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Extraction of Total Phenolic and Flavonoids from Edible Wild and Cultivated Medicinal Mushrooms as Affected by Different Solvents

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ABSTRACT

Extending the knowledge on secondary metabolites and the effect of solvent of extraction in mushrooms, We investigated five mushrooms species; two cultivated edible (Agaricus bisporus (brown) and Agaricus bisporus (white); and three wild medicinal edible mushrooms Trametes versicolor, Ganoderma applanatum and Fomes fomentarius for total phenolic, total flavonoid and condensed tannins content. The study also compared the effect of different solvents (i.e. distilled water, methanol and ethanol) of extraction on phenolic profiles in the mushrooms species. The analyses of the phenolic profiles were carried out using the ferrous tartrate method and concentration calculated using tannic acid equivalence (TAE) for total phenolic content and propylgallate acid equivalence (PGAE) for total flavonoids contents on defrozen fresh weight (FW) basis. Results indicated that type of solvent of extraction and type of mushroom species affects the amount of the secondary metabolites. The total phenolic, flavonoid and condensed tannin content were around 4.07-147.78 mg/mL (TAE) FW and 8.13-138.80 mg/mL (PGAE) FW respectively. Variations were observed between mushrooms species metabolites using different solvent of extraction; thus differences among mushroom were dependent on the solvent used aside the different species of mushrooms studied. The study implies that studies of mushroom metabolites may differ depending on which solvent and cultivar is used. Therefore standard guidelines for testing natural bioactive compounds need developing.

Keywords: Ferrous tartrate, fungi, antioxidants, solvents, effects

INTRODUCTION

Mushrooms are low in fat, contain essential fatty acid, conjugated linoleic acids, high protein, polyunsaturated fatty acid [1], dietary fibre content, have a delicacy flavor and texture which are considered nutritionally, medicinally and economically valuable[1-3]. Apart from the above mentioned prospects of mushrooms, they are regarded as good sources of bioactive compounds in human diets for antioxidants purposes. Mushrooms store secondary metabolites, which includes phenolic compounds like polyketides, terpenes and steroids, just like plants and have pharmacological, nutritional and health applications to consumers [3-6].

Many researchers have shown that the common pathological causes of numerous chronic diseases (e.g. cancer and cardiovascular related diseases) often can be linked to oxidative damage to the cellular components [7-10]. A commonly suggested remedy to the oxidative damage of cellular components during metabolism is to increase

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consumption of richer antioxidant diets. Foods that are considered high in antioxidant activity contain compounds such as phenolics and flavonoids.

Phenolics and flavonoids have been reported to possessed antioxidant, anticancer, antimutagenic, antimicrobial and antiradical properties [11-16]. Phenolics are involved in growth and reproduction and provide plants with resistance to pathogens and predators [17]. Because of the antioxidant properties of phenolics, and flavonoids, they are often added to food products containing lipids and its associated foods, which impede lipid peroxidation and lengthens the shelf life of the product [18].

Since the search for cheaper and more abundant natural sources of antioxidants is increasing worldwide [18], studies are needed to ascertain the most precise and accurate extraction method for analysis of natural antioxidant sources, especially in edible mushrooms to help determine the optimum phenolic and flavonoids content for the human diet.

In this study, we examined the yield in mg/g of total phenolic and total flavonoids in selected edible wild medicinal and cultivated mushrooms with emphasis on the effect of differences in solvent of extractions and mushrooms species.

MATERIALS AND METHODS

Sample Collection and Preservation

The mushrooms studied were commonly cultivated species, *Agaricus bisporus* (brown) and *Agaricus bisporus* (white), obtained from a supermarket in Tuskegee, Alabama and three wild medicinal mushrooms, Turkey-tail (*Trametes versicolor*), Artist Conk (*Ganoderma applanatum*) and Tinder Polypore (*Fomes fomentarius*), obtained from the campus of Tuskegee University. To assess the effect of solvent of extraction on the total phenolic condensed tannins and flavonoids content of medicinal wild and cultivated edible mushrooms. The mushroom species were kept in a frozen condition at -20° C with nitrogen for about 18 months prior to the analysis. Phenolic and flavonoid content were determined within the same day.

Materials

Methanol (spectrophotometric grade) from Sigma, Aldrich, ethanol absolute, 200 proof \geq 99.5% (ACS grade), ferrous sulfate, sodium potassium tartrate tetrahydrate, tannic acid, aluminum chloride hexahydrate, propylgallate acid and sodium nitrite (Sigma Aldrich), and 0.067M pH 7.5 potassium phosphate were all of high performance liquid chromatography (HPLC) grade, which were obtained from Fisher Scientific, USA. Water used was of Millipore quality.

Experimental procedures

Total crude extract of Mushrooms

The extraction was carried out according to [3, 19], with modification. One gram (1.0g) of each mushroom species were extracted with 10 mL of distilled water. The mixture was shaking by a shaker at ambient temperature $(25^{\circ}C)$ for 180 minutes then cold for 10 min. The filtrate was filtered with whatman No. 1 filter paper and then used for analysis of total phenolic (TP), and total flavonoid (TF) contents. Each solvent extraction was carried out in triplicate. Methanol and ethanol solvents were also used in a similar manner as mentioned above to extract the mushrooms species for quantification of the total phenolics, flavonoids and condensed tannins via Spectrophotometer.

Total phenolic (TP) Analysis

The ferrous tartrate method was used for total phenolic analysis following the method of [19-22] with slight modification. A 1.0 mL of each mushroom extract was transferred into a 25.0 mL volumetric flask to react with 5 mL dyeing solution (1g ferrous sulfate and 5g sodium potassium tartrate tetrahydrate dissolved in 1000 mL distilled water), 4mL of distilled water and 15 mL of buffer (0.067M potassium phosphate, pH7.5) were added. The absorbance at 540 nm was measured after 20 minutes of mixing at ambient temperature for colour formation (purple/violent) using a Thermo Spectromic GENESYS 20 spectrophotometer, a blank solution prepared with distilled water replacing the mushroom extract was also assay.

The content of TP was calculated by using tannic acid calibration standard curve with concentration ranged from 50 mg/g to 500 mg/mL (R^2 = 0.999 see in figure 1). Results are reported as mg of tannic acid equivalence per g of fresh weight (TAE/g FW) basis.

Determination of total flavonoids (TP)

For flavonoids with 3', 4' dihydroxy-substitued structures are often react with the NaNO₂- Al (NO₃)₂- NaOH [23]. The method is basically based on reaction between aluminum ion with the hydroxyl groups after oxidation and nitrosylation. In this reaction the Al ion chelates with the carbonyl groups found in position 3' and 4' and with the addition of a strong base (NaOH) to form red chelates which enhance the determination of the flavonoids(Zhu et al.2010). Based on this chemistry of flavanoids, propylgallate acid (propyl 3,4,5-trihydroxybenzoate) was used as the equivalence in the estimation of the total flavonoids.

TF was determined by colourimetric method as described in [3, 24, 25] with slight modification. Briefly, 250μ L of sample was mixed with 1.25ml of deionized water and 75µl of a 5% NaNO₂ solution. After 6 min, 150 µl of a 10% AlCl₃.6H₂O solution was added to the mixture. The mixture was incubated at room temperature for 5min, then 0.5 ml of 1M NaOH and 2.5 mL of deionised water were added. The mixture was then thoroughly vortexed and the absorbance of the light pink colour was measured at 510nm against the blank using Genesys 20 spectrophotometer. Propylgallate acid was used for the calibration curve with a concentration range of 50-500 mg/mL (R²=0.999 see in figure 2) and analyzed as above. Results were expressed as mg propylgallate acid equivalent (PGAE)/mL FW. All experiments were carried out in triplicate.

Statistical analysis

The study was conducted as a completely randomized design arranged in a 5 X 3 factorial. The data were analyzed using SAS (SAS Institute, Inc., Cary, NC) and results were expressed as means plus standard errors. For dependent variables where the interaction was not deemed significant, Turkey's multiple comparisons were carried out to evaluate differences among the main effects. When a significant interaction was detected, comparisons were made within mushroom species, Significant levels were defined using p<0.05.

RESULTS AND DISCUSSION

The total phenolic and total flavonoids content of both the cultivated and wild edible medicinal mushrooms are presented in Table 1. The comparison between different solvents of extraction is also reported in Table 2. To our knowledge, no equivalent data have been reported for these species of mushrooms comparing variation in the extraction methodology, and the type of cultivars. The total phenolic, and flavonoids contents for *Agaricus bisporus* white, *Agaricus bisporus* brown, *Ganoderma applanatum*, *Trametes versicolor* and *Fomes fomentarius* were 4.07 to 147.78±5.21 mg/mL (TP), and 8.13 to 138.80±6.51mg/mL (TF) FW basis respectively. Variations were observed across species in TP, and TF contents between the two cultivated edible medicinal mushrooms and the three wild edible medicinal mushrooms. The amounts of total phenolic were low as compared to previous data from literature of wild mushrooms (*lentinus ciliatus* (801.08mg/GAE/100g DW), *Schizophyllum commune* (427.31mg GAE/100g DW), *Hygrocybe conica* (442.37mg GAE/100g DW) and a cultivated species *Pleurotus ostreatus* (1046.87mg/GAE/100g) [3], 45.6mg/100g for commercial champignon and wild champignon to be 308.3mg CAE/100g FW [8]. The difference perhaps could be attributed to genetic factors (different species). In addition, differences in particle size, type of phenolic complex mixtures, solvent concentration, extraction time and temperature, solvent to solid ratio, moisture content of mushrooms and pH contributed to the differences between the studies [3, 25, 26, 27, 28].

The current paper also investigated the effect of different solvents on TP, and TF contents in these mushrooms (Table 2). It was realized that within mushrooms species differences in TP and TF existed with the use of varying solvents. This could be attributed to the polarity of the solvents [3, 29], the type of phenolic, and flavonoids mixtures present in each mushroom species. This conjecture is supported by other authors in *Pistacia atlacia* [30] and *Punica granatum* L. [10,18,] that the efficiency of phenolics extraction depends on the type of phenol extracted in a plant.

From Table 2. Methanol was considered to be the best solvent for extraction of TP and TF in (*G. applanatum and F. fomentarius*), whereas in *T. versicolor* species distilled water was regarded as the best solvent in the extraction process. Water was also observed to be the best solvent for extraction of TP and TF in *Agaricus bisporus* brown and white kind compared to ethanol and methanol. However, there was a strange observation in the white brand of

Agaricus species where ethanol produced high value than water and methanol. These findings were in agreement with previous studies in edible wild mushrooms, *Vateria indica* and *Macrosolen parasiticus* (L.) Danser reported by [3, 31-33]. Similar findings have been reported in tea TP, catechin and caffeine by [36]. The effectiveness of solvent in extractions of TP, and TF may also depend on the moisture content and particle size of the mushroom species studied. It is known that some solvent is not effective in isolating certain compounds when large particle size is used within a short reaction time and temperature [3] because of the solvent inability to permeate the tissue. The moisture content could also affect the extraction ability because of high water content in sample, which could dilute the concentration of the TP and TF content in such tissues resulting in low absorbance readings or below detection limit. Since the coloumetric analysis depends solely on the intensity of the complex form. The lesser the color the lower the absorbance might be and could affect the total yield of the phenolic profile(s).

Another explanation to the low TP, and TF contents compared to other mushrooms species in literature could be attributed to the formation of protein–phenolic and protein-flavonoid complexes which can limit the extractability of TP and TF in the mushrooms. This is supported by studies in conifer foliage [35]. It is also interesting to point out that during storage under low temperatures anthocyanins are produced in higher amounts in plants that contain them in their system. This conjecture was observed in strawberries and grapes [36] which could be the contributing factor to what is been observed in this mushrooms stored for a while prior to the analysis.

The study indicates that studies evaluating mushroom species for metabolites may rank the species differently depending on which solvent is used. Standard guidelines need developing, but more information is needed on the factors and their interactions that affect the determinations. Standard reagents for phenolic and flavonoids determination should be selected with caution, in that most do not provide a sufficiently high fit for the calibration curve. *Ganoderma applanatum* was observed to be a potential candidate rich of phenolic and flavonoids could be useful in pharmaceutical and food industry for drugs and additives production. Further studies are ongoing to evaluate the effect of long term storage and solvent of extraction on total phenolic and total flavonoid content in similar mushrooms species.



Figure 1. Total phenolic content determination using tannic acid as standard





Table 1. Comparison oftotal phenolic contentand total flavonoid content of edible cultivated and wild medicinal mushrooms species.

Name of mushrooms	TP (mg TAE)/mL FW	TF (mg PGAE)/mL FW
Fomes fomentarius	10.74 ^c	35.02 ^{bc}
Ganodermaapplanatum	53.09 ^a	84.49 ^a
Trametesversicolor	23.70 ^b	30.58°
Agaricusbisporus brown	20.25 ^{bc}	9.91°
Agaricusbisporuswhite	15.56 ^{bc}	41.69 ^b
Standard Error (SEM)	3.01	3.76

Different letter superscript in the same column indicates significant differences in TP, and TF among mushrooms species at p<0.05.

 Table 2. Effect of different solvent types on total phenolic content and total flavonoid content of cultivated and wild medicinal mushrooms species in mg/ mL of TA/PGA Equivalent.

Name of mushrooms	Total phenolic content			Total flavonoids content		
	Dil. H ₂ O	Ethanol	Methanol	Dil. H ₂ O	Ethanol	Methanol
Fomes fomentarius	7.78 ^{cd}	11.48 ^a	12.96 ^{bc}	34.80 ^b	32.13 ^c	38.13 ^b
Ganodermaapplanatum	4.45 ^d	7.04 ^a	147.78 ^a	11.20 ^c	103.47 ^a	138.80 ^a
TrametesVersicolor	52.59 ^a	14.44 ^a	4.07 ^c	74.13 ^a	8.13 ^d	9.47 ^d
Agaricusbisporus brown	33.33 ^b	4.45 ^a	22.96 ^b	32.13 ^b	nd	14.13 ^{cd}
Agaricusbisporus white	22.22 ^{bc}	7.78 ^a	16.67 ^{bc}	40.80^{b}	54.13 ^b	30.13 ^{bc}
Standard Error (SEM)	5.21	5.21	5.21	6.51	6.51	6.51

Results are expressed as means plus standard Error. Different superscripts within the same columns of individual mushroom type denote significant different at (p<0.05). nd means it was below detection limit.

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