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Comparative Evaluation of *Schizophyllum commune* Extracts as Potential Cosmeceutical Bio-Ingredient

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Abstract **– An increasing number of mushroom species have recently been exploited for their potential application in the cosmeceuticals industry. However, the use of mushroom as an ingredient in cosmeceutical products is still limited. Therefore, more research is needed to fully explore the maximum potential of mushroom extracts for skin health. *Schizophyllum commune* is a cultivated edible mushroom known to contain biologically active compounds beneficial to skin health. In this present study, *S. commune* extracts were tested and compared for their functional activities. Biological components, such as the phenolic, polysaccharide and β-glucan contents were evaluated. Cosmeceutical-related functionalities were determined, by assessing the antioxidant and anti-pigmentation activities. The results signified that the extracts of *S. commune* fruiting bodies have the potential to be developed as bio-ingredient in cosmeceutical or skincare products. However, further in depth studies should be undertaken to confirm the capability of mushroom extracts as cosmeceutical agents, such as the optimization of the extraction method and *in vitro* assays.**

Keywords **– Cosmeceutical, Extraction, Mushroom, *S. Commune.***

I. Introduction

Protecting and preserving the skin by using cosmeceuticals has risen drastically over the past years. Cosmeceuticals are products demonstrating medical-like benefits for the improvement of skin's function, structure and appearance. Natural or organic ingredients for cosmeceutical and skin care products are on the rise, due to their strong protective and defensive roles against oxidative damage caused by free radicals, which may lead to premature aging and hyperpigmentation. There is an increasing number of commercially available cosmeceutical and skin care products containing mushroom extracts or their bioactive compounds. Among the most popular mushrooms used in skin care products are Shiitake (*Lentinula edodes*), Reishi (*Ganoderma lucidum*) and Cordyceps (*Cordyceps sinensis*). Therefore, it is of great interest to explore other mushroom species to contribute in cosmeceutical industry as potential active ingredients.

Schizophyllum commune or 'split gill' mushroom, is popular mostly due to schizophyllan, a polysaccharide that possesses multifunctional biological properties, such as anti-tumor, immunomodulator, anti-inflammatory, as well as antimicrobial properties [1] – [2]. The present research was aim to evaluate and compare the cosmeceutical potential between different types of *S. commune* extracts, through the assessment of antioxidant and anti-pigmentation activities, as well as the biological components, such as the total phenolic content (TPC) and polysaccharide content.

II. Materials and Methods

A. *Material and Preparation of Extracts*

Schizophyllum commune fruiting bodies were cleaned and lyophilized in a vacuum freeze dryer. Powdered samples (10 g) were extracted with 200 mL of methanol, ethanol, 70% ethanol and 70% methanol, respectively, at room temperature for 24 h, by using a shaker. The aqueous suspensions were centrifuged and filtered. The supernatant was concentrated using a rotary evaporator, to obtain the crude extracts. Ten mg of crude extract was dissolved in 1 mL distilled water and, then, stored at -20°C for further analyses.

B. *Biological Component Assays*

Total Polysaccharide Content

The total polysaccharide content was determined, according to the phenol-sulfuric acid assay [3]. A 0.1-g aliquot of the crude mushroom extract was diluted with 1 mL distilled water in a test tube, 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.

Total Phenolic Content

Determination of total phenolic content was carried out according to the Folin-Ciocalteu method. An aliquot (1 mL) of each sample was added to the Folin-Ciocalteu reagent (5 mL) followed by 7.5% sodium carbonate solution (4 mL) and stand to react for 2 h in the dark. The absorbance at 765 nm was measured using a spectrophotometer.

Total Glucan and ß-Glucan Content

Evaluation of total glucan and ß-glucan contents was performed by using the Mushroom and Yeast ß-glucan kit (Megazyme). Prepared mushroom extract (0.1 mL) was mixed with 0.1 mL exo-1, 3-ß-glucanase (20 U/mL) and ß glucosidase (4 U/mL) mixture and incubated in a 40°C water bath. After for 60 min, 3 mL of glucose oxidase was added to each tube, followed by incubation 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, and then mixed with 3 mL of glucose oxidase, followed by incubation at 40°C for 20 min. The absorbance of the α-glucan aliquot was read at 510 nm. The ß glucan content was calculated by subtraction of total glucan and α-glucan.

C. *Biological Activity Assays*

2, 2- Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Mushroom extract (150 µL) was mixed with the freshly prepared DPPH working solution (2850 µL) followed by incubation in the dark for 30 min. The following equation was used to determine the percentage of scavenging activity:

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

Where,

A = Absorbance of blank.

B = Absorbance of sample.

Ferric Reducing Antioxidant Power (FRAP) Activity

The FRAP assay was done as described elsewhere [4]. 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 spectrophotometer. Ferrous sulfate was used as the reference standard.

Superoxide Anion (SOA) Scavenging Activity

The SOA scavenging assay was performed using a SOD Assay Kit-WST (Sigma) and a 96-well plate. 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. Twenty µL of WST working solution was added to each well followed by the addition of 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:

$$\%\ SOA\ scavenging\ activity = \left\{\frac{[(A\text{-}B)\text{-}(C\text{-}D)]}{A\text{-}B}\right\} x\ 100$$

Where,

A = Absorbance of blank 1 (solution with enzyme).

B = Absorbance of blank 3 (solution without enzyme).

C = Absorbance of sample solution with enzyme.

D = Absorbance of blank 2 (sample without enzyme).

Anti-Pigmentation 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 as previously described [5]. 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 to determine the quantity of dopachrome produced. Reference standard used in this assay was kojic acid. Inhibition of tyrosinase was calculated using the following equation:

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

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.

D. *Statistical Analysis*

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

III. Results and Discussions

A. *Biological Components of Mushroom Extracts*

Extraction yield

In this study, four different types of extraction solvents were selected to determine and compare their capability to extract compounds that contribute to the cosmeceutical-related activities of *S. commune*. Freeze-drying or lyophilization was used to dry the mushroom fruit bodies, thereby, providing a consistent moisture removal, to ensure that the samples were in a similar form before the extraction process. Besides water, solvent extraction is one of the most frequently used techniques for extraction of bioactive compounds. However, no single solvent is capable of optimally extracting all bioactive compounds from all samples. Biological activities extensively differed among mushroom species because of the vast variety of compounds with distinctive solubility in the extraction solvents used [6]. The extraction yields are displayed in Table 1.

Table 1. Extraction yield[1] (%) of *S. commune* fruit bodies using various solvents

Extract			
Methanol	70% Methanol	Ethanol	70% Ethanol
16.24 ± 0.96^{b}	20.72 ± 1.05^{a}	4.87 ± 0.45^{c}	22.02 ± 1.11^{a}

[1]Each value is expressed as mean ± SD. Values with the same superscript letter are not significantly different at $p<0.05$.

The mixture of organic solvent and water gave a higher extraction yield compared to the organic solvent alone. The result is consistent with a previous report [7], which stated that a mixture of organic solvent and water significantly increases the extraction yield.

TPC, total Polysaccharide, total Glucan and B-Glucan Content

Phenolic compounds are a large group of biologically active metabolites present in mushroom species. Besides their antioxidant activities, phenolics exhibit anti-inflammatory, anti-bacterial and anti-hyperglycemic properties, among others [8]. The TPC, total

polysaccharide, total glucan and β-glucan are shown in Table 2 and 3. The results indicated that 70% ethanol was the best solvent for extraction of phenolics from *S. commune* mushroom, due to the significantly ($p<0.05$) higher phenolics content than the other tested extracts.

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

Extract	TPC[1] (mg GAE/g extract)	Total polysaccharide content[1] (mg GE/g extract)
Methanol	8.95 ± 0.46[b]	76.14 ± 1.87[a]
70% Methanol	8.10 ± 0.25[b]	38.60 ± 0.01[b]
Ethanol	6.95 ± 0.36[c]	39.87 ± 2.85[b]
70% Ethanol	9.49 ± 0.38[a]	75.68 ± 9.07[a]

[1]Each value is expressed as mean±SD. Values in the column with the same superscript letter are not significantly different at $p<0.05$.

Conversely, the 70% methanol extract of *S. commune* showed a higher total polysaccharide content among the extracts. However, the difference between the methanol and 70% ethanol extracts was not significant ($p>0.05$). The descending order of total glucan content was methanol > 70% methanol > ethanol > 70% ethanol. A similar trend was observed for the β-glucan content, which ranged from 6.08−11.02%.

Table 3. Total glucan and β-glucan contents of *S. commune* extracts

Extract	Total glucan content (% w/w)	β-Glucan content (% w/w)
Methanol	11.85	11.02
70% Methanol	10.01	9.34
Ethanol	8.57	7.93
70% Ethanol	7.66	6.08

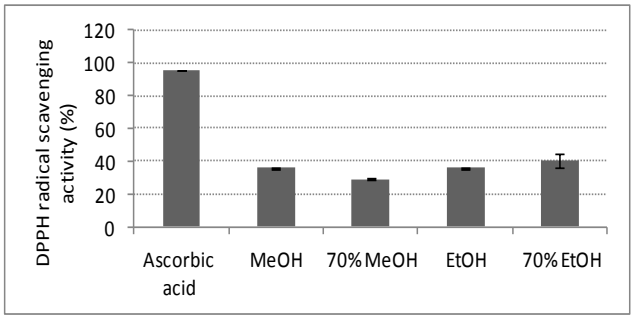
[1]Each value is expressed as mean±SD. Values in the column with the same superscript letter are not significantly different at $p<0.05$.

The differences in response among the various mushroom extracts were due to the broad variation in the physicochemical properties of the polysaccharides, such as sugar composition, molar weights, and structures. Previous study [9] indicated that the effectiveness of the solvent in extracting bioactive compounds might depend on the moisture content and particle size of the mushroom species studied. Some solvents are not effective in isolating certain compounds from large particle sizes because of the solvent inability to permeate the tissue within a short extraction time and at low temperature [10].

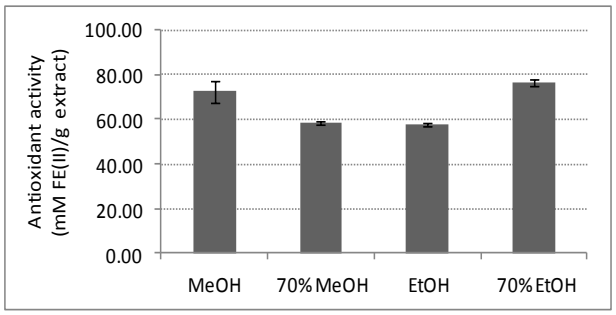
B. *Antioxidant Activity*

Skin is the largest target of oxidative stress caused by free radicals from the exposure to ultraviolet (UV) radiation. Exposed tissues are susceptible to biological damage caused by the formation of reactive oxygen species, which lead to premature aging and over-production of melanin in the skin. Therefore, extracts or compounds with antioxidative property, are crucial in cosmeceuticals and skin care formulations. In this study, methanol, ethanol and their 70% aqueous solutions, respectively, were chosen for the extraction of *S. commune* because the antioxidant activities of extracts may be affected by extraction solvent, test system used and many other factors [11]. Based on the results (Figure 1), the aqueous ethanol extract of *S. commune* tended to show stronger antioxidant capabilities than the other extracts. However, no single extract could be regarded as superior in antioxidative capability because the various extracts displayed distinct antioxidant mechanisms according to the antioxidant assays conducted in this study.

(A)



(B)



(C)

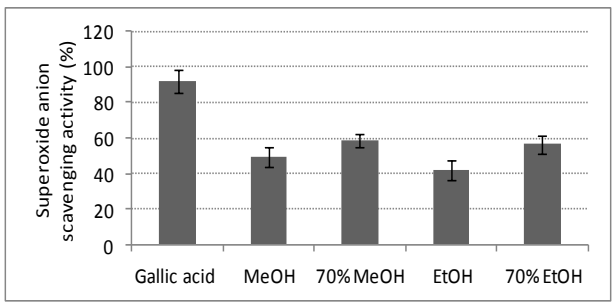


Fig. 1. Antioxidant activities of mushroom extracts as measured by different antioxidant assays; (A) DPPH radical scavenging activity, (B) ferric reducing antioxidant activity, and (C) superoxide anion scavenging activity.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable purple solution in methanol [12]. Upon reaction with antioxidant molecules, the DPPH radical is scavenged through the donation of hydrogen atom or electron, resulted in the bleaching of the DPPH methanol solution. As shown in Fig. 1, the 70% ethanol extract of *S. commune* demonstrated moderate DPPH radical scavenging activity, with the value of 40.36%, while the lowest was 29.17%, exhibited by the 70% methanol extract. 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 was evaluated using the FRAP assay. The results (Fig. 1B) showed that similarly to DPPH, the aqueous ethanol extracts exhibited the strongest reducing ability, among the extracts, with 76.61 ± 1.79 mM Fe(II)/g crude extract.

The methanol extract of *S. commune* showed equally strong activity, with 72.64 ± 5.01 mM Fe(II)/g crude extract. The results suggested that some components within these two extracts were strong reducing agents. The SOA generates oxidative stress, induced by the production of hydroxyl radicals and singlet oxygen [13]. Thus, it is beneficial to find extracts or bioactive compounds that can scavenge SOA and, subsequently, stop the oxidative stress from occurring. The *S. commune* extracts displayed moderate SOA scavenging activity of between 40 to 58% (Fig. 1C). Furthermore, the aqueous solvent extracts exhibited a higher activity than the absolute solvent extracts.

There are many types of compounds present in the mushrooms that contribute to the antioxidant activity of mushroom extracts. Polysaccharides, such as β-glucans, are considered to be one of the most important functional components in mushrooms [14]. It has been previously stated [15] that mushroom polysaccharide-protein complexes show important biological activities, such as anti-tumor, immunomodulatory, as well as antioxidant properties. Phenolic compounds, such as phenolic acids, are known to be powerful antioxidant due to the scavenging ability of their hydroxyl groups [16]. However, as previously explained [17], the quantitative and qualitative nature of the phenolic content might also play a significant role in the variations of antioxidant activity of the tested extracts. According to a research [18], antioxidant compounds perform by means of mixed mechanisms that combine different types of antioxidation. Also, the antioxidant activity of an extract is mostly due to the additive and synergistic effect between various kinds of compounds present in the extract.

C. *Anti-pigmentation Activity*

Tyrosinase (phenol oxidase) is known to be a key enzyme for melanin biosynthesis [19]. Overproduction of melanin may lead to hyperpigmentation, which can cause the skin to appear darker. Therefore, the use of tyrosinase inhibitors is becoming critically essential as skin-whitening agents in the skincare and cosmeceutical products.

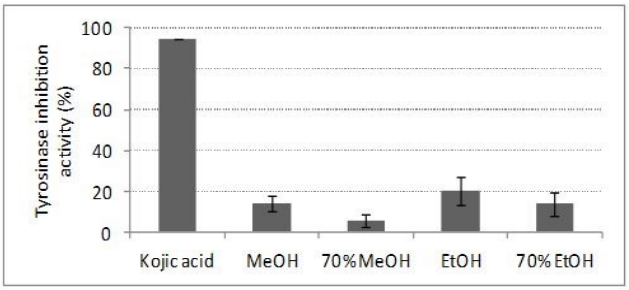


Fig. 2. Tyrosinase inhibition activities of *S. commune* extracts

As depicted in Fig. 2, at 10 mg/mL, all extracts exhibited tyrosinase inhibition activity in the range of 5.56 to 20.37%. These values were relatively low compared to the positive control used in this study, which was kojic acid, at 100 μg/mL. The ethanol extract exhibited the highest inhibition, but it was not significantly different (p>0.05) than the aqueous ethanol extract. There are limited reports on anti-tyrosinase activity of mushroom extract. Alam et al. [5] has reported anti-tyrosinase activity of 11.36 to 59.56% from the methanolic extract of *Pleurotus ostreatus*. Anti-tyrosinase activity in mushroom extracts can attributed to the many types of bioactive compounds such as phenolic compounds, peptides, sterols as well as glucans.

IV. Conclusions

Our results suggest that *S. commune* is a potential source of antioxidants and anti-pigmentation agents and could be used as a natural bio-ingredient in cosmeceutical products. Further investigations of the optimum extraction procedure are required to isolate the elements of the mushroom that show a broad spectrum of cosmeceutical-related activities. An *in vitro* study, using related skin cells should also be undertaken, to fully understand the mechanisms of the anti-pigmentation activity of mushroom extracts.

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Ms. Abd. Razak is one of the executive committee members (co-opted) of The Malaysian Society for Microbiology (MSM) since 2014. She is an active member of organizing committee for MSM's symposia and congresses around Malaysia.