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Effects of different extracting conditions on anti-tyrosinase and antioxidant activities of *Schizophyllum commune* fruit bodies

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ABSTRACT

A current trend in cosmetic and cosmeceutical science is the search for edible ingredients with effective skin-repairing actives. *Schizophyllum commune* is a cultivated edible mushroom known to contain compounds that possess potent biological properties beneficial to skin health. However, research on the development of locally cultivated *S. commune* as a cosmeceutical ingredient is lacking. The objective of this preliminary study was to evaluate the effect of extraction conditions on the cosmeceutical properties of *S. commune* extract. Fruit bodies were extracted using two different temperatures; 4 °C and 30 °C at three different extraction times; 1, 12 and 24 h, using water as extracting solvent. Anti-pigmentation activity measured by a tyrosinase inhibition assay, as well as antioxidant activities, were evaluated. Biological components such as total phenolic, polysaccharide, and glucan content were also assessed. The results revealed that a 1 h extraction time at 4 °C or 30 °C produced extracts with the strongest anti-pigmentation effect, with the value of 94.2 and 95.4% respectively. At 4 °C, shorter extraction time yielded better ferric-reducing and DPPH-radical scavenging antioxidant activities, while results were varied at 30 °C. Based on our results, the optimal conditions for effective cosmeceutical properties in *S. commune* extract was extraction at 30 °C for 1 h. Further research on optimization of the extraction method and in-vitro efficacy test using cell lines should be commenced to thoroughly explore the potential of these mushroom extracts as cosmeceutical agents.

1. Introduction

Natural or organic ingredients for cosmeceutical and skin care products are currently in trend, due to the rise of consumer disinclination for synthetic ingredients and products. Natural ingredients have potent protective and defensive roles against oxidative damage caused by free radicals, which often lead to premature aging and hyperpigmentation of the skin. One of the potential sources for natural and organic cosmeceutical ingredients are edible mushrooms. Mushrooms exhibit various health-promoting properties such as anti-tumour, anti-inflammatory, anti-bacterial, and immuno-modulating activities (Vieira et al., 2012). There is a growing number of cosmeceutical and skin care products containing mushroom extracts, or their bioactive compounds, that are commercially available throughout the world. Medicinal mushrooms such as *Ganoderma lucidum* and *Cordyceps sinensis* have long been used in cosmeceutical products, most commonly as anti-aging agents.

Schizophyllum commune is a well-known edible white-rot fungus that is capable of producing many types of secondary metabolites (Yi and Don, 2012). Locally known as the 'Kukur' mushroom, *S. commune* or 'split gill' mushroom is popular due to an exopolysaccharide known as schizophyllan, which is used in the cosmeceutical industry as a thickener for cosmetic lotions (Hao et al., 2010). It is also used as an anti-aging and hydrating agent for skin (Wu et al., 2016). Apart from schizophyllan, other compounds from *S. commune* have the potential to be exploited in cosmeceutical and cosmetics industries. To evaluate this potential, extracts containing bioactive compounds from *S. commune* should be investigated for their cosmeceutical-related biological properties, such as their antioxidant and tyrosinase inhibition activities. The tyrosinase inhibition activity of a compound or extract serves as a significant indicator of its potential skin anti-pigmentation or whitening capacity. Antioxidant compounds protect the skin from premature aging due to both intrinsic (genetic and epigenetic mechanisms) and extrinsic (UV rays and other environmental hazards) factors (Farage et al., 2008). Extracts with both anti-tyrosinase and antioxidant compounds can be employed as a bio-ingredient not only in cosmeceuticals,

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but in many other health industries. In this context, investigating mushroom extracts possessing both tyrosinase inhibitors and antioxidant compounds represents an important step in the search for versatile, safe, and effective bio-ingredients.

Varying extraction conditions such as solvent type, solvent concentration, temperature, time, solvent to solid ratio, particle size of materials, and extraction pH can influence the extraction efficiency (Chirinos et al., 2007). Correct extraction time may lead to both a time and cost-saving, and therefore is one of the crucial factors in the extraction process. Studies have shown that extending the extraction time may lead to a decreased content of certain bioactive compounds. Increased temperature enhances the solubility of solutes but some compounds, such as phenolics, may be denatured if the temperature goes beyond a certain value (Spigno et al., 2007). For example, a cold-water extract of *Grifola frondosa* ('Maitake' mushroom) exhibited better anti-hepatoma activity than a hot-water extract because of the differences in the polysaccharide and protein content between the two (Lin et al., 2016). The diversity seen in the respective biological activities is caused by the differing amounts of bioactive compounds in the extract. Reports on the cosmeceutical properties of locally cultivated *S. commune* in Malaysia are still scarce and limited work has been reported on the optimal extraction conditions for cosmeceutical-related activities. Thus, the present preliminary research aimed to evaluate the effects of extraction temperature and time on the cosmeceutical properties of *S. commune* extracts.

2. Materials and methods

2.1. Chemicals and fresh materials

Kukur mushrooms (*S. commune*) were purchased from Coconut Island Resources, Kedah, Malaysia. All solvent and chemicals used were of analytical grade. The de-ionized water used throughout the experiments was obtained from Milli-Q water purification system (Millipore, USA).

2.2. Extraction and experimental design

In this study, two sets of single factor experiments were used to determine the optimum conditions for *S. commune* extraction. Two settings of temperature, 4 °C and 30 °C were used. In each setting, the temperature was kept constant and time was varied between 1, 12, and 24 h. Comparisons were made on all data from both experimental settings.

Schizophyllum commune fruiting bodies were cleaned and lyophilised in a vacuum freeze dryer (Christ Alpha 1–4 LD Plus). Two grams of dry powder were weighed in conical flasks using an analytical balance (Mettler Toledo, B204-S) and extracted at either 4 °C or 30 °C and for 1, 12, or 24 h. Water (20 mL) was used as the extracting solvent. The mixture was then shaken in a refrigerated incubator shaker (Innova® 42R, New Brunswick USA) at the designated temperature and time. The extracts then underwent centrifugation, and later filtration using Whatman No.1 filter paper to obtain a clear solution of crude extract. The crude extract was concentrated using a freeze dryer and stored at room temperature. All extractions were carried out in triplicate. For analysis, 10 mg of crude extract was dissolved in 1 mL distilled water. Prior to analysis, the suspended extract was stored at −20 °C.

The optimal extraction setting was selected according to the values of tyrosinase inhibition and antioxidant activities.

2.3. Biological component assays

2.3.1. Total polysaccharide content

The total polysaccharide content was determined using a phenol sulphuric acid assay (Pawar and Mello, 2011). Briefly, crude mushroom extract (0.1 g) was diluted with 1 mL distilled water, followed by 1 mL of 5% (v/v) phenol solution and 5 mL of concentrated sulfuric acid. The mixtures were allowed to stand at room temperature for 10 min. The absorbance of each aliquot was recorded using a spectrophotometer (Cary WinUV, Agilent, USA) at 490 nm. Glucose was use as a reference standard for the assay.

2.3.2. Total phenolic content

Determination of total phenolic content was carried out according to a method by Okmen et al. (2009) with some modifications. An aliquot (1 mL) of each sample was added to 5 mL of Folin-Ciocalteu reagent (Merck, USA) followed by 7.5% sodium carbonate solution (4 mL) and left to react for 2 h in the dark. The absorbance at 765 nm was measured using a UV-Vis spectrophotometer (VARIAN, Cary 50). The calibration curve was plotted by using 0–200 ppm gallic acid as a standard. Results are expressed as mg/g gallic acid equivalent (GAE).

2.3.3. Total glucan, α-glucan, and β-glucan content

Evaluation of total glucan, α-glucan, and β-glucan content was performed using the Mushroom and Yeast β-glucan kit (Megazyme). The assay was conducted according to the manufacturer's protocols. Prepared mushroom extract (0.1 mL) was mixed with 0.1 mL exo-1,3-β-glucanase (20 U/mL) and ß glucosidase (4 U/mL) mixture and incubated at 40 °C in a water bath. After 60 min, 3 mL of glucose oxidase was added to each tube and incubated for 20 min. The absorbance of the aliquots was read at 510 nm for measurement of total glucan content. For α-glucan, 0.1 mL of mushroom extract was added to 0.1 mL sodium acetate buffer, mixed with 3 mL of glucose oxidase, and incubated at 40 °C for 20 min. The absorbance of the α-glucan aliquot was read at 510 nm. The β-glucan content was calculated by subtraction of α-glucan from total glucan.

2.4. Biological activity assays

2.4.1. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The assay was carried out with reference to a method by Thaipong et al. (2006). Mushroom extract (150 µL) was mixed with freshly prepared DPPH working solution (2850 µL) followed by incubation in the dark for 30 min. Absorbance was measured at 515 nm using a spectrophotometer (VARIAN, Cary 50). Ascorbic acid was used as a positive control in this assay. The following equation was used to determine the percentage of scavenging activity:

$$DPPH\ radical\ scavenging\ activity\ (\%) = [(A - B)/A]x\ 100$$

Where,

A = absorbance of blank.

B = absorbance of sample.

2.4.2. Reducing effect on ferrous ion (FRAP)

The FRAP assay was done by following a procedure as described by Benzie and Strain (1999). An aliquot (150 µL) of the sample was reacted with FRAP working solution (2850 µL) for 30 min in the dark. The absorbance at 593 nm was measured using a UV-Vis spectrophotometer. The standard curve was constructed using 0–2000 µM $FeSO_4$ solution. The ferric reducing antioxidant activity was calculated from a ferrous calibration curve and the result was expressed as mM/g ferrous equivalent (FE).

2.4.3. Scavenging effect on superoxide anion (SOA)

The SOA scavenging assay was performed using a SOD Assay Kit-WST (Sigma) and conducted according to the manufacturer's protocols in 96-well plates. An aliquot (20 µL) of mushroom extract was added to the sample and $blank_2$ wells while double-distilled water (20 µL) was added to $blank_1$ and $blank_3$ wells. WST working solution (20 µL) was added to each well followed by the addition of the enzyme working solution (20 µL) to sample and $blank_1$ wells. Then the plate was incubated for 20 min at 37 °C. The absorbance was read at 450 nm using a

microplate reader. Calculation of SOA scavenging activity was done according to the following equation:

$$SOA\ scavenging\ activity\ (\%) = \{[A\ blank1 - A\ blank3) - (A\ sample - A\ blank2)] / (A\ blank1 - A\ blank3)\}\ x\ 100$$

2.4.4. Tyrosinase inhibition activity

The anti-pigmentation potential of each mushroom extract was evaluated by measuring their tyrosinase inhibition activity. Evaluation was performed using 3,4-dihydroxyphenylalanine (L-DOPA) as the substrate (Alam et al., 2010). A mixture of 40 µL sample solution, 80 µL of 0.1 M phosphate buffer (pH 6.8) and 40 µL of mushroom tyrosinase (31 U/mL) was prepared. Sample and blank solutions, with and without enzyme, were also prepared. Ten mM L-DOPA solution (40 µL) was added to each sample and the blank. The final mixtures were allowed to react at 25 °C in the dark for 5 min. The absorbance of reaction mixtures was read at 475 nm suing a microplate reader (VERSA max, Molecular Devices) to determine the quantity of dopachrome produced. Reference standard used in this assay was 100 µg/mL kojic acid. Inhibition of tyrosinase was calculated using the following equation:

$$\%\ Tyrosinase\ inhibition = \left\{\frac{[(A - B) - (C - D)]}{A - B}\right\} x\ 100$$

Where,

A = absorbance of blank solution with enzyme.
B = absorbance of blank solution without enzyme.
C = absorbance of sample solution with enzyme.
D = absorbance of sample solution without enzyme.

2.5. Statistical analysis

Mean values and standard deviations were calculated from data obtained through triplicate experiments. One-way analysis of variance (ANOVA) was conducted using Minitab Statistical Software (version 14) to determine the significance of the experimental data. Differences between means were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of extraction temperature and time on extraction yield

Extraction is the first important step in the recovery of bioactive compounds from mushroom and plant raw materials. There are many factors that can influence the extraction efficiency, such as solvent type, solvent concentration, temperature, time, sample particle size, and solid to solvent ratio (Chirinos et al., 2007). In this preliminary study, we used a one-factor-one-time approach or single factor experiment to assess the effect of extraction temperature and time on the anti-tyrosinase and antioxidant activity of *S. commune* fruit bodies. Freeze-drying or lyophilisation was used to dry the mushroom fruit bodies to provide consistent moisture removal, and to ensure that the samples were similar before the extraction process. The extraction yields are shown in Table 1. Generally, extraction at cold temperatures resulted in a slightly higher extraction yield compared to extraction at 30 °C. At 4 °C, a marginal difference in extraction yields was observed between extraction times. Extending extraction time to 24 h significantly ($p < 0.05$) decreased the extraction yield in experiments conducted at 30 °C.

3.2. Effect of extraction temperature and time on biological component content

Phenolic compounds are a large group of biologically active metabolites present in mushroom species. Flavonoids, phenolic acids, lignans, tannins, and stilbenes are the subgroups of phenolic compounds. According to Puttaraju et al. (2006), phenolic compounds act as antioxidants, anti-inflammatory agents, and anti-tumour agents. On the other hand, many of these health-promoting properties have also been attributed to the polysaccharide content in mushrooms. Polysaccharides are divided into two categories, which are homopolysaccharides (such as β-glucan) and heteropolysaccharides. The total phenolic, total polysaccharide, total glucan, α-glucan, and β-glucan content of *S. commune* extracts are displayed in Tables 2 and 3.

Table 1
Extraction yield[1] (%) of *S. commune* fruit bodies under different extracting conditions.

Temperature (°C)	Time (h)	Yield (%)[1]
4	1	16.06 ± 1.26[ab]
	12	17.79 ± 1.21[a]
	24	16.34 ± 1.53[ab]
30	1	15.27 ± 1.82[b]
	12	14.91 ± 0.71[bc]
	24	12.94 ± 0.46[c]

[1]Each value is expressed as mean ± SD. Means in the column that do not share a superscript letter are significantly different at $p < 0.05$ by one-way ANOVA.

At a fixed 4 °C temperature setting, the total phenolic content decreased with a longer extraction time and the reverse was noted for extractions done at 30 °C. A significantly higher ($p < 0.05$) phenolic content was observed when the extraction temperature was set at 30 °C. This result was approaching the optimal temperature recorded by Yim et al. (2011) for maximal phenolic content from extraction of the same mushroom species. It was also observed that phenolic content was not significantly ($p > 0.05$) changed beyond a 12 h extraction time. This circumstance may be explained by Silva et al. (2007), who stated that according to Fick's second law of diffusion, final equilibrium between the solute in the sample and in the solution of extraction solvent will be reached at a certain time. Mild heating can soften plant tissues and weaken the cell wall and so assist the release of bound phenolic compounds (Spigno et al., 2007). Our results indicated that both temperature and time played equally significant roles in extraction of phenolic compounds from mushrooms. Previous studies have reported that prolonged extraction time would lead to a decrease in the phenolic content of extracts (Chew et al., 2011; Chirinos et al., 2007). According to Chan et al. (2009), exposure to light and oxygen resulted in oxidation of phenolic compounds, which may occur with a prolonged extraction time.

As shown in Table 2, the variety of polysaccharide content was insignificant ($p > 0.05$) between tested extraction temperature and time. At both fixed extraction temperature settings, the polysaccharide content was relatively unchanged regardless of extraction time. This result was similar to a study conducted by Yuan et al. (2017) who observed an unchanged yield of polysaccharide from the wild mushroom *Russula griseocarnosa* after a 4-h extraction time, comparable observations were

Table 2
Total phenolic content (TPC) and total polysaccharide content of *S. commune* extracts.

Temperature (°C)	Time (h)	Total phenolic content (mg GAE/g extract)	Total polysaccharide content (mg GE/g extract)
4	1	33.44 ± 2.23[c]	38.71 ± 2.74[a]
	12	31.48 ± 1.16[cd]	42.48 ± 1.71[a]
	24	30.73 ± 0.99[d]	43.40 ± 2.91[a]
30	1	37.17 ± 0.49[b]	43.48 ± 3.52[a]
	12	39.92 ± 0.36[a]	38.63 ± 3.45[a]
	24	41.07 ± 0.60[a]	40.72 ± 6.41[a]

[1]Each value is expressed as mean ± SD. Means in the column that do not share a superscript letter are significantly different at $p < 0.05$ by one-way ANOVA.

Table 3
Total glucan, α-glucan, and β-glucan content of *S. commune* extracts.

Temperature (°C)	Time (h)	Total glucan content (%)	α-glucan content (%)	β-glucan content (%)
4	1	5.74 ± 0.43^{c}	2.11 ± 0.11^{c}	3.62 ± 0.32^{c}
	12	7.92 ± 0.04^{b}	4.11 ± 0.10^{a}	3.81 ± 0.09^{c}
	24	6.22 ± 0.22^{c}	3.49 ± 0.22^{b}	2.73 ± 0.01^{d}
30	1	8.88 ± 0.62^{a}	0.91 ± 0.32^{d}	7.97 ± 0.39^{a}
	12	7.37 ± 0.07^{b}	0.78 ± 0.01^{d}	6.60 ± 0.07^{b}
	24	7.49 ± 0.06^{b}	0.78 ± 0.01^{d}	6.72 ± 0.07^{b}

[1]Each value is expressed as mean ± SD. Means in the column that do not share a superscript letter are significantly different at $p < 0.05$ by one-way ANOVA.

also reported by Zou et al. (2015). The optimum temperature for polysaccharide extraction from mushroom fruit bodies was reported to be between 90 and 100 °C (Mo et al., 2013; Zang, 2013).

In mushrooms, the content and ratio of saccharide components, for example β-glucan, are determined by factors such as genetics and cultivar (Rop et al., 2009). Glucans are polysaccharides built of monosaccharides linked by alpha and beta type glycosidic bonds, with a complex chemical structure that performs varied physiological functions (Sobieralski et al., 2012). Beta-glucans are the basic components of mushroom cell walls. Due to their many biological attributes, such as antioxidant, anti-inflammatory, anti-tumour, and anti-microbial activities, β-glucans can be use in a wide variety of industrial applications including pharmaceuticals and cosmetics (Papaspyridi et al., 2018). According to Yuan et al. (2017), a high extraction temperature is needed to sufficiently break the cell wall structure and subsequently release the polysaccharide compounds from the cells.

Generally, higher total glucan and β-glucan content was detected in *S. commune* extracted at 30 °C, whereas extraction at 4 °C produced extracts with significantly ($p < 0.05$) higher α-glucan content (Table 3). Longer extraction time did not necessarily increase the content of extractable β-glucans once equilibrium had been reached after 12 h of extraction at 30 °C. At an extraction temperature of 4 °C, total glucan, α-glucan, and β-glucan content increased from 1 h to 12 h, but then significantly ($p < 0.05$) decreased when extraction time reached 24 h. Our results imply that temperature and time, in the range set in this study, greatly affected the content of bio-active components in *S. commune* fruit body extract.

3.3. Effect of extraction temperature and time on antioxidant activities

Being the largest organ of the human body, skin cells are constantly exposed to oxidative stress from exogenous and endogenous sources (Godic et al., 2014). The production of free radicals, as a result of oxidative stress, increases with exposure to environmental factors such as UV rays, air pollutants, radiation, and smoke (Bowe, 2013). Exposed skin tissues are susceptible to biological damage that can lead to various dermatological disorders such as premature aging, coarse texture, dryness, acne vulgaris, and hyper-pigmentation. The human body produces antioxidants to neutralise free radicals, but exogenous antioxidants are essential to support the body's self-protection process (Sonam and Guleria, 2017). As described by Khlebnikov et al. (2007), antioxidants are defined as "any substances that directly scavenges reactive oxygen species (ROS) or indirectly acts to up-regulate antioxidant defences or inhibit ROS production". Antioxidants protect the skin by protecting the cell membranes from oxidative stress, scavenging reactive oxygen species, increasing collagen synthesis, and in many other ways (Pai et al., 2014). Thus extracts, or compounds with potent anti-oxidative properties, are one of the key components in effective anti-aging and whitening formulations of cosmeceuticals and skin care products. Another reason for the incorporation of antioxidant compounds or extracts in cosmeceutical formulations is to preserve and protect the formulations from auto-oxidation caused by exposure to air and chemical degradation.

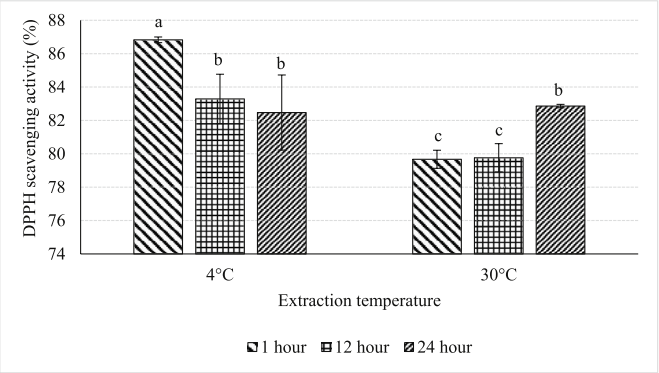

Fig. 1. DPPH radical scavenging activity of extracts from different extraction conditions.

According to Petlevski et al. (2013), it is useful to utilise several types of assays to assess the antioxidant activity of a sample due to the different mechanisms or modes of the established methods. In this study, the antioxidant activity of crude extracts produced from different extraction conditions was evaluated by using several test systems i.e. DPPH-radical scavenging, ferric-reducing, and superoxide anion inhibition assays. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable purple solution in methanol (Boonsong et al., 2016). The DPPH radical is scavenged upon reaction with antioxidant compounds, through the donation of a hydrogen atom or electron, which results in the bleaching of the DPPH methanol solution. The scavenging activity of *S. commune* extracts on DPPH radicals are displayed in Fig. 1. At 4 °C, the antioxidant activity was significantly ($p < 0.05$) decreased when extraction time was extended from 1 to 12 and, subsequently, 24 h. On the other hand, at 30 °C DPPH radical scavenging activity was slightly increased when extraction time reached 12 h, followed by substantial increment at 24 h. Extraction at 4 °C produced extracts with significantly higher ($p < 0.05$) DPPH-radical scavenging activity than at 30 °C. The results indicated that extraction temperature played a more critical role on the DPPH-radical scavenging activity of the produced *S. commune* extracts. Corresponding outcomes were observed in a study conducted by Yim et al. (2011).

Apart from radical scavenging activity, the antioxidant activities of certain mushroom extracts have been related to their reducing potential. The reducing potential of the *S. commune* extracts were evaluated using the FRAP assay. The results (Fig. 2) showed that a shorter extraction time was found to favour the extraction of compounds that contribute to the ferric-reducing activity of *S. commune* extracts. Extraction at 4 °C produced extracts with no significant difference in ferric reducing antioxidant activity between the extraction time settings. This

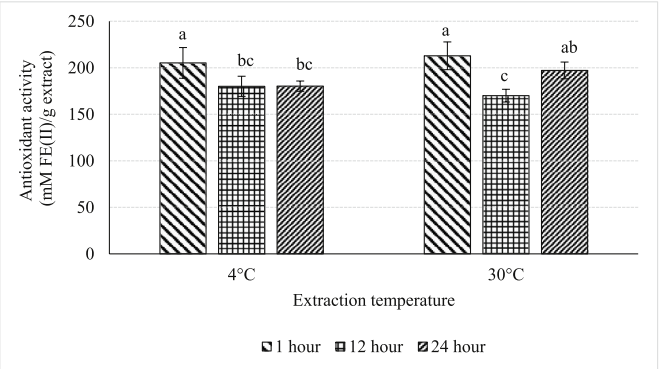

Fig. 2. Ferric-reducing antioxidant activity of extracts from different extraction conditions.

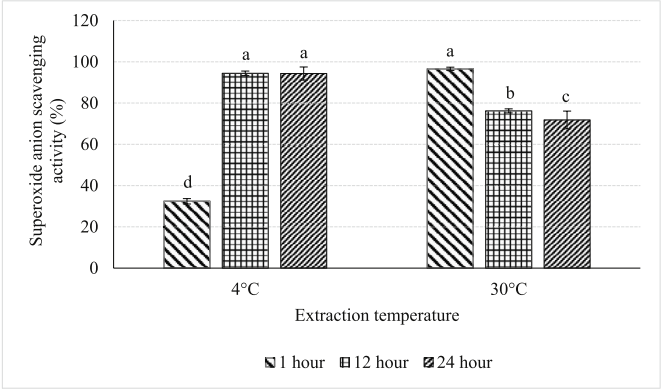

Fig. 3. Superoxide anion scavenging activity of extracts from different extraction conditions.

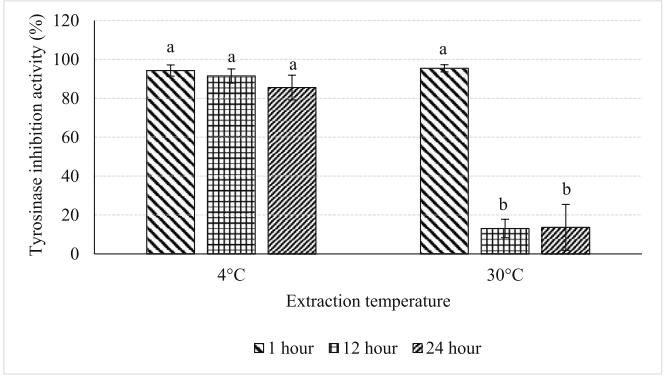

Fig. 4. Tyrosinase inhibition activity of extracts from different extraction conditions.

heavily implies that time had a greater role than temperature within the range settings conducted in this study (see Fig. 3).

Superoxide anion (SOA) is a reactive oxygen species (ROS). Oxidative stress resulted from the imbalance between production and removal of ROS (Yeh et al., 2005). Thus, it is beneficial to find extracts or bioactive compounds that can scavenge SOA and, subsequently, stop the oxidative stress from occurring. At 30 °C, a shorter extraction time was observed to favour the production of *S. commune* extract with a higher SOA scavenging activity. At a 30 °C extraction temperature, the SOA scavenging activity was significantly ($p < 0.05$) decreased from 1 h to 12 h, and 24 h. On the contrary, SOA scavenging activity was considerably increased from 1 h to 12 h, but plateaued beyond 12 h up to 24 h when the extraction was conducted at 4 °C. Based on these results, it can be suggested that both extraction temperature and time notably affected the SOA scavenging activity of *S. commune* extracts.

Positive and negative correlations (data not shown) were found between antioxidant activities (DPPH, FRAP and SOA) of *S. commune* extracts with total phenolic, total polysaccharide and β-glucan content, respectively. However, the findings were statistically insignificant ($p > 0.05$, data not shown). This circumstance can be explained by the fact that the antioxidant activity of an extract is mostly due to the additive and synergistic effect between various kinds of compounds from different classes or groups, present in the extract. In order to obtain conclusive and significant correlations between biological activities and bioactive compounds content, larger experiment size should be conducted to acquire larger sets of data.

The antioxidant activity of mushroom extracts can be attributed to many types of compounds present in the mushroom fruit bodies. As reported by Zou et al. (2015), one of the most important functional components in mushrooms are polysaccharides. Previous studies have revealed that mushroom polysaccharide-protein complexes show important biological activities, such as anti-tumour, immunomodulatory, as well as antioxidant properties (Liu et al., 1997). Phenolic compounds, such as phenolic acids and flavonoids, are known to be potent antioxidants due to the scavenging ability of their hydroxyl groups (Devi et al., 2014). The quantitative, as well as qualitative, characteristics of the phenolic content (Aljadi and Kamaruddin, 2004), as well as other compounds, play a significant role in the variations of antioxidant activity of the tested extracts.

3.4. Effect of extraction temperature and time on tyrosinase inhibition activity

Tyrosinase (phenol oxidase) is known to be a key enzyme for melanin biosynthesis (Momtaz et al., 2008). Overproduction of melanin may lead to hyperpigmentation, which can cause the skin to appear darker. The use of tyrosinase inhibitors is becoming essential in skin-whitening agents and in other skincare and cosmeceutical products. Many antioxidant compounds, such as polyphenols, may also function as tyrosinase inhibitors (Chang, 2009). As depicted in Fig. 4, at an extraction temperature of 30 °C, extraction time contributed substantially to the tyrosinase inhibition activity of the crude extracts. On the contrary, at 4 °C, extraction time exhibited only a minor effect on the tyrosinase inhibition activity of extracts. These results indicate that extraction temperature and time played an equally significant role on the tyrosinase inhibition activity of *S. commune* extracts. At a 30 °C extraction temperature, the decrease of tyrosinase inhibition ability with the increase of extraction time observed in the study may be due to the degradation of anti-tyrosinase compounds during a longer extraction time. As explained by Silva et al. (2007), thermo-sensitive antioxidant compounds, particularly flavonoids, may be degraded with a longer extraction time and higher temperature. It is widely claimed that compounds with antioxidant activity show anti-aging, anti-inflammatory, and anti-pigmentation/whitening activities (Choi et al., 2008). Antioxidant activity may be one of the tyrosinase inhibition mechanisms, as deduced by Yoon et al. (2011). Reactive oxygen species (ROS) formed from exposure to UV rays enhances melanin biosynthesis. Therefore, ROS scavengers or inhibitors may reduce pigmentation or darkening of the skin by inhibiting tyrosinase.

Tyrosinase inhibition activity of *S. commune* extracted at 30 °C were correlated with its total phenolic, total polysaccharide and β-glucan content (with $r = 0.96$, $r = 0.91$ and $r = 0.99$, respectively, data not shown). However, the correlations were statistically insignificant ($p > 0.05$, data not shown). This proves that, similar to antioxidants, tyrosinase inhibition activity in an extract can be mostly attributed to the synergism between compounds present in the extract (Kim and Uyama, 2005). Many studies have reported anti-tyrosinase activity from mushroom extracts, for example, methanol, hot water and acetone extracts (Huang et al., 2014; Yoon et al., 2011). Research on the relationship of mushroom polysaccharide with tyrosinase inhibition activity is scarce. On the other hand, phenolic compounds such as gallic, *p*-coumaric and kojic acid are some of the compounds exists in mushrooms that have been reported to exert potent tyrosinase inhibition activity (Taofiq et al., 2016).

4. Conclusions

The strong antioxidant and anti-tyrosinase effects of *S. commune* extracts produced in this study may bring various benefits to health and wellness, and have the potential to be applied in cosmeceuticals and skin care products. Such products could help to treat and prevent premature aging, as well as pigmentation disorders. However, based on the results of our study, it is difficult to determine the best extraction conditions to obtain *S. commune* extracts with the highest antioxidant activities, due to the varying results from the different antioxidant assays conducted in the study. Regardless of the extraction time,

extraction at 4 °C resulted in high anti-tyrosinase activity, but the result was comparable to the extract obtained at 30 °C for 1 h. Therefore, a possible approach for further study would be to optimise the extraction process using statistical software, which would include factorial and response surface methodologies, with the desired cosmeceutical-related property, such as tyrosinase inhibition activity, as the targeted response.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101116.

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