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FULL PAPER

Scavenging activities of *Schizophyllum commune* Fr. extracts against DPPH radicals, extractable materials and a TLC qualitative of the mushroom extracts

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Abstract

A fresh split gill mushroom or locally known as cendawan kukur (*Schizophyllum commune* Fr.) (SC) was obtained from Terengganu, Malaysia. The SC samples were dried in an oven and extracted with several polarities of solvent such as water, ethanol, dichloromethane and hexane. Then the SC extracts were tested for their scavenging activity against the DDPH radical models. The scavenging activities were calculated as a percentage of DDPH radicals discolouration and measured as scavenging activities of the *S. commune* extracts. The finding observed the highest scavenging activities resulted from SC-H_2O (85.40%), followed by SC-ethanol (54.91%), SC-dichloromethane (0.46%) extracts and no observation of scavenging activities showed by a non-polar extract (SC-Hexane). In this finding, the scavenging activity of SC-H_2O extract against DDPH radicals was found more potent at 85.40% and it showed the highest activities as compared to the other SC-DCM and SC-EtOH extracts. In the next observation, the scavenging activities of the single SC-water extract against free radical DDPH were observed for another 30 mins by an interval of 5 mins increment after the incubation time. As a result, the scavenging activities of SC-H_2O extract against DDPH free radicals maintained its activities between 85.40-85.76% within 30 mins. These observed that the SC-H_2O extracts presented a highly potent antioxidant as compared to other SC extracts in this model. Finally, an additional HPTLC method was used as a preliminary observation of the SC extract separation profiles. The result of SC water extracts (polar extract) observed the same Rf value (0.25) of the unknown compound as compared to the SC-EtOH and SC-DCM extracts, and this spot compound was not observed in the non-polar extract of SC-Hexane. The polar and mid-polar extractable materials were more abundant in TLC separations and are related to the % TSS and strength of the mushroom aroma in the extracts. These preliminary studies will be continued for the *S. commune* phytochemicals discovery and the development of a new product of *S. commune* flavour extracts.

1. Introduction

Mushrooms are natural sources that have great potential as nutritional, functional foods and sources of physiological benefits and non-toxic medicines (Wasser and Weis, 1999). *Schizophyllum commune* is a species of fungus in the genus of *Schizophyllum*. A *Schizophyllum commune* is one of the widest fungi distributed as common mushrooms (Kuo, 2003) and easily grows on rotten wood and plants. The edible and medicinal fungi or mushrooms have been increasing in their applications in either functional foods or nutraceutical products because of their proven nutritive and medicinal properties. According to Wu (2014), polysaccharides (PS) and PS-protein (PSP) complexes are major constituents in edible fungi and contain several

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bioactivity properties such as immunomodulation, antitumor, antioxidant, antiviral and prebiotic.

Many researchers are currently focusing in their studies on the antioxidant properties of *S. commune* (Mirfat *et al.,* 2010; Emsen *et al.,* 2017; Dang Lelamurni *et al.,* 2018), and there are many great findings on the antioxidant materials that have been discovered from our natural living materials such as plants and fungi. A measurement of scavenging activities of food or plant extracts against DDPH radicals is the simplest methodology to determine a potent antioxidant in the sample extracts. The extractable samples scavenging activities against the DDPH radicals are calculated as a percentage of the radical's discolouration and measured as scavenging activities of the *S. commune* extracts against the radicals. DDPH radicals are free radicals that can accept a negative or positive charge to become a stable and colourless molecule (Soares *et al.*, 1997) and it is widely used to access radical scavenging activities from several matrices of materials.

In this research, evaluation of scavenging activities against DDPH radicals, determination of sugar profiles and extractable materials as total soluble solid (TSS) percentage, physical observations of the aroma and appearance, and a preliminary observation on an HPTLC, are fully observed onto the four polarity extracts of the *S. commune*. An additional discussion is also focused on *S. commune* or *cendawan kukur* extracts as the new potential of high antioxidant mushroom seasoning.

2. Materials and methods

2.1 Experimental design

In Figure 1, fresh *S. commune* (split gill mushrooms) or locally known as cendawan kukur was obtained from Fatisha Agro Farm (M) Sdn Bhd. Al-Muktafi Billah Shah, Terengganu Malaysia. The fresh samples of *S. commune* were dried in an oven (Memmert) at 45-55°C for 24 hrs then the samples continued to dry for another 1 hr until fully dried at 60°C. The moisture content was taken to ensure the sample was less than 10% moist was obtained. Then the samples were packed in proper packaging and frozen prior to analysis. In the same Figure 1, 10 g of sample was weighed and transferred into a 250 mL volumetric flask and 100 mL of solution (distilled water, ethanol, dichloromethane and hexane) was transferred into the flask and closed properly with an aluminium foil and the mixtures were mixed and left to stand in a cold extraction condition for 1 week. Then, the *S. commune* extracts were filtered using a Whatman no.1, and the filtrate was dried using a rotary evaporator (Heidolph, Germany) and dried extractable materials were collected and re-diluted with the same solution of distilled water, ethanol, dichloromethane and hexane for further analysis.

2.2 Determination of antioxidant activity by DDPH radicals scavenging assay

A volume of 2.90 mL of 0.2 mM DDPH radicals (in methanol) was added to each test tube containing 100 µL of *S. commune* extracts and mix. The test tubes were then kept in a dark cabinet for 30 mins. The absorbance was measured at a wavelength of 515 nm. The assays were performed in triplicate. The radical scavenging activity was calculated as a percentage of DDPH radicals discolouration based on the formula:

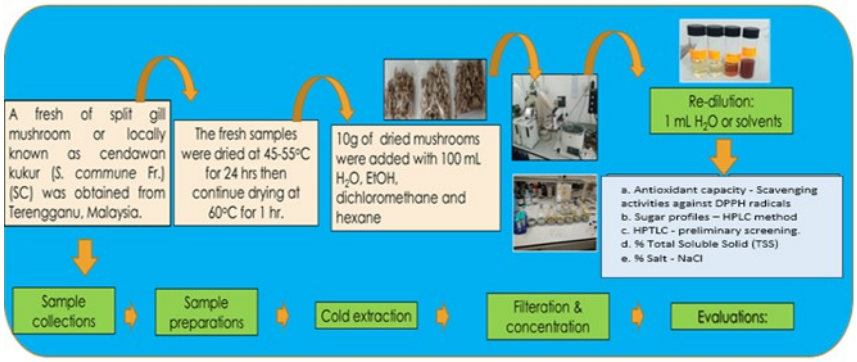
$$SA\ (\%) = 100 - (100\ (Aa - Ac)/Ab)$$

Where Aa is the absorbance of the system (mushroom extracts) and DDPH radicals, SA is referred to scavenging activities, Ab is an absorbance of the system with only the DDPH radicals, and Ac is the absorbance of the system with only the mushroom extracts.

2.3 Analysis of sugar profiles

The method was referred to Malaysian Standard MS 2683:2017 (Department of Standards Malaysia, 2017). The sugar standards (Fluka brand) used in HPLC calibration were sucrose, glucose, fructose and maltose. Approximately 25 mL of methanol was transferred into a

a. Fresh *S. commune*



b. Experimental design

Figure 1. Fresh of *S. commune* (a) and the experimental design of the research (b)

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100 mL volumetric flask, and then the sugar standards (fructose 2.000 g, glucose 1.500 g, sucrose 0.250 g and maltose 0.150 g) were added into each volumetric flask and marked up to 100 mL with deionized water. Five grams of mushroom samples were diluted as mentioned previously. The standards and samples were filtered through a membrane filter (a pore size of 0.45 µm) and a 10 µL filtrate was used for injection and separation in a Waters HPLC system with an amine-modified silica gel column (5 µm particle size) at a column temperature of 30°C. The flow rate of the mobile phase of acetonitrile: water (80:20, v/v) was set at 1.3 mL/min.

2.4 Preparation of TLC and separation techniques

Approximately 2 mL of the sample solution using a syringe were loaded on a pre-coated TLC plate (Silica gel 60 F_{254} sheets 20 × 20 cm, 0.5 mm thickness, Merck Darmstadt, Germany) using an automatic sampler. The plate was developed up to a distance of the length of the TLC plate (mm) in a glass chamber that was pre-saturated with a mobile phase petroleum ether: ethyl acetate (90:10 v/v) and 1% acetic acid for approximately 30 mins. The plate was fixed on a scanner stage and scanning was done at 254 nm and 366 nm. The distance between the TLC spots was measured and the retention factor (Rf) values were calculated using the following calculation:

Rf value = Distance moved by the compound/ Distance moved by the solvent front

2.5 Determination of total soluble solid

An Atago 3810 (PAL-1) Digital Pocket Refractometer range of 0-53% is ideal for determining % total soluble solid (TSS) or % Brix concentrations in fruits and beverages, cutting oils and organic samples. The refractometer is focused on the determination of the percentage of TSS content of the *S. commune* extract as prepared in Figure 1.

2.6 Determination of salt

The percentage of NaCl salt content in SC-H_2O extract was detected using a Master S28M Atago hand refractometer. The percentage salt range of the refractometer used is between 0.6 ~ 28%.

3. Results and discussion

In Table 1, the result observed the highest scavenging activities resulted from an SC-H_2O extract (85.40%), followed by SC-EtOH (54.91%), SC-dichloromethane (0.46%) extracts and no observation of scavenging activities showed by a non-polar extract (SC-Hexane-upper layer). According to Emsen *et al.* (2017), the IC_{50} values of the *S. commune* chloroform extract gave the highest DDPH radicals scavenging and metal chelating activities at 7.652 mg/mL and 6.590 mg/mL respectively. The IC_{50} values of the water extracts were recorded at 86.712 mg/mL with less scavenging activity on the DDPH radicals. In this finding, the water extract of the *S. commune* is observed higher antioxidant capacities. Dang Lelamurni *et al.* (2018) reported that a 70% ethanol extract of *S. commune* demonstrated a moderate DDPH radical scavenging activity, with a value of 40.36% activity, while the 70% methanol extract gave the lowest 29.17% activities. These findings supported that a more polar solvent used resulted in higher DDPH radical scavenging activity of the *S. commune* extract as compared to the mid-polar and non-polar solvents used.

In Table 1, a percentage of total soluble solid (% TSS) of SC-H_2O extract presenting up to 8.10% TSS and having a very strong balsamic and dried mushroom aroma, and presents a dark brownish solution. The SC-EtOH (19.87% TSS) gave a mild mushroom aroma, while an SC-DCM extract (50.73% TSS) gave a fresh mushroom aroma; and SC-Hexane extracts (5.73% TSS) had a light odour of mushroom aroma. The *S. commune* dichloromethane extract gave the highest % TSS in the extract, followed by ethanol and water extracts. Thus these observations can be summarized that the % TSS amount of the extractable solid of *S. commune* in the extracts was increased in order by dichloromethane> ethanol> water and less in the hexane portion. As reported by Magwazaa and Opara (2015), a total soluble solid (TSS) can be measured either using a Brix scale

Table 1. Physical properties and scavenging activities of *S. commune* extracts against DDPH free radicals

S. commune Extracts	*S. commune* extract - appearance and aroma	Total soluble solid (TSS) of the *S. commune extract* (%)[1]	Salt content (%)[2]	Percentage of DDPH radicals (%)[3]
SC-Hexane (clear phase)	Clear and odourless	5.733±0.058	-	0.00
SC-DCM	Slightly yellowish clear and fresh mushroom aroma	50.733±0.289	-	0.46
SC-EtOH	Slightly yellowish clear and mild mushroom aroma	19.867±0.153	-	54.91
SC-H_20	Dull brownish and very strong balsamic mushroom aroma	8.100±0.000	6.50±0.00	85.40

[1]triplicate readings, [2]triplicate readings, and [3]scavenging activities after 30 mins incubation time

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hydrometer or a refractometer; and it can be reported as "degrees Brix" (◦Brix) which is equivalent to the percentage (%). The sugars and acids, together with small amounts of dissolved vitamins, fructans, proteins, pigments, phenolics, and minerals, are commonly referred to as soluble solids. A refractometer method quantifies the number of dissolved solids which includes sugars, proteins and organic acids; and it is not a totally accurate method for total sugar content (Chope *et al*., 2006).

The total salt content in an SC-H_2O sample (Table 1) was detected at 6.50% of NaCl salt. The water-soluble extract of these gill mushrooms presents a commercial potential for the production of a new mushroom seasoning or flavour due to its balsamic and mushroom aroma and high antioxidant capacity. In Figure 2, the scavenging activities of the single SC-H_2O extract against DDPH radicals were observed for 30 mins by interval increments of 5 mins after the incubation time, as a result, the scavenging activities of SC-H_2O extract against DDPH radicals were extended activities between 85.40-85.76% for 30 mins. These findings observed the SC-H_2O extracts presenting a very potent antioxidant of 85% scavenging activities against the DDPH radicals.

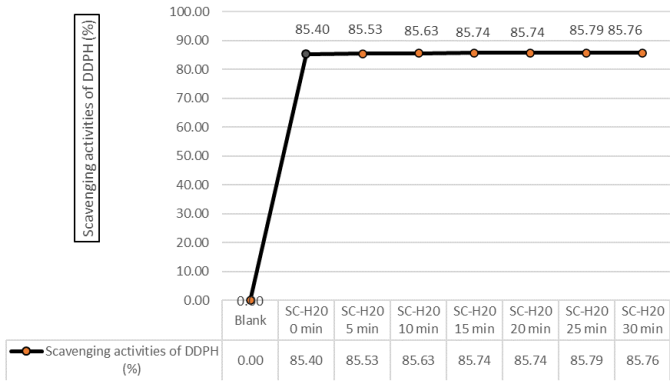

Figure 2. Percentage of scavenging activities of SC-H_2O extracts against DDPH radicals (by incubation period)

As shown in Figure 3, two speciality sugars, maltose and glucose sugars were present in the HPLC chromatogram of the dried-fresh *S. commune* sample at *Rt* 9.564 mins and 16.364 mins respectively. These sugars give the sweet properties of the fresh and dried *S. commune*, and enhance the taste and aroma in the *S. commune* seasoning products. The amount of glucose and maltose was observed at 1.89% and 3.42% (d/w) respectively. The *S. commune* was reported at 2.1 and 5.1% glucose (d/w) if the mushroom is growing in the soybean hulls and distilled dried grains, however the glucose content in the mushroom can be increased up to 20.2 and 23.5% in the untreated and pre-treated corn fibre medium respectively (Sutivisedsak *et al.*, 2014). In this research, the *S. commune* samples collected were inoculated and grown on the wood dust and their sugar profiles were varied as compared to the above findings.

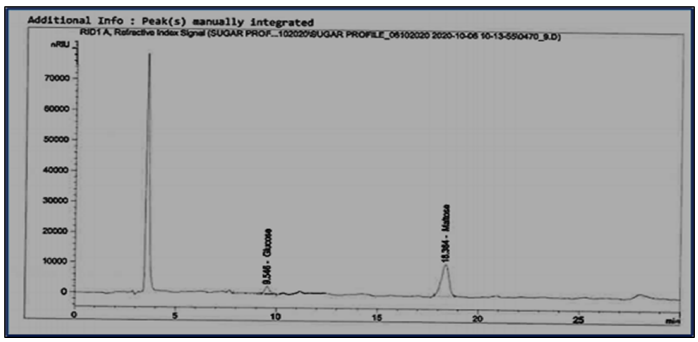

Figure 3. Sugar profiles of oven dried *S. commune* (from fresh samples)

A qualitative TLC analysis was conducted for the *S. commune* extracts. In Figure 4 (c), a TLC result of SC-H_2O extract observed the same *Rf* value (0.25) of the interesting compound that was also recorded by SC-EtOH and SC-DCM extracts respectively, and this compound was not observed in a non-polar of SC-Hexane extract. As a comparison, Appiah *et al.* (2017) illustrated a TLC separation of 'S' (methanolic extract of *S. commune*) eluted by chloroform mobile phase and the result gave a TLC band separation was similar to the TLC band separation of *S. commune* ethanolic extract (SC-EtOH) as in Figure 4(c). They also reported the presence of several compounds such as tannins, flavonoids, triterpenoids, alkaloids, glycosides and anthraquinones as secondary metabolites in the *S. commune* extract. As mentioned in Table 1, the % TSS and aroma of the SC-DCM, SC-EtOH and SC-H_2O extracts were higher and more aromatic than the SC-Hexane sample. Therefore, it resulted in less extract material in an SC-hexane sample. Related research done

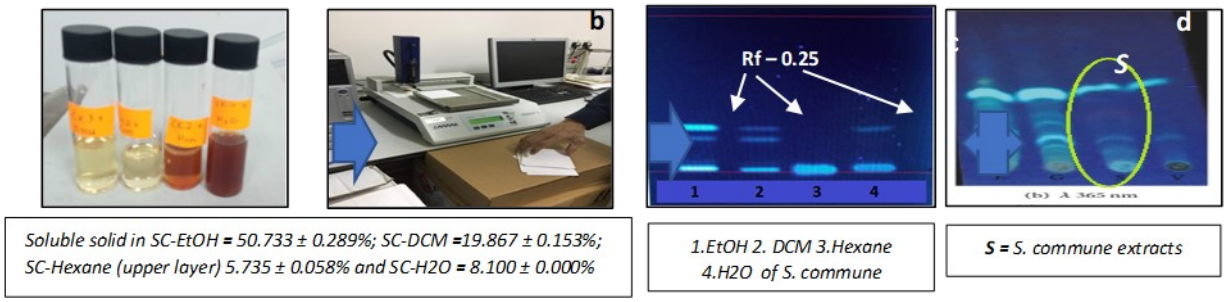

Figure 4. HPTLC screening method used as a preliminary observation of the SC extracts separation profiles and the TLC reference. (a) *S. commune* extracts, (b) HPLTC instruments, (c) A TLC of *S. commune* extracts, and (d) A TLC reference. Source: Appiah *et al*. (2017).

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by Acharya *et al.* (2016) showed a quantification of some major bioactive compounds present in the *S. commune* extract as follows: phenol > flavonoid > ascorbic acid > β-carotene > lycopene. These showed that the non-polar extract materials of the *S. commune* (hexane extract) were found less than the polar and mid polar portions (as water, ethanol and dichloromethane). These preliminary studies will be continued for *S. commune* fundamental phytochemicals discovery.

4. Conclusion

The water extract shows the best scavenging activities against DDPH free radicals as compared to other extraction and it has good properties of balsamic and dried mushroom odour and presents a brownish colour and is suitable for the development of new functional food seasoning. Further R&D should be done by the MARDI researcher to commercialise these functional *S. commune* water extract products.

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