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Antioxidant Properties and Antimicrobial Activity in the Extracts of Two Edible Mushroom, *Pleurotus sajor caju* and *Schizophyllum commune*

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Abstract

Extracts of two edible mushrooms, *Pleurotus sajor-caju* (commercial) and *Schizophyllum commune* (wild) were used to compare the antioxidant and antimicrobial properties. Aqueous and three types of organic solvents, like 50% of ethanol, methanol and acetone extracts were used in trial. DPPH scavenging activity in *P. sajor-caju* extract was determined in the range of 53.13% to 85.08%, whereas extracts of *S. commune* were observed in the range of 54.11% to 97.19% at a concentration of 5 mg/ml. The highest DPPH scavenging activity of 97.19% was observed in ethanol extract of *S. commune* (97.19%), higher than butyl hydroxytoluene (BTH). Half effective concentration (EC_{50}) in extracts of *P. sajor-caju* was found in the range of 1.47 to 4.23 mg/ml and that of *S. commune* in the range of 1.52 to 4.52 mg/ml. The reducing power of *P. sajor caju* aqueous concentration extract was found to be the closest of 3.353 (700 nm) that of antioxidant activity to BHT (3.445) at 2 mg/ml concentration. The best reducing power EC_{50} was obtained in *P. sajor caju* aqueous extract (0.09 mg/ml), but in *S. commune* with acetone extract (0.22 mg/ml). Minimum inhibition concentration (MIC) was compared in extracts of mushrooms in various *Vibrio* species. All extracts were able to inhibit *V. harveyi* growth with MIC of lower than 1.25 mg/ml. In aqueous and methanol extracts of current study showed that bacteria inhibition activity occurred at the concentration of <1.25 mg/ml to 10 mg/ml. Aqueous extract of *P. sajor-caju* was able to act as reducing agent as functional as the commercial antioxidant agent, BHT. Crude extracts of *P. sajor-caju* and *S. commune* were observed to contain antibacterial potential against these mentioned *Vibrio* bacteria.

Keywords

Mushrooms, Extracts, Antioxidant, Antimicrobial, Vibrio, Bacteria

1. Introduction

Pleurotus sajor caju, a commercially grown and naturally grown *Schizophyllum commune* are popular edible mushrooms in Sabah (East Malaysia). Taste and smell of these two types of mushrooms are different. *Schizophyllum commune* is a wild edible mushroom that belongs to the family Schizophyllaceae [1]. Although *S. com*mune growing conditions and substrates are different from commercially grown mushrooms, the nutritional value of this mushroom is as good as commercial mushrooms [2].

Mushroom was proven to be safer, environmentally friendly, pollution free, improve disease resistance, enhance immunity and decrease mortality [3]. Extracts of mushroom are in concentrated fom also gives beneficial effects while being used. There were two common solvents used for mushrooms extractions as which are ethanol and aqueous extract. *Pleurotus sajor-caju* extract was known for its carbohydrate content, particularly polysaccharide, is soluble in hot water [4]. The mentioned polysaccharide is actively studied for its nutraceutical properties to combat cancer [5], anti-inflammatory [6], anti-obesity and prevent oxidative stress [7]. In addition, this mushroom crude extract revealed antioxidant activity and anti-proliferative activity in aqueous extraction [8]. Crude extract of *S. commune* was found as good as oxidative stabilizer and free radical scavenging agent to prolong the shelf life of lipid food products [9]. Other traces of antioxidant compounds of S. commune are flavonoid, beta-carotene and lycopene [10]. Schizophyllan has long been identified as medicinal active compound used for cancer research and development in Japan [11].

Methanol extract of *P. sajor-caju* exhibited anti-bacteria properties that are able to inhibit growth of gram positive and negative bacteria like *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus luteus* [12]. In *Schizophyllum commune* methanol extract, antimicrobial activity was reported significant to clinical importance bacteria namely *B. cereus*, *B. subtilis*, *S. aureus*, *Pseudomonas aeuroginosa*, *Proteus vulgaris*, *E. coli*, *Streptococcus mutans*, *S. sanguis*, *S. mitis*, *Shigella* sp., *S. flexneri*, *Plesiomonas shigelloides*, *Salmonella typhi*, and *Enterobacter faecalis* [13]. In methanol extract of *S. commune* had indicated active antimicrobial inhibitory effect towards gram positive bacteria and fungus [14]. So far, there is limited information on antioxidant and antimicrobial activity in *S. commune* extract other than methanol. This study will provide information of the chemical selectivity in four extractions from *P. sajor-caju* and *S. commune* mushroom. The purpose of this research was to compare the antioxidant and antimicrobial activity of commercially cultured *P. sajor-caju* and wild variety of *S. commune* mushrooms ex-

tracted with aqueous and three types of organic solvents.

2. Materials and Methods

Sample preparation and extractions used Detail of two types of mushroom collection, drying and making fine powder were mentioned in previous research article. Three types of organic solvents and aqueous extraction were used in the preparation of extracts from two types of mushroom. The detail of extraction techniques and solvents used in the extracts were also stated in other published article [15].

2.1. Antioxidant Activity

2.1.1. Scavenging Effect on 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical

The scavenging activity of the mushrooms extracts on DPPH radical, a free radical was measured according to the method of [16]. Absorbance of the color was read in 517 nm wavelength. Methanol was used as blank while BHT was used as a standard. Mixture without extract was used as the negative standard and butyl hydroxytoluene (BHT) was used as positive standard. Inhibition of free radical DPPH was calculated with formula:

$$\text{Scavenging activity}(\%) = \left(A_1 - A_2 / A_0\right) \times 100$$

where, A_0 is the absorbance of the DPPH without the sample and A_1 is the absorbance of the sample with DPPH, and A_2 is the absorbance of the blank only.

EC_{50} value (mg/mL) was defined as the half effective concentration. The EC50 value (mg/mL) of DPPH scavenging activity was determined at 50% of various concentration of extract scavenging activity against DPPH from the plotted graph.

2.1.2. Reducing Power Assay

The reducing power was determined according to the method described in research [17]. Absorbance at 700 nm was measured against a blank (distilled water). BHT was used as the positive control. The EC50 value represented the concentration of the reducing power activity at 0.5 (700 nm).

2.1.3. Antimicrobial Activity

The minimum inhibition concentration (MIC) was determined with the technique described by researchers [18] in broth micro-dilution method using 96-well micro-titer plates. Three marine bacteria tested in this study were *V. harveyi*, V. parahaemolyticus and *V. anguillarum*. Five two-fold serial dilutions of concentration ranging from 1.25 to 10 mg/mL of extracts were prepared from 100 mg/mL of mushroom extract stock. In each well of a 96-well plate, 90 µL of nutrient broth, 100 µL of mushroom extract solution (1.25 - 10 mg/mL) and 50 µL of bacterial suspension (10^8 CFU/mL) were added and mixed. Well without mushroom extract was used as blank. After incubation at 37˚C for 24 hours, the absorbance of each incubation was read at 750 nm with a micro-plate reader. The absorbance values of blank and bacteria solution with mushroom extract

were compared. The lower absorbance value than blank indicated as positive antimicrobial activity while similar or higher absorbance value than blank indicated as negative. MIC was determined by the lowest concentration of mushroom extract exhibited inhibition activity against bacteria growth.

2.2. Statistical Analysis

Microsoft Excel 2010 was used to calculate the means and standard deviations for all multiple measurements as well as to generate graphs. Antioxidant activities (EC50) results were analysed using analysis of variance to test the mean value and the comparisons of the mean values were done by using turkey test in software program SPSS version 22. The level of significance setting was $p < 0.05$.

3. Results

3.1. Antioxidant Activity of Varies Mushrooms Extracts

3.1.1. DPPH Radical Scavenging Activity

At concentration of 5 mg/ml, DPPH scavenging activity in *P. sajor-caju* extract observed in the range of 53.13% to 85.08%, while in *S. commune* range values (54.11% to 97.19%) observed higher than *P. sajor-caju* extract (Figure 1). The highest DPPH scavenging activity percentage was observed in ethanol extract of *S. commune* (97.19%) was higher than BHT (94.24%). The least activity was observed in acetone extracts of both mushrooms (*P. sajor-caju* = 53.13% and *S. commune* = 54.11%).

The average half effective concentration (EC_{50}) of P. sajor caju was determined in the range of 1.47 to 4.23 mg/ml and the range of 1.52 to 4.52 mg/ml was observed in *S. commune* (Table 1). The best EC_{50} was seen in aqueous extract of *P. sajor-caju* (1.47 mg/ml) and aqueous extract of *S. commune* (1.52 mg/ml), which were significant different ($p < 0.05$) compared to other extracts. Acetone extracts of *P. sajor-caju* and *S. commune* were significantly different ($p < 0.05$) the highest

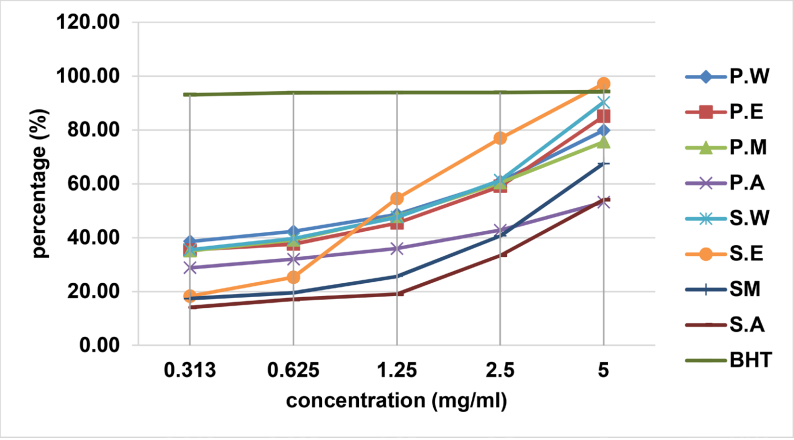

Figure 1. DPPH activity (%) of *Pleurotus sajor-caju* and *Schizophyllum commune* in different extraction solvent and concentration. (P = *P. sajor-caju*, S = *S. commune*, W: aqueous extract, E: ethanol extract, M: methanol extract, A: acetone extract and BHT: butylated hydroxytoluene).

EC_{50} value indicating the weakest DPPH scavenging capability.

3.1.2. Reducing Power

Antioxidant activity (reducing power) of extract was found to increase with 0.5 mg/ml increment of concentration (Figure 2). Among all extracts, *P. sajor-caju* aqueous concentration at 2 mg/ml had the closest values of 3.353 (700 nm) antioxidant activity to BHT (3.445). The least reducing power reaction was seen in *P. sajor-caju* ethanol extract.

The value of EC50 in *P. sajor-caju* extract was obtained in the range of 0.09 mg/ml to 0.81 mg/ml, while in extract of *S. commune* extract the range values was from 0.22 mg/ml to 0.5 mg/ml. The best reducing power EC_{50} was determined in *P. sajor-caju* aqueous extract (0.09 mg/ml) and acetone extract (0.22 mg/ml) of *S. commune* with significant different of $p < 0.05$ (Table 2).

Table 1. EC_{50} (mg/ml) of DPPH scavenging activity from *Pleurotus sajor-caju* and *Schizophyllum commune* in various extracts (mean ± sd).

Solvents used	*Pleurotus sajor caju*	*Schizophyllum commune*
Aqueous	1.47 ± 0.02^{a}	1.52 ± 0.07^{a}
Ethanol	1.70 ± 0.07^{b}	1.67 ± 0.01^{a}
Methanol	1.75 ± 0.02^{b}	3.40 ± 0.10^{b}
Acetone	4.23 ± 0.37^{c}	4.52 ± 0.03^{c}

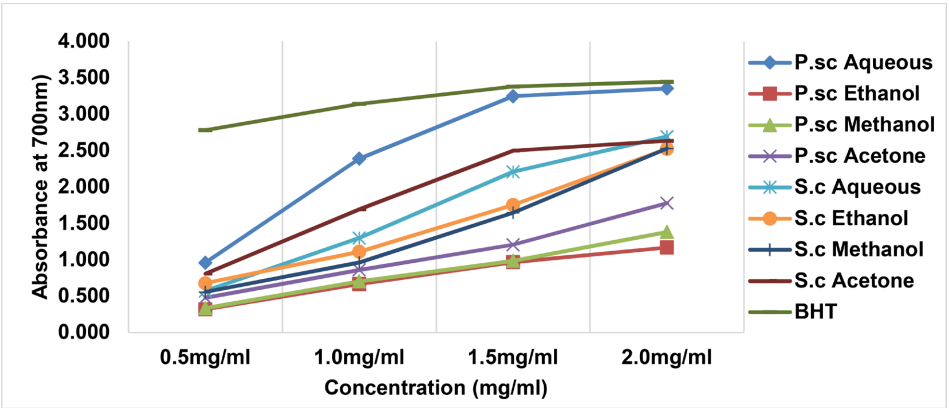

Figure 2. The Absorbance value of *Pleurotus sajor-caju* and *Schizophyllum commune* in different extraction solvent and concentration.

Table 2. EC50 (mg/ml) of reducing power activity from *Pleurotus sajor-caju* and *Schizophyllum commune* in various extracts (mean ± sd).

Solvents used	*Pleurotus sajor-caju*	*Schizophyllum commune*
Aqueous	0.09 ± 0.06^{a}	0.41 ± 0.01^{b}
Ethanol	0.81 ± 0.03^{d}	0.42 ± 0.02^{b}
Methanol	0.73 ± 0.01^{c}	0.50 ± 0.11^{c}
Acetone	0.58 ± 0.04^{b}	0.22 ± 0.03^{a}

3.1.3. Antimicrobial Activity

All extracts were able to inhibit *V. harveyi* growth with Minimum Inhibition Concentration (MIC) of lower than 1.25 mg/ml. MIC value was determined in *V. parahaemolyticus* < 1.25 mg/ml and *V. anguillarum* was observed 10 mg/ml and >10 mg/ml in *P. sajor caju* extracts. *S. commune* extracts obtained MIC value of 2.5 mg/ml and 5 mg/ml in *V. parahaemolyticus* and 5 mg/ml and 10 mg/ml in *V. anguilarium*. Methanol and acetone extracts of *P. sajor-caju* were unable to show inhibitory activity against *V. anguillarum* (Table 3).

4. Discussion

4.1. DPPH Radical Scavenging Activity

Radical scavenging activity is essential to protect tissue damage from deleterious effect of lipid oxidation. *S. commune* extracted in hot water (autoclaved 121˚C) obtained 79.5% of scavenging activity at 10 mg/mL [19]. Better scavenging activity of 90.27% was obtained at 5 mg/mL of *S. commune* aqueous extract (room temperature) in current study.

The lower effective concentration (EC_{50}) indicates the better DPPH scavenging capability of the extract. In this study mushroom extraction was conducted under room temperature for three days. Aqueous extract of *P. sajor-ca*ju and *S. commune* was obtained the lowest of EC_{50} in 1.47 mg/mL and 1.52 mg/mL, respectively. Other *P. sajor-caju* studies obtained EC_{50} of 13.38 mg/ml observed in in 24 hours aqueous extraction [20]. The EC_{50} result of aqueous extract in *S. commune* reported of 0.8 mg/mL using mushroom over solvent ratio of 1:20 (w/v) [19], comparable to the current aqueous extraction effect. On the other hand, *S. commune* was not able to obtain any EC_{50} value within the concentration of 0 to 20 mg/mL in 70% methanol extract with the dry mushroom:solvent ratio of 1:5 (w/v) [21]. Current study using mushroom:solvent ratio of 1:10 (w/v) obtained better EC_{50} value (3.4 mg/mL) in *S. commune* 50% methanol extract. Ethanol extract of this study obtained EC_{50} value of 1.7 mg/mL (*P. sajor-caju*) and 1.67 mg/mL (*S. commune*). Ethanol extract of *S. commune p*rocessed for

Table 3. Minimum Inhibition concentration (mg/ml) of antimicrobial activity from *Pleurotus sajor-caju* and *Schizophyllum commune* in various extracts.

Mushroom	Extracts	*V. harveyi*	*V. parahaemolyticus*	*V. anguillarum*
Pleurotus sajor-caju	Aqueous	<1.25	<1.25	10.0
	Ethanol	<1.25	<1.25	10.0
	Methanol	<1.25	<1.25	>10.0
	Acetone	<1.25	<1.25	>10.0
Schizophyllum commune	Aqueous	<1.25	5.0	10.0
	Ethanol	<1.25	5.0	5.0
	Methanol	<1.25	2.5	5.0
	Acetone	<1.25	2.5	5.0

two days with twice ethanol extraction obtained 0.883 mg/mL EC_{50} value [10] better than this study ethanol extract of *S. commune*.

4.2. Reducing Power

The reducing power of extract exhibited medium antioxidant activity at 0.5 mg/mL and demonstrated the highest activity at the concentration of 2 mg/mL. In this study, at 2 mg/mL (abs = 2.23) of *P. sajor-caju* aqueous extract was able to act as reducing agent as functional as the commercial antioxidant agent, BHT. Boil aqueous extract of *P. sajor-caju* was reported 0.37 (700 nm) of reducing power intensity at 0.5 mg/mL extract [22] observed to obtained higher of 0.96 (700 nm) at 0.5 mg/mL of P. *sajor-caju* aqueous crude extract in the current study. The best EC_{50} in aqueous extraction of 1.295 mg/mL and 2.237 mg/mL were obtained in *P. sajor-caju* and S. commune, respectively. EC_{50} of *S. commune* was found vast difference in different extractions which were >20 mg/mL in methanol extract [21], 7.6 mg/mL in hot water extract [19] and 0.825 mg/mL in ethanol extract [10]. Antioxidant activity was higher when mushroom was re-extracted twice with ethanol solvent [10]. In addition, aqueous extract of mushroom was expected with high content of phenolic compounds responsible with reductive capabilities [12].

4.3. Antimicrobial Activity

Mushroom *P. sajor-caju* was reported to be able to inhibit growth of gram positive and negative bacteria using methanol extract [12]. *P.sajor-caju* extracted in aqueous extract at 4˚C overnight fractionated ribonuclease produced 50% inhibition concentration (IC50) of 51 ± 6 μM, 186 ± 12 μM and 34 ± 4 μM to *Pseudomonas aeruginosa*, *P. fluorescens* and *S. aureus*, respectively [23]. The MIC value obtained from *S. commune* methanol extract was 500 μg/mL concentration against gram positive bacterias, *S. aureus* and *B. subtilis* [14]. The above authors also mentioned that *S. commune* methanol extract revealed better antimicrobial activity than aqueous extract and ethanol extract. *V. harveyi*, *V. parahaemolyticus* and *V. anguillarum* are clinically important gram negative bacteria lead to vibriosis in marine life [24]. Up to date, *V. harveyi* has developed resistance to 16 antibiotics, and this alarmed the search for natural alternatives to provide protection against this bacteria [25]. In this work, crude extracts of *P. sajor-caju* and *S. commune* were observed to contain antibacterial potential against these species of bacteria. Methanol extract of sea grass (*Thalassia hemprichii*) was able to obtain MIC value of 1.56 mg/mL against *V. harveyi* [26]. In this study, *Vibrio harveyi* was observed sensitive to all types of mushrooms extract at minimum inhibition concentration of <1.25 mg/mL. *V. parahaemolyticus* was seen vulnerable to *P. sajor-caju* extracts antimicrobial activity at <1.25 mg/mL concentration. The poorest MIC obtained in *V. anguillarum* observed no antimicrobial activity at 10 mg/mL with *P. sajor-caju* methanol and acetone extracts. Methanol extract in betel (Piper betle), a traditional medicinal plant possess strong antimicrobial properties demonstrated inhibitory effect towards *V.*

parahaemolyticus at lower than 0.1 mg/disk but needed a little higher concentration used to inhibit *V. anguillarum* growth at 0.1 mg/disk [24]. *S. commune* aqueous extract prepared in boiling water contained no bacteria inhibition activity whereas methanol extract displayed inhibition activity towards gram negative bacteria growth at the concentration of 2 mg/mL [13]. In aqueous and methanol extracts of current study showed that bacteria inhibition activity occurred at the concentration of <1.25 mg/mL to 10 mg/mL.

5. Conclusion

Aqueous extract exhibited the higher antioxidant activities by containing higher reluctant agent and free radical scavenging capability. However different types of extracts have least effect on antimicrobial activity. *V. harveyi* was susceptible to all extracts while *V. parahaemolyticus* and *V. anguillarum* were susceptible to *P. sajor-caju* and *S. commune* extracts, respectively.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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