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Bioassay-guided fractionation of mushroom (*Schizophyllum commune*) extract using vacuum column chromatography

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Abstract

This study was carried out to evaluate the tyrosinase inhibition activity, which represents anti-pigmentation/browning property and bioactive compounds of sub-fractions of *Schizophyllum commune* extracts. In this study, two types of crude extracts, namely K4C and K30C, were subjected to chromatographic fractionation in vacuum column chromatography packed with silica gel. The columns were stepwise eluted with different ratios of solvent acetone: methanol (A:M) to obtain several fractions. Anti-pigmentation activity was measured by tyrosinase inhibition assay. The fraction with the highest activity for K4C extract was 7A:3M (30.46±3.22%) while fraction 100M (34.32±2.14%) was the highest for K30C extract. Biological components such as organic acids, amino acids, as well as phenolic acids were also assessed by ultra and high-performance liquid chromatography (UPLC and HPLC). The results revealed that the amounts of bioactive compounds were varied; the major organic acids detected were citric acid (313.142±1.069 mg/L), tartaric acid (37.656±8.807 mg/L) and L-malic (23.748±2.565 mg/L) in the fraction K4C-7A:3M compared to fraction K30C-100M. The same fraction showed the highest amount of amino acids while phenolic acids were detected in low amounts in K4C -7A:3M and K30C-100M fractions. The fraction with the highest biological activity has the potential to be used as a bio-ingredient in food applications and nutricosmetic product formulations. The potential product derived from this project may diversify mushroom-based downstream products available in Malaysia, and subsequently further expand our mushroom industry.

1. Introduction

Mushroom is one of the important commodities under the National Agro-Food Policy in Malaysia. Our local mushroom industry is lacking downstream processing and products based on locally-cultivated mushrooms. Therefore, in an effort to improve the current state of Malaysia's mushroom industry, solutions involving major changes or innovations should be implemented immediately. One of the many solutions to this problem is to create new products based on local mushrooms. Research towards developing mushroom-based bio-ingredients and other products should be undertaken in order to fully utilize the potential of these mushrooms. Currently, local mushroom downstream products are focused on producing food products such as burger patties and sausages only.

Mushrooms possess many health benefits that not only contribute to the food industry but can also be utilized by the wellness industry. With the current increase in awareness, consumers' preference is now shifted to food containing natural bioactive ingredients from natural resources without unnecessary synthetic ingredients. Substances contributing to the multi-activities can be explored and exploited in many natural raw materials, especially those of plant origin (Abd. Razak *et al.,* 2016). Mushrooms have great potential to be used as functional and effective ingredients in food/ nutricosmetic products due to their many bioactive metabolites. Bioactive metabolites in mushrooms including polysaccharides, phenolic, flavonoids, terpenes, alkaloids and many more can be isolated from mushroom fruiting bodies (Mehmet and Sevda, 2009). These vast arrays of bioactive compounds contribute to the many nutraceutical-related functionalities such as anti-oxidant, anti-ageing, anti-inflammatory and anti-pigmentation. It is estimated that 50% of annual 5 million metric tons of cultivated mushrooms contain

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functional or medicinal properties, which may be utilized as a source of biologically and physiologically active substances (Lavanya and Subhashini, 2013).

Schizophyllum commune (Cendawan Kukur) is a cultivated edible mushroom commonly known to contain compounds that possess potent biological properties beneficial to health. Research on biological activities and development of locally-cultivated *S. commune* as functional food or nutricosmetic products is scarce. South-East Asian countries have been known to have rich sources of medicinal mushrooms. Some medicinal mushrooms are widely used for therapeutic properties and some of them have not been thoroughly studied yet (Wasser, 2002). Our previous research has found two types of water extracts from *S. commune* with high anti-pigmentation and antioxidant properties namely K4C and K30C. From the results of our preliminary *in-vitro* study using human skin cells, these two optimized extracts were proven to positively inhibit melanin production in melanoma skin cells.

Based on our findings, it is important to continue conducting further experiments in order to identify potential bioactive compounds and to better understand the nutricosmetic functions of these extracts, especially as tyrosinase inhibitors, before utilizing the extracts/ fractions as bio-ingredient in food product formulations. Nutricosmetics are products and ingredients that act as nutritional supplements in the beauty industry such as skincare, nails, and hair natural beauty. These products contain bioactive ingredients such as metabolites, minerals, dairy products, and phytonutrients and they work from the inside to promote beauty from within. Currently, this is the latest trend in the market and some product components such as collagen, peptides, proteins, vitamins, carotenes, minerals, and omega-3 fatty acids are reported. Today, consumers are cautious with the selection of food products that they introduce into their bodies, and there is also an increasing demand for natural products able to enhance one's health and beauty without harmful side effects and significant traction before use (Dini and Laneri, 2019).

Tyrosinase (copper-containing enzyme), is the primary rate-limiting enzyme responsible for the conversion of substrate tyrosine into melanin by melanocytes (Garcia-Borron and Solano, 2002). This situation can occur in food and human skin. Melanin is the major pigment responsible for skin colour. Human skin is constantly exposed to UV radiation that influences both skin function and structure. Body overexposure to UV radiation causes the overproduction of melanin in the skin, due to the enhanced activity of the tyrosinase enzyme (Taofiq, Gonzalez-Paramas, Martin, e*t al*., 2016). The most common approach to discovery of the skin anti-pigmentation is by determination of tyrosinase inhibitory pproperties due to the involvement of tyrosinase as the key enzyme in melanogenesis (Park *et al*., 2015).

Tyrosinase causes enzymatic browning in raw fruits, vegetables, and beverages. Browning is an undesirable reaction that is responsible for a less attractive appearance and loss of nutritional quality. This problem has encouraged researchers to seek new potent tyrosinase inhibitors from natural and synthetic origins with desirable efficacy and safety, and for improving overall food quality (Loizzo *et al*., 2012). Extracts or compounds that possess multiple functionalities are highly valuable as they can address several issues present in food products simultaneously, such as browning and nutrition loss.

Bioassay-guided fractionation is the best approach to fractionate the crude extracts with subsequent profiling of bioactive compounds. Bioassay-guided fractionation integrates the processes of separation of compounds in a mixture, using various analytical methods, with results obtained from biological testing. The process begins with testing an extract to confirm its activity, followed by crude separation of the compounds in the matrix and testing the crude fractions. Further fractionation is carried out on the fractions that are found to be active at a certain concentration threshold, whereas the inactive fractions are set aside or discarded (Jamil *et al*., 2012).

The aim of this research was to evaluate the anti-pigmentation activity and bioactive compounds of sub-fractions from *S. commune* extracts. The present study adopted bioassay-guided fractionation to fractionate the crude water extracts of *S. commune*, followed by the determination of the best fraction with the highest anti-pigmentation activity as well as the profiling of bioactive compounds. The concentrations of metabolites were analyzed using ultra and high-performance liquid chromatography (UPLC and HPLC).

2. Materials and methods

2.1 Mobile phase and column preparation

Thin-layer chromatography (TLC) was performed to determine the mobile phase separation of crude water extracts. TLC was performed on aluminium sheets pre-coated with silica gel, and crude water extracts were spotted using a capillary tube (2-5 µL) and allowed the plates to dry. The plate was developed in 5 solvent mixtures, hexane: ethyl acetate (H:E), hexane: acetone (H:A), ethyl acetate: acetone (E:A), acetone: methanol (A:M) and methanol: water (M:W) as the solvent system. The developed plates were dried then sprayed with H_2SO_4 and visualized under a UV illuminator. A small

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swab of cotton wool was placed at the bottom of the column. Dry Li Chroprep RP-18 silica gel was then dissolved in acetone. The gel slurry was swirled to remove any fine particles before being poured into the column. The stopcock was then opened to allow the gel to be evenly packed. The column was then washed with acetone once. Both columns were prepared with the same procedure. The silica gel packed would occupy three-quarters of the column length.

2.2 Sample preparation

The crude water extracts of *S. commune* were dissolved in a small amount of distilled water followed by acetone. Dry Li Chroprep RP-18 silica powder was then added to the sample. The sample solutions were then left to dry completely in the laminar flow hood. The silica gel coated with crude extracts (dry packed) was subjected to chromatographic fractionation in a vacuum column prepared previously.

2.3 Fractionation of crude water extracts of Schizophyllum commune

The dried packed samples were inserted into the column containing approximately 1 mL of acetone above the packed gel. The sample was swirled around with the remaining acetone to remove trapped air bubbles and ensure even packing. Next, 600 mL of acetone was added gently into the column without disturbing the surface of the packed column. The stopcock was then opened and the eluted sample was collected in a 250 mL conical flask with an appropriate label. The solvent was continuously topped up until only 1 cm was left above the surface of the column. The column was stepwise-eluted with different concentrations of solvent, acetone: methanol with ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) and this continued until the last solvent, 100% methanol to obtain several fractions. Each fraction thus obtained, was evaporated to dryness by a rotary evaporator (Buchi, Canada). The fractions were subjected to an anti-pigmentation assay to determine the most active fraction(s).

2.4 Tyrosinase inhibition activity

Tyrosinase inhibition assay was performed to determine the anti-pigmentation or anti-browning effect of the sample by using the dopachrome method with L-3,4-dihydroxyphenylalanine (L- DOPA) as the substrate according to the method by Alam *et al*. (2010) with minor modifications. A mixture of the sample (40 µL), 0.1 M phosphate buffer pH 6.8 (80 µL) and 31 U/mL mushroom tyrosinase (40 µL) was prepared in a 96-well plate. Then, the mixture was incubated at 25°C in the dark for 5 mins. Then, 40 µL of 10 mM L-DOPA solution was added to the mixture and incubated again for 10 mins. Next, the absorbance was measured at 475 nm using the microplate reader (Versamax, Molecular Devices). Each sample was accompanied by a blank containing all components except L-DOPA. The reference inhibitor used in this assay was kojic acid at the concentration of 100 µg/mL. Tyrosinase inhibition activity was calculated using the following equation:

$$\% \text{ inhibition} = \{[(A - B) - (C - D)] / (A - B)\} \times 100$$

Where A = absorbance of blank solution with enzyme; B = absorbance of blank solution without enzyme; C = absorbance of sample solution with enzyme and D = absorbance of sample solution without enzyme

2.5 Determination of bioactive compounds in active fraction(s) using UPLC and HPLC

The highly active partitioned fractions of both crude extracts were analyzed by UPLC and HPLC to identify and quantify the composition of bioactive compounds. Active fractions (5 mg) were dissolved in 5 mL deionized water and filtered with 0.22 µm pore size nylon membrane. Then, samples were injected for the determination of amino acid, organic acid and phenolic acid composition with some modifications.

2.5.1 Organic acid composition

High-performance liquid chromatography (HPLC) Alliance Separation Module (Waters 2695) equipped with a photodiode array detector (Waters 2996) was used to quantify lactic acid. Approximately 10 µL of the sample were injected and separated using Synergi™ 4 µm Hydro-RP 80 A column (250 × 4.6 mm) with a flow rate of 0.6 mL/min and column temperature of 30°C. The mobile phase consisted of mobile phase A (KH_2PO_4) and mobile phase B (water) with a gradient elution program as follows: 100% A maintained from 0.00 to 30 min; followed by a linear gradient of A from 100 to 0% at 30 to 31 mins; 0% of A remained from 31-45 mins before proceeding to a linear gradient of A from 0 to 100% at 45 to 46 mins and remained constant in flow rate of 100% A at 46-55 mins. The absorbance was read at the wavelength of 190 nm. Known quantities of individual standard compounds were used to construct the calibration curves to determine the number of organic acids in the samples.

2.5.2 Amino acid composition

Amino acid content was analyzed using UPLC according to a method done previously with modifications (Danial *et al*., 2015). Briefly, 10 µL of the sample was derivatized using 70 µL of AccQ-Tag™ Ultra and dissolved together with borate buffer and 20 µL of AccQ-Tag™ Fluor agent. The mixture was heated

at 55°C for 10 mins. 1 µL of the mixture was injected into the UPLC system. An AccQ-Tag™ Ultra column (2.1 mm × 100 mm, 1.7 µm) was used with a flow rate of 0.7 mL/min and a column temperature of 55°C. The reading was done using wavelength at 260 nm. The elution solvent system was comprised of (A) ACN:FA: Ammonium (10:6:84) and (B) ACN:FA (98:2). Gradient elution was conducted as follows: 0 to 0.54 min, maintained at 99.9% A; 0.54 to 5.74 min, linear gradient from 99.9 to 90.9% A; 5.74 to 7.74 mins, linear gradient from 90.9 to 78.8% A; 7.74 to 8.50 mins, linear gradient from 78.8 to 40.4% A and then held for 0.3 min at 40.4% A; 8.80 to 8.90 mins, linear gradient from 40.4 to 99.9% A and then maintained at 99.9% for another 2.1 mins. Quantification was made using calibration curves obtained by injecting known amounts of pure compounds as external standards.

2.5.3 Phenolic acid composition

Free phenolic acids in samples were determined using HPLC Alliance Separation Module (Waters 2695) according to the method by Robbins and Bean, 2004 with some modifications. A 10 µL aliquot of sample solution was separated using a reverse-phase analytical column (150 mm × 4.6 mm XBridge C18, 3.5 µm, Waters) with the temperature controlled at 25°C. The mobile phase consisted of 0.1% formic acid (A) and methanol (B) with the flow rate set at 0.7 mL/min. Peak identification was made by comparing retention times and UV spectra at 280 nm and 325 nm with authentic compounds. Quantification was made using calibration curves obtained by injecting known amounts of pure compounds as external standards. All analyses were done in triplicates.

2.6 Statistical analysis

Mean values and standard deviations were calculated from the data obtained from triplicate experiments. One-way analysis of variance (ANOVA) was conducted with Tukey's post hoc test at a significance level of $p<0.05$ using Minitab (version 19) Statistical Software. All data were presented as mean±standard deviation (SD).

3. Results and discussion

3.1 Bioassay-guided fractionation of crude water K4C and K30C extracts

Initial screenings of potential locally-cultivated mushrooms as a source of food/nutricosmetics bio-ingredient with different crude extraction methods were conducted. This resulted in two types of the most potential crude water extracts from *S. commune*. The results of our preliminary study showed that—these potential extracts possess strong anti-pigmentation activity, as measured by the capability of these extracts to inhibit the tyrosinase enzyme, which is involved in the production of melanin. The same extracts also exhibited high antioxidant activity, as measured by their ability to scavenge DPPH radicals and superoxide anion, as well as reducing ferric (FRAP). The potential *S. commune* extracts were subjected to optimization of the extraction procedure and resulted in two optimized extracts namely K4C and K30C (data not shown due to intellectual property protection). From the results of our *in-vitro* study using human skin cells, the optimized crude extracts were proven to positively inhibit melanin production in melanoma skin cells.

In order to determine the active fraction, bioassay-guided fractionation and subsequent profiling of bioactive compounds were commenced (Figure 1). Bioassay-guided fractionation is a protocol to sub-fraction or isolates a pure chemical agent from its natural origin, step-by-step separation of extracted components based on differences in their bioassay-guided fractionation physicochemical properties, and assessing the biological activity, followed by the next round of separation and assaying. Typically, such work is initiated after a given crude extract is deemed active in a particular *in vitro* assay.

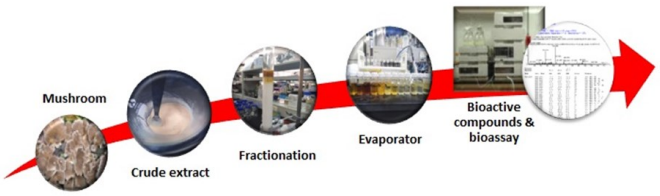

Figure 1. Process bioassay-guided fractionation of *Schizophyllum commune*

Before bioassay-guided fractionation is performed, the selection of solvent for the compound separation process should be identified using thin-layer chromatography (TLC). TLC results indicated that the mixture of acetone: methanol (A:M) gave the best mobile phase separation and was selected for bioassay-guided fractionation. TLC is one of the simplest, fastest, easiest and least expensive of several chromatographic techniques. The crude water extracts of *S. commune* were then partitioned on silica gel Li Chroprep RP-18 by vacuum column chromatography using a gradient elution; acetone and methanol as mentioned previously. A total of eleven fractions were obtained from each crude extract.

3.2 Tyrosinase inhibition activity of fractions K4C and K30C

Tyrosinase (phenol oxidase) is known to be a key enzyme involved in the formation of melanin in our skin

– a process called melanogenesis as well as the enzymatic browning of food such as fruits and vegetables. Hyperpigmentation disorders are characterized by darker skin appearance, light to dark brown spots, irregular grey patches on the face, neck and trunk, and pale brown to dark brown spots on the skin. Several factors such as exposure to UV radiation and the release of αmelanocyte-stimulating hormone trigger the over-secretion of melanin from melanocytes that causes hyperpigmentation (Ali *et al*., 2015).

Loizzo *et al*. (2012) reported that appearance is one of the attributes that are considered by consumers when choosing a food product. Among them, colour is a critical determinant for the appearance of fruits, vegetables and crustaceans. Browning usually impairs the colour attribute together with sensory properties such as flavour and texture (softening).

Tyrosinase inhibitor compounds or extracts/fractions are often sought as an anti-pigmentation and anti-browning agent in food, medicine and nutricosmetics industries. Nutricosmetics is a dietary and nutritional supplement product intended to produce a visual appearance benefit and improve the health of the skin (Luque de Castro, 2011). Haruta-Ono *et al*. (2012) was reported that oral ingestion of dietary products improves the water-holding capacity of the skin and the barrier function.

In order to prevent pigmentation and browning, tyrosinase inhibitors from natural sources such as plants, mushrooms, rhizomes or marine algae can be used not only for food applications but also as skin anti-pigmentation agents. Kojic acid, among other well-known tyrosinase inhibitors such as hydroquinone and azelaic acid, has been reported and tested as a cosmetic and pharmaceutical ingredient to be used to prevent melanin overproduction in epidermal layers of the skin (Miyazawa *et al*., 2003). Previous studies also reported the enhancement of tyrosinase inhibition activity of samples through fungal fermentation (Jamaluddin *et al*., 2014; Wu *et al*., 2018).

Tyrosinase inhibition activity of crude water extract and fractions of *S. commune* were summarized in Table 1. All crude water extracts and fractions (10 mg/mL) exhibited tyrosinase inhibition activity in the range of 3.78 – 34.32%. These values were relatively low compared to the positive control, which is kojic acid (97.16±0.61%) and crude water extracts, K4C (98.27±1.49%) and K30C (95.11±0.49%). The percentage of tyrosinase inhibition of all fractions was significantly (p<0.05) lower than in crude water extracts. The fraction K4C-7A:3M (30.46±3.22%) and K30C-100M (34.32±2.14%) exhibited the highest inhibition, but it was not significantly different (p>0.05) than the other fractions. The inhibition of these enzymes by a sample is contributed by many types of compounds in the extracts and the synergism that exists between the compounds as well as their respective concentration (Caesar and Cech, 2019). Therefore, it can be speculated that both crude extracts from *S. commune* contained a mixture of compounds that functioned better at inhibiting tyrosinase activity, compared to their respective fractions.

Table 1. Anti-tyrosinase activity of crude extract and fractions of K4C and K30C

Fractions	Extract K4C (%)	Extract K30C (%)
Crude	98.27±1.49^{a}	95.11±0.49^{a}
100A	N.D.	N.D.
9A:1M	4.05±0.58^{e}	N.D.
8A:2M	8.39±4.74^{e}	13.75±2.15^{de}
7A:3M	30.46±3.22^{b}	13.40±1.03^{de}
6A:4M	30.12±8.11^{b}	5.84±3.62^{f}
5A:5M	21.43±3.22^{bc}	7.90±3.62^{ef}
4A:6M	9.40±1.16^{de}	24.06±3.62^{c}
3A:7M	30.12±3.22^{b}	N.D.
2A:8M	19.09±1.53^{cd}	3.78±1.19^{f}
1A:9M	7.72±2.01^{e}	19.59±1.03^{cd}
100M	25.41±3.03^{bc}	34.32±2.14^{b}
Kojic acid	97.16±0.61^{a}	

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (P<0.05). N.D.: Not detected.

Although numerous investigations have been carried out on the biological activities of water extracts from other mushroom species, research regarding the nutricosmetic potential of mushrooms, especially *S. commune* - such as tyrosinase inhibition activity is still limited (Taofiq, Gonzalez-Paramas, Martin, e*t al*., 2016). Nannapaneni *et al*. (2016) reported that extracts from *S. commune* recorded the minimum tyrosinase inhibitory activity when eluted with all three solvents methanol (24.65%), acetone (19.22%), and water (11.57%). Previous studies reported that ethanolic extracts from *Pleurotus ostreatus* and *Lentinula edodes* showed high anti-tyrosinase activity with the value of 0.86±0.07 mg/mL and 0.86±0.07 mg/mL, respectively (Taofiq, Heleno, Calhelha *et al*., 2016). While Alam *et al*. (2010) have reported anti-tyrosinase activity of 11.36 to 59.56% from the methanolic extract of *P. ostreatus*. Meng *et al*. (2011) reported tyrosinase inhibition potential and inhibition of melanin production from the fruiting bodies of *Pleurotus citrinopileatus* extracts including n-hexane, ethyl acetate, and n-butanol soluble extracts showed up to 28.8%, 27.4%, and 41.0% inhibitory activity, respectively. Based on the present study, crude extracts and sub-fractions of K4C-7A:3M and K30C:100M show

promising anti-tyrosinase activity and can be used as potential bio-ingredients in the development of functional food and nutricosmetic product.

3.3 Phytochemicals profiling of active fractions K4C-7A:3M and K30C-100M

There are many types of compounds present in mushrooms that contribute to the various activities of mushroom extracts. In the previous study, it was found that K4C and K30C extracts contain a mixture of bioactive compounds such as amino acids, phenolic acids as well as organic acids. However, the main compound or a group of compounds that contributes to the anti-pigmentation activity of the two extracts is unknown. In this study, we identified the biochemical profile of sub-fractions with the highest anti-pigmentation activity. Ultra and high-performance liquid chromatography (UPLC and HPLC) with photodiode array (PDA) were used to analyze the concentration of bioactive compounds.

As shown in Table 2, the contents of bioactive compounds (organic acid, amino acid and phenolic acid) varied for both active fractions. Several types of organic acids were detected, such as citric acid, tartaric acid, L-malic and oxalic acid. Fraction K4C-7A:3M contained a significantly higher (p<0.05) concentration of organic acids compared to fraction K30C-100M. Citric acid content was found to be the highest in K4C-7A:3M fraction with the value 313.142±1.069 mg/L, followed by tartaric acid (37.656±8.807 mg/L) and L-malic (23.748±2.565 mg/L). While in fraction K30C-100M only L-malic (18.422±1.458) was detected.

The amino acid analysis results also showed the presence of higher amino acid content in the same fraction, K4C-7A:3M. The amino acids in descending order were alanine (2.341±0.002 mg/L), glutamic (2.183±0.030 mg/L), leucine (2.113±0.010 mg/L), methionine (1.774±0.010 mg/L) and threonine (0.607±0.013 mg/L). On the contrary, in fraction K30C-100M, only alanine was detected in a low amount (0.048±0.024 mg/L). As shown in Table 2, phenolic acids were detected in a low amount for both fractions. The results of UPLC analysis on fraction K4C-7A:3M showed the detection of vanillic acid (0.418±0.026 mg/L) and cinnamic acid (0.255±0.091 mg/L) while in fraction K30C-100M, only cinnamic acid (0.282±0.028 mg/L) was detected. Both fractions showed no presence of gallic acid and benzoic acid. Determination of bioactive compounds' content as nutraceutical agents in the potential active fractions is important in order to provide information on the compounds contributing to the fractions' nutraceutical functions.

Table 2. Biochemical profiling of K4C-7A:3M and K30C-100M fractions

Compounds	Fraction K4C-7A:3M (mg/L)	Fraction K30C-100M (mg/L)
Organic acid		
Tartaric	37.656±8.807^{b}	N.D.
Citric	313.142±1.069^{a}	N.D.
Oxalic	1.458±0.023^{c}	N.D.
L-malic	23.748±2.565^{b}	18.422±1.458^{a}
Kojic	N.D.	N.D.
Amino acid		
Alanine	2.341±0.002^{a}	0.048±0.024^{a}
Threonine	0.607±0.013^{e}	N.D.
Glutamic	2.183±0.030d^{b}	N.D.
Methione	1.774±0.010^{d}	N.D.
Leucine	2.113±0.010^{c}	N.D.
Phenolic acid		
Gallic	N.D.	N.D.
Vanillic	0.418±0.026^{a}	N.D.
Benzoic	N.D.	N.D.
Cinnamic	0.255±0.091^{b}	0.282±0.028^{a}

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (P<0.05). N.D.: Not detected.

These findings were contrary to a report of ethanolic extract of *S. commune* by Devi *et al.* (2014), where phenol was present in the highest amount, 12.5±1.4 µg / mg of the sample. Flavonoid (1.78±0.36 µg /mg) and ascorbic acid (2.18 + 0.12 µg /mg) were present in moderate amounts whereas β carotene (0.0136±0.001 µg/mg) and lycopene (0.011±0.007 µg/mg) were present in trace amounts. Based on these findings, the tyrosinase inhibition activity of our samples can be inferred to be dependent more on the profile of bioactive compounds present in the crude water extracts than the total concentration of the sub-fractions. However, further investigation on the flavonoid compositions should be undertaken to prove this hypothesis. It can also be speculated that these results were the outcome of synergistic and additive effects between different tyrosinase inhibitors present in our samples. Wang *et al*. (2018) have demonstrated synergistic and additive effects of tyrosinase inhibition activity between different antioxidant compounds. The concentrations of compounds in samples are also a determining factor for tyrosinase inhibition activities.

Specifically, reactions of amines, amino acids, peptides, and proteins with reducing sugars and vitamin C (nonenzymatic browning, often called Maillard reaction browning), and quinones (enzymatic browning) cause deterioration of food during storage and commercial or domestic processing. The loss of nutritional quality is attributed to the destruction of essential amino acids and a decrease in digestibility and inhibition of proteolytic and glycolytic enzymes. The

production of anti-nutritional and toxic compounds may further reduce the nutritional value and possibly the safety of foods (Mauron, 1990). All these aforementioned medicinal properties of mushroom extracts and related bioactive constituents, reinforce the interest in these natural matrices for cosmetic applications.

4. Conclusion

Two active fractions from each of *S. commune* crude extracts were identified in our study. Although the anti-pigmentation activity of these fractions was lower than the crude extract, the content of biological compounds detected in these fractions indicates the potential for these fractions to be further developed as promising ingredients in nutraceutical products. However, both crude extracts can be recommended for the application in nutricosmetics industry. We believe that the information gathered from this study will eventually lead to the much -needed improvement of our local mushroom as well as the nutraceutical industries. More choices of scientifically proven nutraceutical products will be available to local consumers and more employment opportunities can be created within both industries.

Conflict of interest

The authors declare no conflict of interest.

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