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Research Paper

Phenolic profiles of selected edible wild mushrooms as affected by extraction solvent, time and temperature

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Abstract

Four species of edible wild mushrooms and one cultivated mushroom were selected to investigate how extraction solvent (methanol, ethanol, acetone, water and hexane) affected the yield of phenolic substances, which were comprised of total phenolics (TP), total flavonoids (TF) and condensed tannins (CT). Results showed that water extracts exhibited the highest TP, followed by TF and CT of all edible wild and cultivated mushrooms. TP was chosen as an indicator in response to extraction time (60, 150, 240, 330 and 420 min) and temperature (25, 30, 40, 50 and 60˚C) with water as extraction solvent. Contents of TP with optimal extraction time and temperature were (in descending order): *Pleurotus* sp. (1046.87 mg GAE/100g; 330 min at 40˚C) > *Lentinus ciliatus* (801.08 mg GAE/100g; 150 min at 50˚C) > *Pleurotus ostreatus* (cultivated) (798.55 mg GAE/100g; 330 min at 50˚C) > *Hygrocybe conica* (442.37 mg GAE/100g; 240 min at 60˚C) > *Schizophyllum commune* (427.31 mg GAE/100g; 240 min at 25˚C). Extraction time and temperature with water as extraction solvent were found to have a critical role in extracting total phenolic in edible wild mushrooms.

Keywords: *Pleurotus* sp., *Lentinus ciliatus*, *Schizophyllum commune*, *Hygrocybe conica, Pleurotus ostreatus,* total phenolic, total flavonoid, condensed tannin content, Malaysia

Introduction

Mushrooms with their delicate flavour and texture are recognised as a nutritious food and an important source of biologically active compounds with medicinal values. Generally, mushrooms are low in energy and high in dietary fibre [1], and an excellent source for antioxidants as they accumulate a variety of secondary metabolites, including phenolic compounds [2, 3]. Antioxidants are substances needed in minute quantity which are capable of counteracting with free radicals to prevent oxidative damage. There are about 1200 species of mushroom used in 85 different countries for their gastronomic value and/or medicinal properties [4].

Edible wild mushrooms are often regarded as being nutritionally high and with potential economic value. Many species with medicinal value are widely used in traditional medicine for a broad range of diseases [4]. Some species are regarded as therapeutic food for their anti-carcinogenic, anti-cholesterolaemic and anti-viral properties [1]. Oyster mushrooms (*Pleurotus ostreatus*), shiitake (*Lentinus edodes*), Jew's ear fungus also known as monkey's ear fungus (*Auricularia* sp.) and paddy straw mushroom (*Volvariella volvacea*) have been utilized for a long time in Malaysia by the Malay, Chinese and Indians for their beneficial effects in addition to being a source of food [5].

Sabah, which is located in East Malaysia, is well-known for its diversified natural resources, in particular forestry biodiversity. These rich forest resources give rise to various untapped biological compounds, which include edible wild mushrooms that could be of benefit to human health and as food for the local people. A recent study by Chye *et al*. [6], reported that some wild edible mushrooms found in Sabah were of high nutritional value and exhibited high antioxidant activity.

Extraction conditions are the major concern to enhance the efficiency in order to obtain highest yields of antioxidative compounds from natural resources [7]. These conditions include extraction method, particle size, solvent type, solvent concentration, solvent-to-solid ratio, extraction temperature, extraction time and pH [7, 8, 9]. There is still limited data concerning extraction conditions on phenolic profiles of Sabah wild edible mushrooms. Hence, in this present study, three extraction conditions, which include extraction solvent, time and temperature, were selected to determine their effects on phenolic profiles of the selected edible wild mushrooms.

Materials and Methods

Chemicals and reagents

All the chemicals and reagents were of analytical grade. Ethanol and hexane were purchased from Fisher Scientific (UK), while methanol and acetone were obtained from Merck (Germany). Gallic acid, sodium carbonate anhydrous, aluminium chloride 6-hydrate and vanillin were purchased from Fisher Scientific (UK). Folin-Ciocalteu's phenol reagent, hydrochloric acid and sodium hydroxide were from Merck (Germany). (+)-catechin hydrate and sodium nitrite were purchased from Sigma-Aldrich (St. Louis, MO). Water used was of Millipore quality.

Sample preparation

Four species of edible wild mushrooms comprising *Pleurotus* sp., *Hygrocybe conica, Schizophyllum commune* and *Lentinus ciliatus* were purchased from indigenous people who collect from the forest and sell at *"tamu"* (local market) in Kota Kinabalu, Sabah, Malaysia. For comparison purposes, a commercially cultivated edible mushroom (*Pleurotus ostreatus*; oyster mushroom) was purchased from a local market in Kuala Lumpur. The samples were washed, air dried followed by drying in an oven at 45°C for 24 h. The dried samples were ground to powder using a miller (MF 10 basic; IKA®

Werke, Germany) with 0.5 mm mesh size and vacuum packaged into a nylon-linear low density polyethylene film by using a vacuum packaging machine (DZQ 400/500) prior to analysis.

Experimental design

The current study involved screening for phenolic profiles of edible wild and cultivated mushrooms using single factor experiment to determine the optimal extraction conditions. The extraction conditions involved solvent type, time and temperature by varying one independent variable at a time, while other variables were kept constant.

Solvent type

Five grams of powdered mushrooms were extracted with 50 ml of different solvents (60% (v/v) methanol, 60% (v/v) ethanol, 60% (v/v) acetone, water and absolute hexane) at a ratio of 1:10 (w/v). The mixture was shaken by a shaker (Vision) at constant speed of 150 rpm for 180 min at ambient temperature (25˚C). The mixture was filtered through a Whatman filter paper No. 1. The filtrate was directly used for analysis of total phenolic (TP), total flavonoid (TF) and condensed tannin (CT) contents. The optimal solvent type was determined based on the highest value of either TP, TF or CT.

Extraction time

The optimal solvent type was determined and the samples were extracted with various extraction time (60, 150, 240, 330 and 420 min) at ambient temperature (25˚C). The optimal extraction time was determined based on the highest value of either TP, TF or CT.

Extraction temperature

After determining the optimal solvent type, concentration and extraction time, the samples were extracted with various extraction temperature (25, 30, 40, 50 and 60˚C). The optimal extraction temperature was determined based on the highest value of either TP, TF or CT.

Total phenolic (TP) analysis

The Folin-Ciocalteu method was used for TP analysis following the method of Barros *et al*. [10] and Zhao and Hall [11], with slight modification. A 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu's solution. After 3 minutes, 1 ml of 7.5% sodium carbonate solution was added to the mixture and adjusted to 10 ml with deionized water. The mixture was allowed to stand at room temperature in a dark environment for 90 min. Absorbance was measured against the blank reagent at 725 nm using (XTD 5, Secomam, UK). Gallic acid was used for the calibration curve with a concentration range of 50–1000 µg/ml ($R^2 = 0.99$) and analyzed as above. Results were expressed as mg gallic acid equivalent (GAE)/100g DW. All experiments were performed in triplicate.

Determination of total flavonoid (TF)

TF was determined by a colourimetric method as described in Xu and Chang [12] and Yoo *et al*. [13], with slight modification. Briefly, 0.25 ml of sample was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, then 0.5 ml of 1M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink colour was measured at 510 nm against the blank (XTD 5, Secomam, UK). (+)-catechin was used for the calibration curve with a concentration range of 10–1000 µg/ml (R^2 = 0.99) and analyzed as above. Results were expressed as mg (+)-catechin equivalent (CE)/100g DW. All experiments were carried out in triplicate.

Determination of condensed tannin (CT)

CT content in mushroom crude extracts was determined using a colourimetric method as described in Chavan *et al*. [14] and Xu and Chang [12], with slight modification. A 3 ml of 4% methanol vanillin solution was added to 50 µl sample. Then, 1.5 ml of concentrated HCl was added to the mixture. After mixing well, the mixture was allowed to stand for 15 min at room temperature. Absorbance was measured against the blank reagent at 500 nm (XTD 5, Secomam, UK). The blank was a 5 ml of 4% concentrated HCl in methanol. (+)-catechin was used for the calibration curve and a concentration range of 50–1000 µg/ml (R^2 = 0.99) was prepared and analyzed as above. Results were expressed as mg (+)-catechin equivalent (CE)/100g DW. All experiments were conducted in triplicate.

Statistical analysis

All analyses were performed in triplicate. The data were analyzed by MINITAB version 14 and results were expressed as means ± standard deviations. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons were carried out to test any significant differences among the means. Significant levels were defined using $p<0.05$.

Results and Discussion

Yield of edible wild mushrooms

The yield of four wild edible mushrooms species including *Lentinus ciliatus, S. commune, H. conica, Pleurotus* sp. and cultivated *P. ostreatus* is presented in Table 1. *S. commune* contributed to the highest yield, amounting to 28.13%, followed by *L. ciliatus, Pleurotus* sp. and *P. ostreatus* (24.65%, 14.78% and 10.32%, respectively). Whilst *H conica* has the lowest yield of 9.69%, it is believed that it contains more water soluble substances [15].

Table 1. Yield (%) from edible wild and cultivated mushrooms.

Species	Weight of sample (g)		Yield[a] (%)
	Before drying	After drying	
L. ciliatus	950.55	234.31	24.60
S. commune	620.62	174.58	28.10
H. conica	1380.80	133.80	9.70
Pleurotus sp.	962.92	142.32	14.80
P. ostreatus (cultivated)	1232.45	127.15	10.30

[a](%)Yield = Weight $_{\text{after drying}}$/ Weight $_{\text{before drying}}$ x 100%

Effects of solvent types on phenolic profile

In this present study, five types of solvents namely methanol, ethanol, acetone, water and hexane were used to determine the best extraction yield of phenolic profiles. Alcoholic solvents are commonly used for phenolic extraction, as they give the highest yield of total extract although they are not highly selective for phenols [9]. Higher yield of total phenols are obtained by using polar solvents, for instance, water, methanol and ethanol [7, 16].

Table 2 shows that water contributed to the highest yield of TP, TF and CT under the same extraction conditions (180 min, and 25°C); no values were detected in hexane extraction. Amongst all the species, *Pleurotus* sp. contained the highest TP followed by *P. ostreatus*, *L. ciliatus, S. commune* and *H. conica*. TF appears to be the lowest yield and was found in descending order of: *Pleurotus* sp. > *H. conica* > *S. commune* > *P. ostreatus* > *L. ciliatus*. CT ranks as the second highest yield after TP and *Pleurotus* sp. produced the highest yield followed by *P. ostreatus*, *H. conica*, *L. ciliatus* and *S. commune*.

Table 2. Effect of solvent types on contents of total phenolic, total flavonoid, and condensed tannin of five mushroom species.

Results are expressed as means ± SD. TP: total phenolic; TF: total flavonoid; CT: condensed tannin; ND: not detected.
[a-e]Different superscripts within the columns denote significantly different (p<0.05).

	TP (mg GAE/100g)	**TF (mg CE/100g)**	**CT (mg CE/100g)**
L. ciliatus			
Methanol (60%, v/v)	388.62 ± 2.97^{c}	20.44 ± 1.64^{c}	34.33 ± 3.03^{d}
Ethanol (60%, v/v)	401.58 ± 3.91^{b}	32.67 ± 1.80^{b}	39.33 ± 2.58^{c}
Acetone (60%, v/v)	324.14 ± 5.22^{d}	15.95 ± 1.32^{d}	51.00 ± 3.87^{b}
Water	641.65 ± 11.73^{a}	37.71 ± 0.48^{a}	118.92 ± 5.39^{a}
Hexane	ND	ND	ND
S. commune			
Methanol (60%, v/v)	170.66 ± 6.18^{bc}	19.06 ± 1.10^{c}	28.08 ± 2.46^{c}
Ethanol (60%, v/v)	175.71 ± 3.82^{b}	21.69 ± 1.11^{b}	28.50 ± 2.74^{c}
Acetone (60%, v/v)	163.50 ± 5.48^{c}	19.19 ± 0.34^{c}	34.75 ± 2.62^{b}
Water	461.65 ± 40.50^{a}	73.73 ± 1.84^{a}	115.58 ± 6.00^{a}
Hexane	ND	ND	ND
H. conica			
Methanol (60%, v/v)	215.94 ± 2.42^{c}	36.79 ± 0.92^{c}	25.17 ± 1.44^{d}
Ethanol (60%, v/v)	226.97 ± 2.47^{b}	42.67 ± 2.55^{bc}	46.83 ± 3.82^{c}
Acetone (60%, v/v)	206.60 ± 2.67^{d}	47.48 ± 1.12^{b}	73.50 ± 5.00^{b}
Water	311.23 ± 1.29^{a}	87.30 ± 1.97^{a}	130.17 ± 3.82^{a}
Hexane	ND	ND	ND
Pleurotus sp.			
Methanol (60%, v/v)	603.10 ± 6.72^{b}	75.68 ± 2.80^{b}	107.67 ± 5.16^{d}
Ethanol (60%, v/v)	578.18 ± 4.33^{c}	72.21 ± 4.97^{b}	113.92 ± 2.92^{c}
Acetone (60%, v/v)	457.14 ± 12.82^{d}	57.11 ± 4.09^{c}	126.83 ± 7.85^{b}
Water	901.75 ± 2.27^{a}	184.80 ± 1.47^{a}	224.75 ± 6.47^{a}
Hexane	ND	ND	ND
P. ostreatus (cultivated)			
Methanol (60%, v/v)	506.30 ± 5.76^{c}	10.35 ± 0.49^{c}	32.67 ± 2.58^{c}
Ethanol (60%, v/v)	549.06 ± 4.58^{b}	15.40 ± 0.77^{b}	52.25 ± 2.09^{b}
Acetone (60%, v/v)	493.50 ± 12.48^{c}	5.35 ± 0.42^{d}	55.58 ± 5.34^{b}
Water	723.30 ± 4.12^{a}	43.22 ± 0.93^{a}	144.33 ± 13.57^{a}
Hexane	ND	ND	ND

Solvent concentration is one of the major investigated factors in the extraction of antioxidative compounds. A mixture of solvents, in particular aqueous alcohol, are preferred to using a mono-component solvent system, as they are more effective at extracting antioxidative compounds [9]. In this study, although water extraction produced highest yield of phenolic profile, different concentrations (20-100%) of alcoholic solvents (either methanol or ethanol which ranked second highest after water depending on species) on TP as indicator was carried out for comparison purposes.

Table 3 shows the TP of all solvents with 20% (v/v) concentration contributed to the highest yields and were significantly higher ($p<0.05$) when compared to other concentrations. A comparison on TP between water extract and 20% (v/v) solvent concentration was performed, results indicating that water gives a significantly higher value ($p<0.05$) compared to 20% methanol (for *Pleurotus* sp.) and 20% ethanol (for *L. ciliatus, S. commune, H. conica*, and *P. ostreatus*) (Table 4). The results indicated that phenolic compounds from edible wild and cultivated mushrooms might present a moderately to polar profile.

Table 3. Total phenolics in different solvent concentrations of five mushroom species.

Species	TP (mg GAE/100g, DW)				
	20%, v/v	40%, v/v	60%, v/v	80%, v/v	100%, v/v
L. ciliatus	592.83 ± 11.23^a	491.31 ± 2.78^b	410.84 ± 3.30^c	339.29 ± 2.69^d	144.01 ± 2.82^e
S. commune	302.64 ± 5.70^a	236.99 ± 1.82^b	168.38 ± 0.85^c	94.31 ± 1.30^d	19.31 ± 0.93^e
H. conica	298.77 ± 1.03^a	291.03 ± 1.79^b	244.14 ± 1.50^c	159.12 ± 1.52^d	81.01 ± 1.60^e
Pleurotus sp.	870.94 ± 1.74^a	779.70 ± 0.85^b	652.09 ± 3.46^c	480.88 ± 2.61^d	216.90 ± 3.24^e
P. ostreatus (cultivated)	652.76 ± 2.96^a	604.95 ± 1.89^b	545.52 ± 3.92^c	374.81 ± 3.54^d	175.32 ± 4.21^e

Results are expressed as means ± SD. TP: total phenolic; TF: total flavonoid; CT: condensed tannin.
$^{a-e}$Different superscripts within each species denote significantly different ($p<0.05$).

Table 4. Comparison of water and 20% concentration of solvent on total phenolics of five mushroom species.

Species	Water	Solvent 20% (v/v)
L. ciliatus	641.65 ± 11.73*	592.83 ± 11.23
S. commune	461.65 ± 40.50*	302.64 ± 5.70
H. conica	311.23 ± 1.29*	298.77 ± 1.03
Pleurotus sp.	901.75 ± 2.27*	870.94 ± 1.74
P. ostreatus (cultivated)	723.30 ± 4.12*	625.76 ± 2.96

Results are expressed as means ± SD. TP: total phenolic; TF: total flavonoid; CT: condensed tannin.
Ethanol was used as extraction solvent for all species except *P.* sp. (methanol).
*For each species, water extract denotes significantly higher ($p<0.05$).

Effect of extraction time on total phenolics

The effect of extraction time (60-420 min) with water as extracting solvent at 25ºC on TP is shown in Figure 1. *Pleurotus* sp. gave highest TP yield followed by (in descending order) *P. ostreatus* (oyster mushroom) > *L. ciliatus* > *S. commune* > *H. conica*. For *P.* sp., TP at 330 min (971.78 ± 2.36 mg GAE/100g DW) was significantly higher ($p<0.05$) than other extraction time; *P. ostreatus* was also found highest at 330 min (760.34 ± 1.77 mg GAE/100g DW) followed by 420 min (756.63 ± 2.27 mg GAE/100g DW), with no significant difference observed. TP extracted at these two times was significantly higher ($p<0.05$) when compared to 60, 150 and 240 min. Thus, extraction time of 330 min was considered optimal for both *Pleurotus* sp. and *P. ostreatus*. For *L. ciliatus*, comparable TP values were observed at 60, 150 and 420 min, and all were significantly higher than 240 and 330 min (p<0.05). Hence, 150 min with highest TP was deemed to be the optimal extraction time for *L. ciliatus*. For both *S. commune* and *H. conica*, two highest points at 240 min and 420 min were found with no significant difference (p>0.05). Thus, 240 min was chosen as the optimal extraction time for both species, with highest TP yield and economical concern.

The extraction time for phenolic compounds varies from a few minutes to 24 hours, e.g. one day is used to extract phenolic compounds from red currant marc [17], and not more than 3 hours are employed to extract the dried sage, *Salvia officinalis* [18]. Spigno *et al.* [9] explained that in order to be cost effective in the manufacturing sector, one of the determinants in cost reduction is to optimize the extraction time.

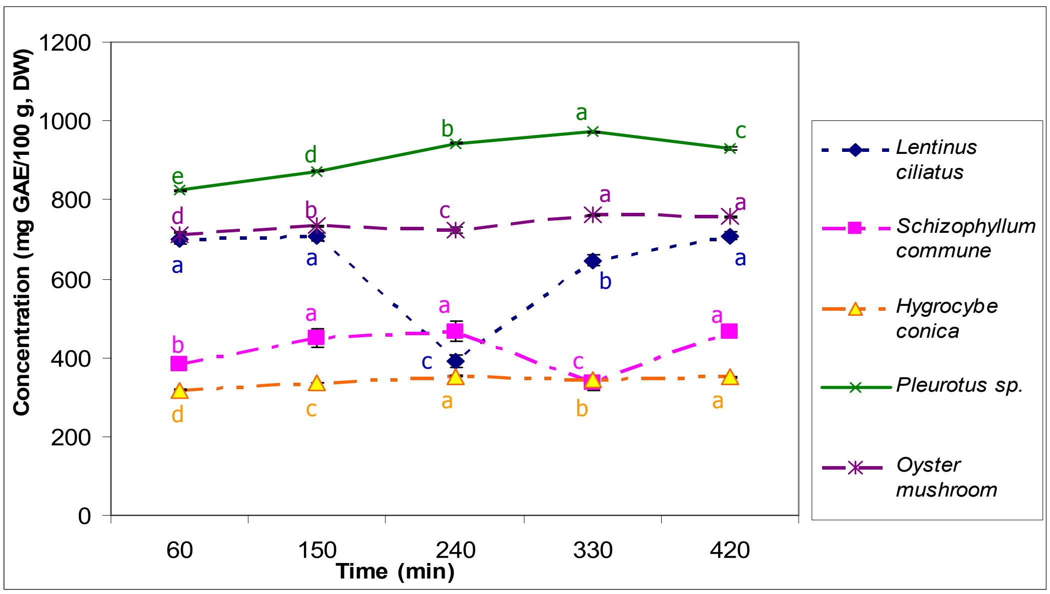

Figure 1. The total phenolic contents of wild edible and cultivated mushrooms extracted using different extraction times.
Different letters within each species denote significantly different (p<0.05).

Effect of extraction temperature on total phenolics
The effect of extraction temperature on TP is shown in Figure 2. A similar trend was observed as per extraction time, where *Pleurotus* sp. gave highest TP yield followed by (in descending order) *P. ostreatus* (oyster mushroom) > *L. ciliatus* > *S. commune* > *H. conica*. As for *Pleurotus* sp. with 330 min extraction time, TP at 40˚C (1046.03 ± 1.49 mg GAE/100g DW) was significantly higher (p<0.05) than other extraction temperatures. For both *L. ciliatus* and *P. ostreatus* (with extraction time of 150 min and 330 min, respectively), highest TP was found at 50˚C (801.08 ± 7.30 and 798.55 ± 4.76 mg GAE/100g DW, respectively) and was significantly higher (p<0.05) than other temperatures. There were no significant differences in TP among 25, 30, 40 and 50˚C in *S. commune* extracted at 240 min, where highest yield (427.31 ± 8.69 mg GAE/100g DW) was observed at room temperature (25˚C). Elevated TP with increased temperature was observed in *H. conica* (extraction time, 240 min) with highest TP at 60˚C and was significantly higher when compared with other temperatures (p<0.05); 65˚C was then used to verify whether more yield of TP was liberated above 60˚C. However, TP at 65˚C

showed a slight decrease (data not shown in Figure 2), thus 60˚C was chosen as the optimal extraction temperature for *H. conica*.

Extraction temperature is one of the major concerns as it would impact on the yield of extraction [19], and increased temperature promotes the solubility of solute as well as the diffusion coefficient [7, 9]. However, an increased temperature may denature certain bioactive compounds in particular phenolic compounds [9]. As supported by Juntachote *et al*. [20], extraction yield of lemon grass decreased with an increase in temperature. However, different samples may involve different processing steps that may affect the overall extraction yields according to Kim *et al*. [21]. The effect of temperature cannot be generalised as it strongly depends on the type of compounds with different characteristics [9]. Similar trends have been reported by Herodez *et al*. [22], where the yield of extracted carnosic acid increases with a temperature increase from 0 to 20˚C, but decreases thereafter. From the present study, the optimal temperature for all species was not more than 50˚C, except for *H. conica* at 60˚C.

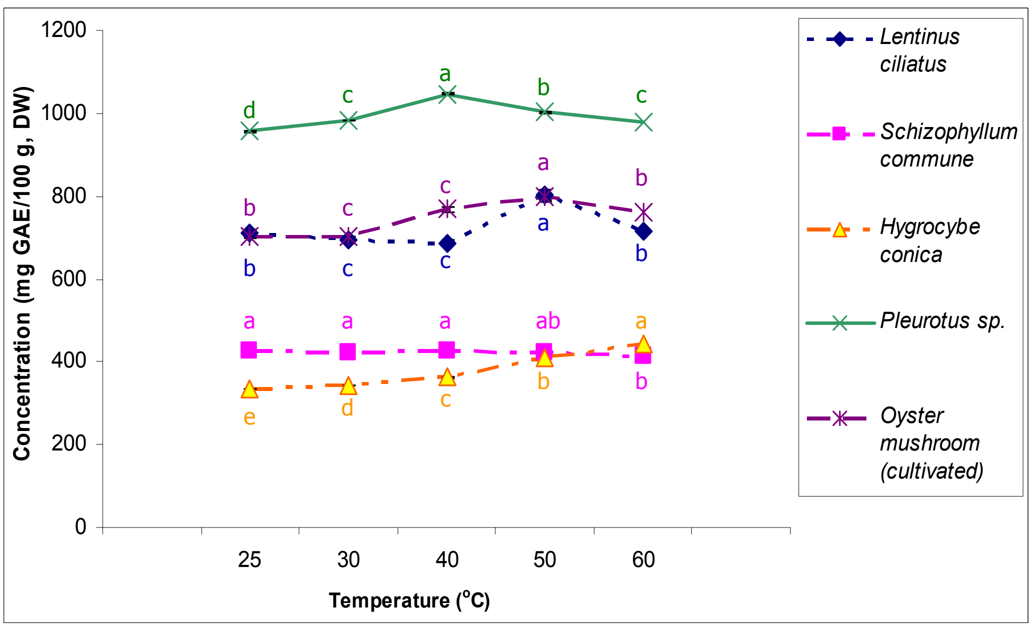

Figure 2. Effect of extraction temperature on the extraction of total phenolic contents of wild edible and cultivated mushrooms.
Different letters within each species denote significantly different ($p<0.05$).

Conclusion

Extraction parameters, including solvent type and concentration, extraction time and temperature are an important consideration when screening for phenolic profiles. In this present study, water extracts exhibited the highest TP, TF and CT of all edible wild and cultivated mushrooms. Water was used as the extraction solvent and TP as indicator in response to extraction time (60, 150, 240, 330 and 420 min) and temperature (25, 30, 40, 50 and 60˚C). Contents of TP with optimal extraction time and temperature were as follows (in descending order): *Pleurotus* sp. (1046.87 mg GAE/100g; 330 min at 40˚C) > *Lentinus ciliatus* (801.08 mg GAE/100g; 150 min at 50˚C) > *Pleurotus ostreatus* (cultivated) (798.55 mg GAE/100g; 330 min at 50˚C) > *Hygrocybe conica* (442.37 mg GAE/100g; 240 min at 60˚C) > *Schizophyllum commune* (427.31 mg GAE/100g; 240

min at 25˚C). Extraction time and temperature with water as extraction solvent were found to have a critical role in extracting total phenolics in edible wild and cultivated mushrooms, which in turn are believed to possess antioxidant activity with possible food application.

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