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CHEMICAL COMPOSITION AND THE ANTIOXIDANT ACTIVITY OF TWO EDIBLE MUSHROOMS

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ABSTRACT: The proximate composition and minerals contents of two edible mushroom (*Pleurotus ostreatus* and *Agaricus bisporus*) flours used in Egypt were investigated. Also, ethyl acetate, ethanol, methanol and distilled water were used to fractionate soluble compounds from the mushrooms in ascending polarity by sequentially extracting. As well as, total phenolic, total flavonoid compounds and the antioxidants activity for these extracts were investigated. The percentage moisture content ranged between 90.47% in *Pleurotus ostreatus* to 90.55% in *Agaricus bisporus*. The crude protein ranged from 3.25% in *Pleurotus ostreatus* to 3.48% in *Agaricus bisporus*. Total carbohydrates ranged from 5.23% in *Agaricus bisporus* to 5.35%±0.03 in *Pleurotus ostreatus.* The most abundant mineral element is potassium (ranging from 2376.8 to 2820.5 mg/100 g on dry weight base (DW) for *Agaricus bisporus* and *Pleurotus ostreatus,* respectively) followed by Phosphorus (ranging from 537 to 754 mg/100 g on DW for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively). The antioxidant activity of different extracts for two mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*) increased gradually as concentration increases. These results compatible with those results recorded for total phenolic compounds of investigated mushroom. In conclusion, mushrooms, in spite of the great variability observed among species, represent an interesting food item that can contribute to the formulation of a well-balanced diet.

Key words: *Agaricus bisporus*, *Pleurotus ostreatus*, proximate analysis, minerals, total phenolic compounds, antioxidant activity.

INTRODUCTION

Edible mushrooms are valuable healthy foods, having rich source of vitamins, proteins and minerals, especially in potassium and phosphorus. They are also low in calories and fats **(León-Guzmán *et al.,* 1997; Öztürk *et al.,* 2011).** Oyster mushrooms (*Pleurotus ostreatus*) and champignons (*Agaricus bisporus*) are regularly used as raw ingredients in the preparation of various dishes, as well as food stocks, replacing chicken stocks. Champignons are commercially available in three varieties, white, brown and Portobello.

In recent years, amounts of used mushrooms have risen greatly, involving a large number of species, due to continuous developments in cultivation, harvest, postharvest, processing and storage treatments, which facilitates the consumption throughout the year. Along the nutritional properties **(Barros *et al.,* 2007)**. Mushrooms have been demonstrated to possess healthy properties **(Lindequist *et al.,* 2005)** and they have evidence to be efficient as anti-inflammatory, antitumor, antibacterial, antioxidant and antiviral agents **(Dore *et al.,* 2007; Chen *et al.,* 2009; Garcia-Lafuentea *et al.,* 2010).** Recently, they have become increasingly attractive as functional foods due to their potential beneficial effects on human health. Hence, food industry is especially interested in both cultivated and wild edible mushrooms. The most extensively cultivated mushroom worldwide are *Agaricus bisporus* and *Pleurotus ostreatus*. Among the biologically active articles

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present in mushrooms, phenolic compounds have attracted much awareness due to their excellent properties as antioxidant, anti-inflammatory or anti-tumour articles, among others **(Puttaraju *et al.,* 2006)**.

In the present work, two edible mushrooms collected from Egypt (*Pleurotus ostreatus* and *Agaricus bisporus*) were studied for detailed chemical composition. Also, ethyl acetate, ethanol, methanol and distilled water were used to fractionate soluble compounds from the mushrooms in ascending polarity by sequentially extracting. As well as, total phenolic, total flavonoid compounds and the antioxidants activity for these extracts were investigated.

MATERIALS AND METHODS

Mushroom

Oyster mushroom (*Pleurotus ostreatus*) was purchased from Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt. Champignons (*Agaricus bisporus*) was purchased from local market, Zagazig city, Egypt.

Chemical Composition

Proximate analyses

Proximate analysis of the mushrooms, including moisture, ash, crude protein, crude fat and total carbohydrates were determined in triplicate, according to **(Horwitz and Latimer, 2000).** The moisture content was determined by further heating of the dried sample at 105°C overnight until constant weight; the ash content was determined by weighing the incinerated residue obtained at 550°C for 24 hr., the crude protein content (N × 4.38) was determined by the Kjeldahl method **(Braaksma and Schaap, 1996; Okoro and Achuba, 2012)** the crude fat content was determined by Soxhlet extraction with petroleum ether as a solvent **(Fernandes *et al.,* 2014)**. Total carbohydrate content was determined by measuring the absorbance of phenol and concentrated sulfuric acid extracts at 490 nm **(Dubois *et al.,* 1956).**

Mineral analysis

The mushroom sample (1 g) was placed in a porcelain crucible and ashed in a muffle furnace at 500°C for 24 hr. After cooling, the ashed material was dissolved in 2 ml of concentrated HNO_3, and diluted with distilled water up to 25 ml. The solution was then transferred to a suitable container, after it was filtered through filter paper. A blank digest was carried out in the same way. The concentrations of iron (Fe), zinc (Zn), potassium (K), sodium (Na), calcium (Ca), manganese (Mn), copper (Cu), and magnesium (Mg) were determined by a graphite furnace atomic absorption spectrometry (Thermoscientific/Germany). Phosphorus (P) was determined by molybdenum blue spectrophotometry (UV-160A Shimadzu/Japan) according to (**Adejumo and Awosanya, 2005**).

Preparation of Extracts

The mushroom samples (*Pleurotus ostreatus* and *Agaricus bisporus*) were cleaned manually, cut and lyophilized (Thermo-electron Corporation – Heto power dry LL 300 Freeze dryer). Then, the lyophilized powder was defatted using n-hexane. Ethyl acetate, ethanol, methanol and distilled water to fractionate soluble compounds from the mushrooms in ascending polarity by sequentially extracting **(Smolskaitė *et al.,* 2015)**. Twenty grams ground mushrooms were extracted with different solvents (200 ml) using magnetic stirrer at room temperature for 2 hr., followed by filtration through Whatman No.1 filter paper. The residues were re-extracted under the same conditions. The samples were air dried after each solvent extraction and finally the residues were extracted with boiling water during 2 hr. Organic solvents were removed in a vacuum rotary evaporator (BüCHI-water bath-B-480), while water extracts were freeze-dried (Thermo- electron Corporation–Heto power dry LL 300 Freeze dryer). All extracts were kept in a refrigerator until further analysis.

Total Phenolic Compounds (TPCs) Determination

The TPCs were measured with Foline-Ciocalteu reagent as described by **(Singleton *et al.,* 1999)**. One ml of sample (1000 µg in 1ml) was mixed with Folin-Ciocalteu reagent (5 ml, previously diluted with water 1:10, *V/V*) and sodium carbonate (75 g/l, 4 ml). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40°C for colour development. Absorbance

was then measured at 765 nm. Gallic acid was used to obtain the standard curve (20 – 200 µg/ml), and the reduction of Folin-Ciocalteu reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract. The calibration equation for gallic acid was y = 0.001x + 0.0563 (R^2 = 0.9792), where y is absorbance and x is concentration of gallic acid in µg/ml (Fig. 1).

Total Flavonoids (TFs) Determination

Total flavonoids (TFs) were measured according to the method of **(Ordonez *et al.*, 2006)** with some modification. Two ml aliquot of 20 g/l $AlCl_3$ ethanol solution was added to 1 ml of the extract (1000 µg in 1ml solvent). After 60 min, the absorbance at 420 nm was recorded. Quercetin was used to obtain the standard curve (20–200 µg/ml), Total flavonoids contents expressed as quercetin equivalent (QE), which was calculated based on the calibration curve (Fig. 2).

Antioxidant Activity Evaluation (DPPH-assay)

The electron donation ability of each extract was recorded by bleaching of the DPPH purple colored solution according to **(Hatano *et al.*, 1988)**. Five hundred µl of each extract at different concentrations (100, 250, 500, 1000, 1500 and 2000 µg extract/1ml solvent) were added to 2.5 ml of 0.1 mM DPPH dissolved in ethyl acetate or ethanol or methanol according to the solvent used for extraction. After incubation time of 30 min at room temperature, the absorbance was recorded against the control at 517 nm **(Gülçin *et al.*, 2004).** Percentage of antioxidant potential of DPPH radicals was calculated as follow:

Inhibition (%) = [(Abs control-Abs sample)/Abs control] x 100

Where Abs. control is the absorbance of the control and Abs. sample is the absorbance in the presence of mushroom extract.

RESULTS AND DISCUSSION

Proximate Composition

Proximate analyses were carried out on two edible mushroom species: *Pleurotus ostreatus* and *Agaricus bisporus*. They were selected based on their availability. Results of proximate composition are presented in Table 1.

The percentage of moisture content ranged between 90.47% ±0.88 in *Pleurotus ostreatus* to 90.55% ±0.65 in *Agaricus bisporus*. The ash content ranged from 0.43% ±0.004 in *Agaricus bisporus* to 0.65% ±0.002 in *Pleurotus ostreatus*. The crude fat ranged from 0.22% ±0.002 in *Agaricus bisporus* to 0.28% ±0.003 in *Pleurotus ostreatus*. The crude protein ranged from 3.25% ±0.02 in in *Pleurotus ostreatus* to 3.48% ±0.01 in *Agaricus bisporus***.** Total carbohydrates ranged from 5.23% ±0.02 in *Agaricus bisporus* to 5.35% ±0.03 in *Pleurotus ostreatus*. Dry matter content of fresh mushrooms is relatively low, i.e. around 10%, and is mainly composed of carbohydrates, proteins, fibre and minerals **(Wang *et al.*, 2014).** When considering the chemical composition of mushrooms, it is worthwhile to keep in mind that water content is the parameter that is to some degree, variable for fresh mushroom. This is because changing weather conditions can to some degree influence the moisture content of collected fruiting bodies (mushrooms). Fruiting bodies, after collection, also lose moisture easily due to evaporation. There is a consensus that the moisture content of fresh fruiting bodies is 90% **(Chudzyński and Falandysz, 2008).** The proximate composition of mushrooms also varies within and among species, and fruiting body maturity can also play a role. As well as, the environmental factors can have an impact on the abundance of certain compounds in mushrooms.

Mineral Analysis

Table 2 show the mineral contents of the samples analysed. The mineral constituents, are in agreement with other literature data **(Manzi *et al.*, 1999; Manzi *et al.*, 2004),** the most abundant mineral element is potassium (ranging from 2376.8 to 2820.5 mg/100 g on dry weight base for *Agaricus bisporus* and *Pleurotus ostreatus,* respectively) followed by Phosphorus (ranging from 537 to 754 mg/100 g on dry weight base for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively). Iron content was between 16.4 and 39 mg/100 g on dry weight base for *Agaricus bisporus* and *Pleurotus ostreatus,* respectively. Zinc content was between

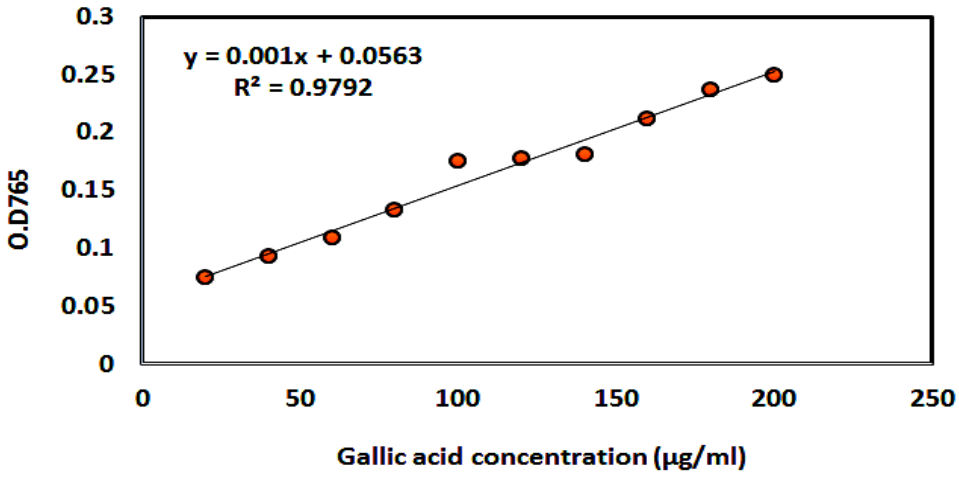

Fig. 1. Standard curve for gallic acid

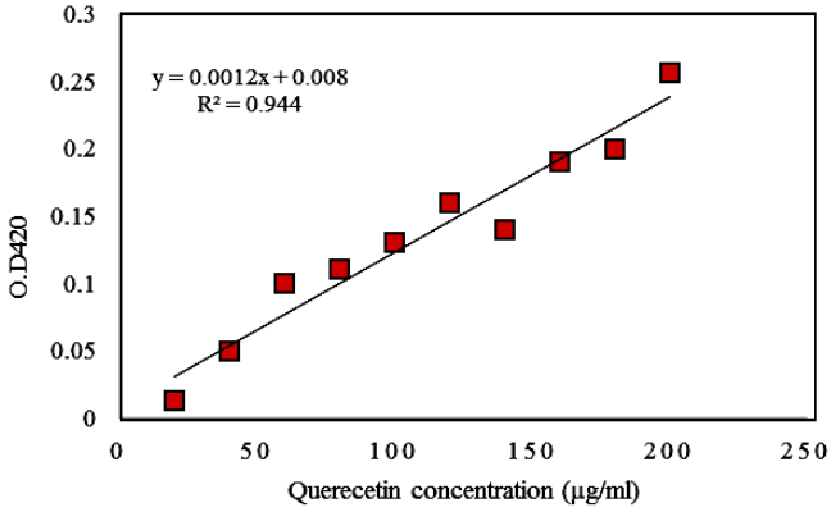

Fig. 2. Standard curve for quercetin

Table 1. Proximate composition of *Pleurotus ostreatus* and *Agaricus bisporus* mushroom species

Parameter	Concentration [a] (g/100 g fresh weight)	
	Pleurotus ostreatus	*Agaricus bisporus*
Moisture	90.47 ±0.88	90.55 ±0.65
Ash	0.65 ±0.002	0.43 ±0.004
Crude fat	0.28 ±0.003	0.22 ±0.002
Crude protein	3.25 ±0.02	3.48 ±0.01
Total carbohydrates	5.35 ±0.03	5.23 ±0.02

[a] Values are mean ± standard deviation of triplicate determinations.

Table 2. Mineral composition of flour from *Pleurotus ostreatus* and *Agaricus bisporus* mushroom species

Mineral	Concentration (mg / 100 g dry matter)	
	Pleurotus ostreatus	***Agaricus bisporus***
Iron	39	16.4
Zinc	3.5	6.1
Potassium	2820.5	2376.8
Sodium	65.73	75.3
Calcium	29.93	42.1
Manganese	3.1	4.4
Copper	4.8	7.6
Magnesium	173.43	120
Phosphorus	537	754

3.5 and 6.1 mg/100 g on dry weight base for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively. Sodium content was between 65.73 and 75.3 mg/100 g on dry weight base for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively. Calcium content was between 29.93 and 42.1 mg/100 g on dry weight base for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively. Magnesium content was between 120 and 173.43 mg/100 g on dry weight base for *Agaricus bisporus* and *Pleurotus ostreatus,* respectively. Copper content was between 4.8 and 7.6 mg/100 g on dry weight base for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively. Manganese levels are not so high in mushrooms (ranging from 3.1 to 4.4 mg/100 g on dry weight base for *Pleurotus ostreatus* and *Agaricus bisporus,* respectively).

Total Phenolic Contents (TPC)

The TPC values are presented in Fig. 3. The results obtained for *Pleurotus ostreatus* mushroom in Folin Ciocalteu assay were 23, 55, 56 and 46 mg GAE/g for ethyl acetate, ethanol, methanol and aqueous extracts, respectively. Likewise, *Agaricus bisporus* mushroom recorded in Folin Ciocalteu assay were 36, 77, 164, and 119 mg GAE/g for ethyl acetate, ethanol, methanol and aqueous extracts, respectively. The results obtained in these investigation were higher than results obtained by **(Yang *et al.,* 2002; Oke and Aslim, 2011; Reis *et al.,* 2012)**.

Total Flavonoid Contents (TFCs)

The TFC values are presented in Fig. 4. The results obtained for *Pleurotus ostreatus* mushroom were 11, 15. 23 and 16 mg QE/g for ethyl acetate, ethanol, methanol, and water extracts, respectively. Likewise, *Agaricus bisporus* mushroom recorded 22, 45, 100 and 92 mg QE/g for ethyl acetate, ethanol, methanol, and water extracts, respectively.

Antioxidants Activity (DPPH assay)

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical is a long-lived organic nitrogen radical with a deep purple color. In the performed method, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine, according to the following equation:

$$Z^{\bullet} + AH \text{----------} ZH + A^{\bullet}$$

Where $Z^{\bullet}$ represents the DPPH radical and the donor molecule is represented by AH. In this reaction, ZH is the reduced form and $A^{\bullet}$ is the free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant **(Molyneux, 2004).** This reduction could be monitored measuring the absorbance decrease at 515-528 nm until the absorbance remains stable

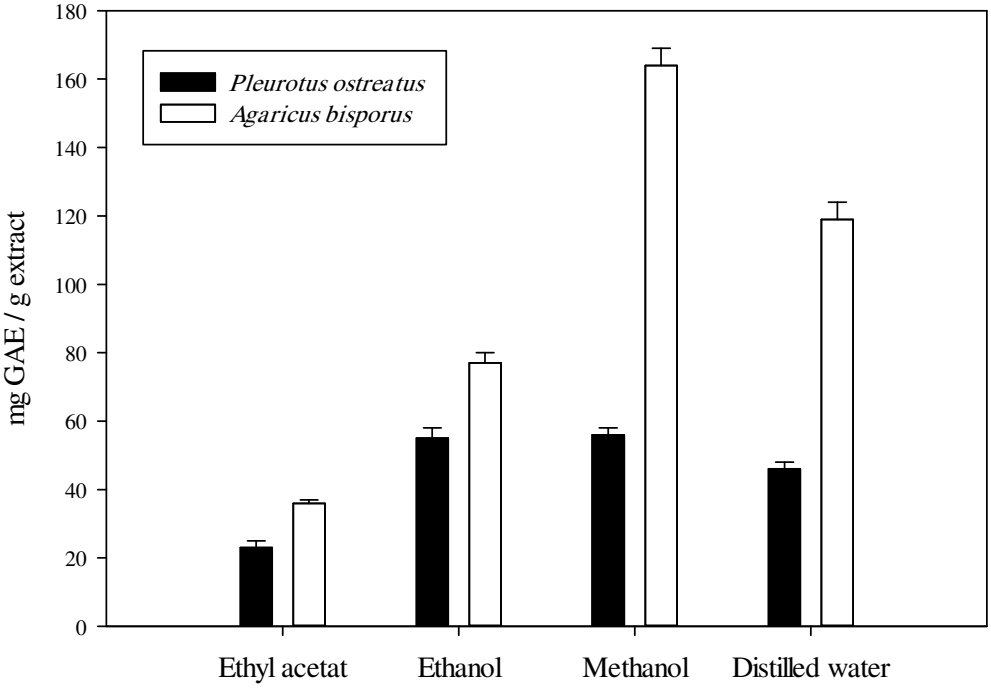

Fig. 3. Total phenolic contents (mg GAE/g extract) for different extracts from *Pleurotus ostreatus* and *Agaricus bisporus* mushroom species

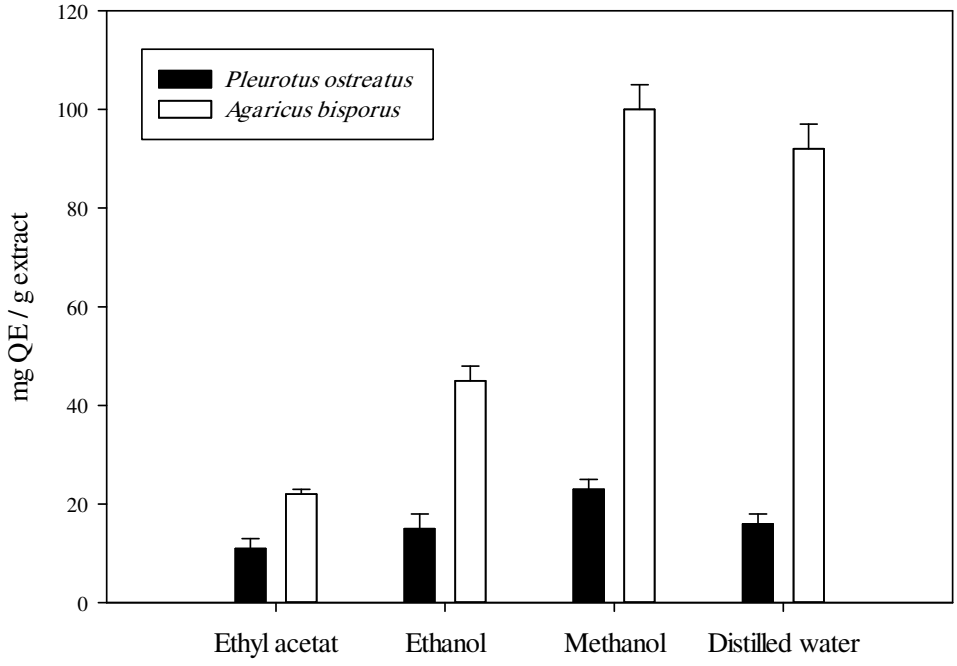

Fig. 4. Total flavonoid contents (mg QE/g extract) for different extracts from *Pleurotus ostreatus* and *Agaricus bisporus* mushroom species

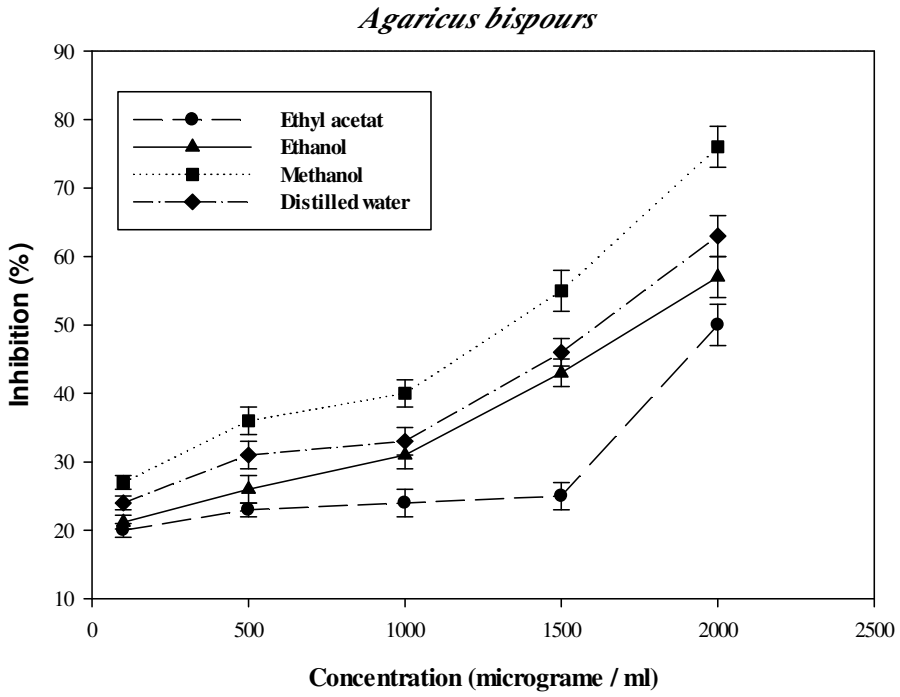

Fig. 5. Antioxidants activity (inhibition %) for different extracts from *Agaricus bisporus* using DPPH assay

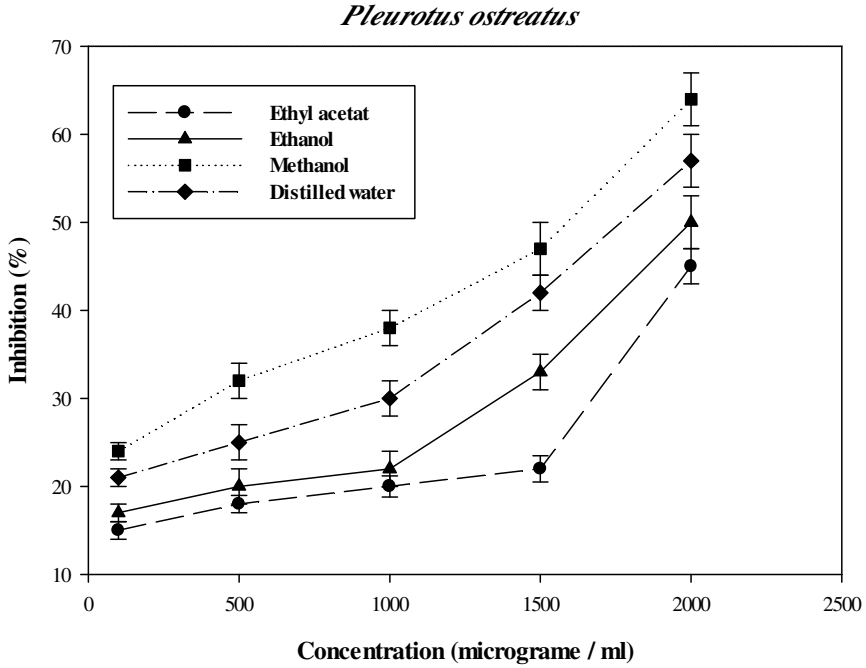

Fig. 6. Antioxidants activity (inhibition %) for different extracts from *Pleurotus ostreatus* using DPPH assay

in organic media **(Karadag *et al.,* 2009),** and free radical scavenging activity can be determined by the discoloration of the DPPH solution **(Ndhlala *et al.,* 2010)**.

Antioxidants activity (% inhibition) for different extracts from *Agaricus bisporus* or *Pleurotus ostreatus* using DPPH assay are presented in Figs. 5 and 6.

Agaricus bisporus was the specie that presented the highest radical scavenging activity compared with *Pleurotus ostreatus.* These results compatible with our results recorded in total phenolic compounds. It can be noted that, the antioxidant activity of different extracts in two mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*) increased gradually with increasing concentration of TPCs and TFCs. These results are in agreement with results obtained by **(Chirinang and Intarapichet, 2009; Tsai *et al.,* 2009; Oke and Aslim, 2011).**

Conclusions

In conclusion, mushrooms, in spite of the great variability observed among species, represent an interesting food item that can contribute to the formulation of a well-balanced diet.

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التركيب الكيميائى والنشاط المضاد للأكسدة لاثنين من فطر عيش الغراب الصالحة للغذاء

أمال صلاح على – على عثمان عثمان – رجب عبدالفتاح المصرى – سيد سليمان السعدنى

قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة الزقازيق – مصر

فى هذا البحث تمت دراسة التركيب الأولى، ومحتوى المعادن لأثنين من فطر عيش الغراب الصالحة للغذاء (بليوروتس أوستراتوس وأغاريكوس بيسبوروس) المستخدمة في مصر. كما تم استخدام الهكسان، خلات الإيثيل، الإيثانول، الميثانول والماء المقطر (استخلاص بالتتابع تصاعديا على حسب درجة القطبية) لتجزئة وفصل المركبات القابلة للذوبان من الفطر فى هذه المذيبات، تم تقييم محتوى هذه المستخلصات من المركبات الفينولية والفلافونويد الكلية ونشاطها المضاد للأكسدة، ووجد أن نسبة الرطوبة بين ٩٠,٤٧% في بليوروتس أوستراتوس إلى ٩٠,٥٥% في أغاريكوس بيسبوروس، تراوح البروتين الخام من ٣,٢٥% في بليوروتس أوستراتوس إلى ٣,٤٨ % في أغاريكوس بيسبوروس، تراوحت الكربوهيدرات الكلية من ٥,٢٣% في أجاريكوس بيسوروس إلى ٥,٣٥ % ± ٠,٠٣ في بليوروتس أوستراتوس، العنصر الأكثر وفرة من المعادن هو البوتاسيوم (يتراوح من ٢٣٧٦,٨ إلى ٢٨٢٠,٥ ملجم/١٠٠ جم من الوزن الجاف لفطر أغاريكوس بيسبوروس و بليوروتس أوستراتوس، على التوالي) يليه الفوسفور (يتراوح من ٥٣٧ إلى ٧٥٤ ملجم/١٠٠ جم من الوزن الجاف لفطر بليوروتس أوستراتوس وأغاريكوس بيسبوروس، على التوالي)، النشاط المضاد للأكسدة من المستخلصات المختلفة لكلا من أغاريكوس بيسبوروس وبليوروتس أوستراتوس يزداد تدريجيا مع زيادة التركيز للمواد الفينولية والفلافونويد حيث ان، هذه النتائج متوافقة مع النتائج المتحصل عليها في المركبات الفينولية الكلية والفلافونويد الكلية، مما يوضح أنه على الرغم من التباين الملحوظ بين الأنواع المختلفة فى محتواها من المركبات الكيميائية ونشاطها كمضادات أكسدة فان المشروم يمثل مادة غذائية جيدة تساهم فى تكوين نظام غذائى متوازن للانسان عموما ولذوى الاحتياجات الخاصة.

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