting body) extract was reported (Mirfat *et al*., 2014). However, the study of *Schizophyllum commune* by product (SCB) extracts as an antimicrobial and antioxidant activity for aquaculture has not been reported. Undesirable mushroom, mushroom stalk, contained a rich source of protein, polysaccharide and antioxidant resulting in application either replacement fish meal protein (Phromkunthong *et al*., 2014; Srichanun *et al*., 2017) or stress reduction by PH fluctuation (Ahmed *et al*., 2017). In addition, the study of spent mushroom substrate from *Cordyceps militaris* at 10 g/kg diet is suggested to improve growth and health status of Nile tilapia (Doan *et al*., 2017). In addition the supplementation of oyster mushroom extract in the rainbow trout diets at 1 and 2% concentration significantly improved hematological parameters and modulates the immune response against *L. arvieae* (Ulukö*y et al*., 2016). The objective was to evaluate the antimicrobial and antioxidant properties of the split mushroom by product (Stalk, SCB) and to provide scientific information on the in vitro antimicrobial activity and antioxidant capacities for further apply in aquaculture.

Materials and methods

Preparation of Mushroom Extracts

Schizophyllum commune by product (SCB) consisting of stalk and cap deterioration were provided from Faculty of Plant Science, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Thailand. SCB were then cut into small pieces and dry by hot air oven at 60^{o}C overnight. Bioactive compounds present in *S. commune* were extracted separately using organic solvents with different polarity; hexane, dichloromethane, ethyl acetate, 2-butanol, ethanol, methanol and water. Dry SCB and each organic solvent with the ration at 1: 2 was mixed well in flask and soaked for 3 days. The mixture was then shaken every 3 hours. Liquid was collected and filtered with Whatman G/C filter paper. Filtrate was concentrate with rotary evaporator at 50 ℃. Dried extracts were kept in vial at 4 ℃ while not in use. The yield of extraction production was calculated as percentage to raw SCB as followed:
Percentage yield = (Weight of extraction gain / weight of raw SCB) x 100

Biological activity

Screening of antimicrobial activity

The extraction was examined the biological activity against pathogenic bacteria that causes important disease in fish culture; *Aeromonas hydrophila* and *Streptococcus agalactiae* by disc diffusion method. The bacterial stocks cultured were prepared in Trypticas soy broth (TSB) at the concentration 10^8 CFU. One hundred microliters of each pathogenic bacteria was spread into TSA plate. Dry SCB extractions were weighted at 0.1 g and dissolved with 5 ml of the organic solvent and diluted at the concentration at 5,000, 10,000 and 20,000 ppm. The disc (6 mm) were then impregnated with 25 µL of each SCB extract and placed on the inoculated agar. The petri dishes were incubated at 33^{o}C and an activity of extracts was estimated by measuring the diameter of inhibition zones after 24 hours of incubation. The inhibition zones were compared with those of commercial antibiotic; Oxytetracyclin (Oxy) and Norfloxacin (Nor) used as positive reference discs (Collins and Lyne, 1987).

Antioxidant activities assays

The ability of free radical scavenging of the SCB extracts was tested by DPPH (1,1-diphenyl-2-picrylhydrazyl radical) radical scavenging assay as described by Shimada *et al*., (1992). The various concentrations of SCB extraction sample; 0.0000, 0.0125, 0.0250, 0.0500 and 0.1000 ppm at the volume of 0.2 mL was mixed with 0.1 mM of DPPH (0.2 ml).The Mixture was shaken vigorously and left to stand at the dark for 30 minutes, and the absorbance was measured at 517 nm. BHT was used as control .The Percentage of DPPH decolorization of the sample was calculated according to the equation: Scavenging activity (%) = [Abs (control) – Abs (standard)] ×100.

Where, Abs (control): Absorbance of DPPH radical + methanol Abs (standard): Absorbance of DPPH radical +extract/standard.

The antioxidant capacity of each SBS extraction was calculated to the IC_{50}, which is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed using linear regression analysis.

Statistical analysis

The experiment was done in triplicate for each substance. The results of IC_{50} of each SCB extractions were compared by one-way ANOVA and differences between means were determined and compared by Duncan multiple ranged test. A difference was considered statistically significant if $p<0.05$.

Results

Yield production of SCB extraction

SCB extracted with water showed the highest yield of extraction followed by methanol, 2-butanol, ethanol, dichloromethane, ethyl acetate and hexane respectively which followed by the relative polarity of each organic solvent (Table 1).

Table 1. Yield production of SCB extraction of each organic solvent

Organic solvent	Yield production (g)	Yield production (%)	Relative polarity
Hexane	1.10	0.50	0.009
Ethyl acetate	1.10	0.50	0.028
Dichloromethane	2.50	1.00	0.309
2-butanol	3.41	1.36	0.506
Ethanol	2.64	1.06	0.654
Methanol	7.97	3.17	0.762
Water	44.96	17.98	1.000

Antimicrobial activity

The antimicrobial activity of SCB extracts was tested against two species of pathogenic bacteria that causes important disease in fish culture; *Aeromonas hydrophila* and *Streptococcus agalactiae.* The result found that the inhibitory to pathogenic bacteria was not observed in all SCB extracted compared to positive disc of commercial antibiotic (oxytetracyclin and norfloxacin) at all concentration even at 20,000 ppm.

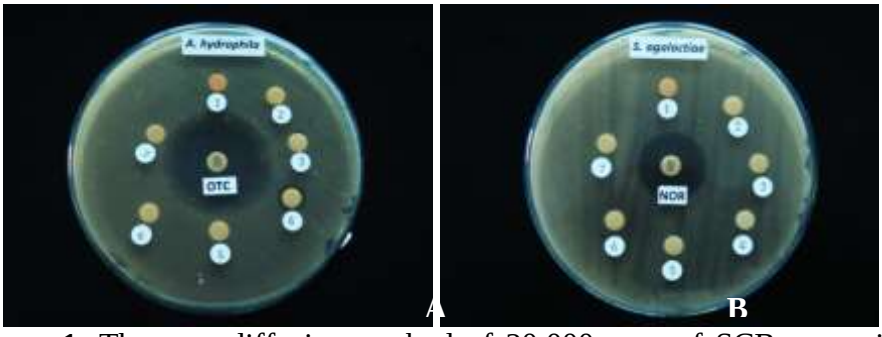

Figure 1. The agar diffusion method of 20,000 ppm of SCB extraction: **(**A) disc- diffusion method of SCB extracts using *A. hydrophila* as test microorganism, (B) disc- diffusion method of SCB extracts using *S. agalactiae* as test microorganism with different organic solvent; 1: water, 2 methanol, 3: ethanol, 4: 2-butanol,5: dichloromethane, 6: ethyl acetate, 7: hexane, OTC: oxytetracycline, NOR: norfloxzacin

Antioxidant capacity of SCB extraction

The antioxidant activity of SCB extraction with different organic solvent were significant ($p<0.05$). All SCB extracts showed the higher scavenging activity than BHT which is a commercial antioxidant (Figure 2).The mushroom extracted with methanol showed the highest activity to radical scavenging activity with no significantly different to 2-butanol extraction followed by dichloromethane, hexane and water. While, mushroom extracted with ethyl acetate and ethanol showed the lowest antioxidant activity ($p<0.05$) (Figure 3).

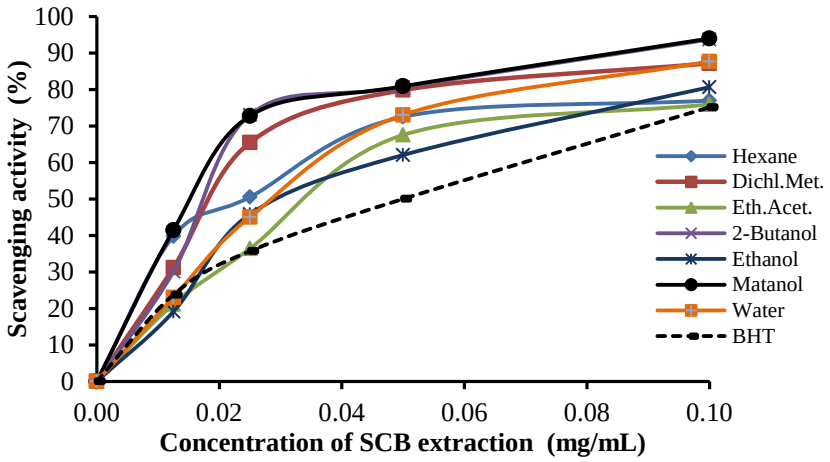

Figure 2. Scavenging activity (%) of DPPH radical of SCB extraction at different concentration

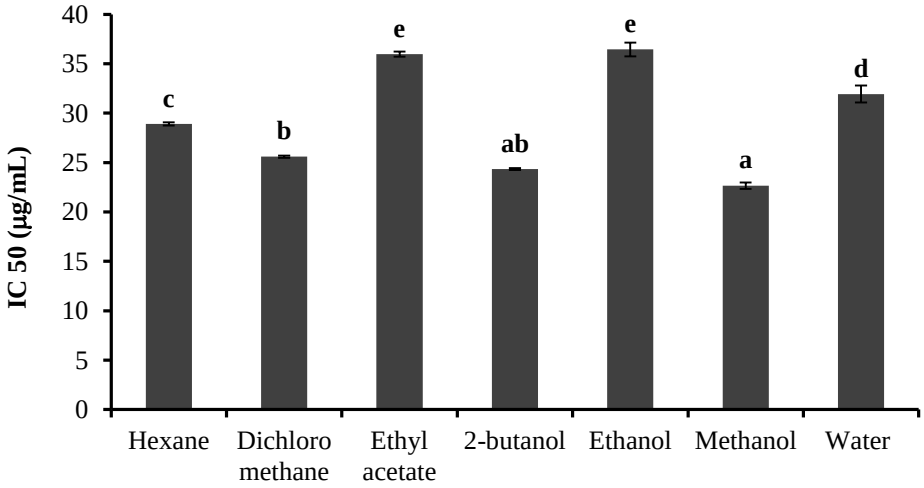

Figure 3. IC_{50} of SCB extraction with different organic solvent

Discussion

The extraction yield of SCB extracts was different between the different polarities of solvent. Water showed the highest yield production (44.96%) followed by methanol extract (7.97%) while, hexane obtained the lowest yield production (0.5). This implied that that most of the soluble components in mushrooms were high in solvent at high polarity (Cheung *et al.*, 2003). The antimicrobial activity to pathogenic bacterial for aquaculture of SCB extracted with all solvent was not observed in this study even at the concentration of 20,000 ppm. This result was not consistent with the study of Mirfat *et al*. (2014) who reported that the crude extracts from fruiting body of *Schizophyllum commun* display the antibacterial activity to *Bacillus cereus, B. subtilis, Staphylococcus aureus, Pseudomonas aeuroginosa, Proteus vulgaris, Escherichia coli, Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis, Shigella* sp., *Shigella flexneri, Plesiomonas shigelloides, Salmonella* sp., *Salmonella typhi,* and *Enterobacter faecalis.* Dichloromethane extract showed the most active against *Streptococcus sanguis* followed by ethyl acetate and methanol. While, the water extract was not observed the inhibition zone. This might be due to the stalk mushroom used in this study may not have any significant antimicrobial activity compound against *A. hydrophila* and *S. agalactiae*.

The antioxidant properties of SCB extracts were observed in that the antioxidant activity of split mushroom extracted with different solvent were significant ($p<0.05$). Split mushroom extracted with methanol showed the most remarkable antioxidant activity to radical scavenging activity with IC_{50} at 23μg/ mL with no significant different with 2-butanol estract. While ethyl acetate and ethanol showed the lowest activity. This result comply with the study of Mirfat *et al*. (2010) reporting that methanol extract showed the highest antioxidant compared to water extract. The authors suggested that the high antioxidant activities are mainly due to its total phenolic content (Mirfat *et al.*, 2010). The IC_{50} of SCB extract with methanol was lower than IC_{50} of fruiting body SC (0.145 mg/mL). This result might be due to the stalk mushroom could contain the high level of total phenolic content. However, the antioxidant capacity is different in mushroom species. Cheung *et al.*, (2003) found that the water extract from Shiitake mushroom (*Lentinus edodes*) showed the most potent radical scavenging activity in that 55.4% in the DPPH radical scavenging method (at 6 mg/mL) which higher than methanol extracts.

It is concluded that the SCB extracted with the highest polarity of solvent gained the high amount of yield production. The SCB extracted with different organic solvent could not inhibit the growth of both pathogenic bacteria.

However, the high capacity of antioxidant was observed. The methanol and 2-butanol extracts showed the highest antioxidant activity. The efficiency of SCB extracts could be further studied in the economic fish diet as a feed additive to increase the ability of fish for oxidative defense that can be beneficial applied in aquaculture and increase the value of agricultural waste.

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