Antioxidant properties of wild edible Mushrooms growing in Al-Jabal Alakhdar region of Libya

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الخصائص المضادة للأكسدة للفطر البري الصالح للأكل المتنامي في منطقة الجبل الأخضر – ليبيا

الملخص:

تهدف هذه الدراسة الى تحديد محتوى مضادات الأكسدة ونشاط مضادات الأكسدة في الفطر البري الصالح للأكل (A. Bispours) الذي يتم تناوله تقليديا في محافظة الجبل الأخضر . لهذا الهدف تم تحليل ست عينات من أغطية الفطر بشكل عشوائي من مناطق مختلفة بما في ذلك الوسيطة ومراوة ووردامه واسلطنه وسيدي الحمري والفائدية لمعرفة أنشطتها المضادة للاكسدة في اختبارات مختلفة.

أشارت نتائج الدراسة إلى أن إجمالي محتويات الفينول نراوحت بين 2.14 الى 4.37 ملجم من مكافيء حمض الجاليك/جم من الوزن الجاف وتراوحت محتويات الفلافونويد من 0.57 الى 1.54 ملجم (+) – مكافيء الكاتكين/جم من الوزن الجاف. علاوة على ذلك أظهر المستخلص الميثانولي لجميع عينات الغطاء نشاط لإزالة الجذور الحرة. تراوحت قدرة التخفيض من 3.40 إلى 7.04 ملجم من مكافيء/BHA جم وزن جاف، في حين كان نشاط الكسح الجذري بواسطة DPPH و BTS في حدود 9.83 إلى 24.26 و 29.51 إلى 52.46 ميكرومول/ TE وزن جاف على التوالي. أظهر مستخلص الفطر الميثانولي لمنطقة الوسيطة أعلى محتوى إجمالي من الفينول والفلافونويد ونشاط مضاد الأكسدة.

الكلمات المفتاحية: – الفطر، الأنشطة المضادة للأكسدة، الفينول الكلي، الفلافونويد، الجبل الأخضر.

Abstract:

The aims of this study were to determine the antioxidant content and antioxidant activity in wild growing edible mushrooms (*A. bisporus*) traditionally eaten in Al-Jabal Alakhdar province. For this purpose: Randomly six mushroom cap samples from different region including Alosita, Marawah, Werdama, Salantah, Sidihamri and Faidiyah were analyzed for their antioxidant activities in different test namely, reducing power and scavenging effect on DPPH and ABTS, in addition, total phenolic and flavonoid contents were determined using Folin–Ciocalteu procedure and the flavonoid aluminium method. Results indicated that total phenolic contents were in the range of 2.14 to 4.37 mg gallic acid equivalent/g dry weight (dw) and flavonoid contents ranged from 0.57 to 1.54 mg (+)-catechin equivalent/g dw. Moreover, methanolic extract for all cap samples showed radicals scavenging activities. The reducing power ranged from 3.40 to 7.04 mg BHA equivalent/g dw, whereas, the radical scavenging activity by DPPH and ABTS was in the range of 9.83 to 24.26 and 29.51 to52.46 µmol TE/g dw respectively.

The methanolic mushroom extract of Alosita region exhibited the highest total phenolic, flavonoid content and antioxidant activity.

Keywords*: - Mushroom, Antioxidant activities, Total phenolic, Flavonoid, Al-Jabal Alakhdar province.*

Introduction:

Humans have regarded edible mushrooms as a significant food source for thousands of years. Numerous researchers have looked into the nutritional worth and therapeutic benefits of edible mushrooms' chemical makeup. Both Eastern and Western nations have gradually boosted their consumption of edible and medicinal mushrooms in recent decades (Ahmed, *et al.*, 2023). Proteins, polyunsaturated fatty acids, polysaccharides, dietary fibers, amino acids, vitamins, and minerals are among the bioactive substances found in edible mushrooms. They contain vital health benefits, including cholesterol-lowering, immune-stimulating, antioxidant, and anticancer qualities (Lopez-Hortas, *et al.*, 2022). Therefore, mushrooms should be regarded as a source of material for pharmacological compounds, functional foods, and food supplements in addition to being a conventional food (Smolskaite, *et al.*, 2015). Thus, studying the antioxidant activity and concentration of antioxidant chemicals in mushrooms and their extracts is a very interesting scientific topic (Vidovic, *et al.*, 2010).

Additionally, digestible mushrooms have been described as beneficial foods that can help prevent conditions like cancer and hypertension. Their chemical makeup is largely responsible for their functional qualities (Manzi, *et al.*, 2001). As a source of physiologically useful medicine and as a functional food, mushrooms have gained popularity. The fruiting body may frequently be generated in a significantly shorter amount of time, which is one benefit of employing mushrooms rather than plants as sources of bioactive chemicals. There have been reports of antioxidant activity in a variety of wild mushroom species, mostly associated with their phenolic content (phenolic acids and flavonoids), followed by tocopherols, ascorbic acid, and carotenoids (Ferreira, *et al.*, 2009).

Wild mushrooms growing in eastern Libya have nutritional, therapeutic, and physiological qualities, but their main constituents and antioxidant composition which would be a good way to evaluate their nutritional value have not been studied. Due to their abundance during the rainy season, people in Libya's Al-Jabal Alakhdar region eat wild edible mushrooms. In order to figure out their potential for human consumption and health, this study was conducted to investigate the antioxidant activity.

Materials and methods:

1. Samples:

Alabraq, Alosita, Asalpiea, Ashnishen, Balanaje, Faidiyah, Marawah, Omar Mukhtar, Salantah, Sidihamri, and Werdama regions are among the areas in Al-Jabal Alakhdar province/Libya where fruiting bodies (cap and stipe) of wild edible mushrooms were collected (Fig.1). The rainy season, which runs from month 9 to month 11 in 2012, is when the collections were completed. For the purpose of diagnosis, the physical and habitat properties of the mushrooms that were identified in the areas were noted and photographed. First, foreign contaminants were removed from the freshly obtained samples. A plastic knife was used to cut off any damaged or dirty (muddy) portions, and

a fine brush was used to clean any smaller particles. The mushrooms were subsequently identified by Dr. Abdolmageed Kamara, Department of Plant Pathology/Alexandria University Egypt as listed in Table (1). For the purpose of determining moisture content, approximately 10 g of each sample were taken right away. prior to the analysis began, the collected materials were stored in already cleaned polyethylene bottles after being cut into small pieces and dried for 24 hours at 105°C. The dried samples were then ground into a fine powder using an agate homogenizer.

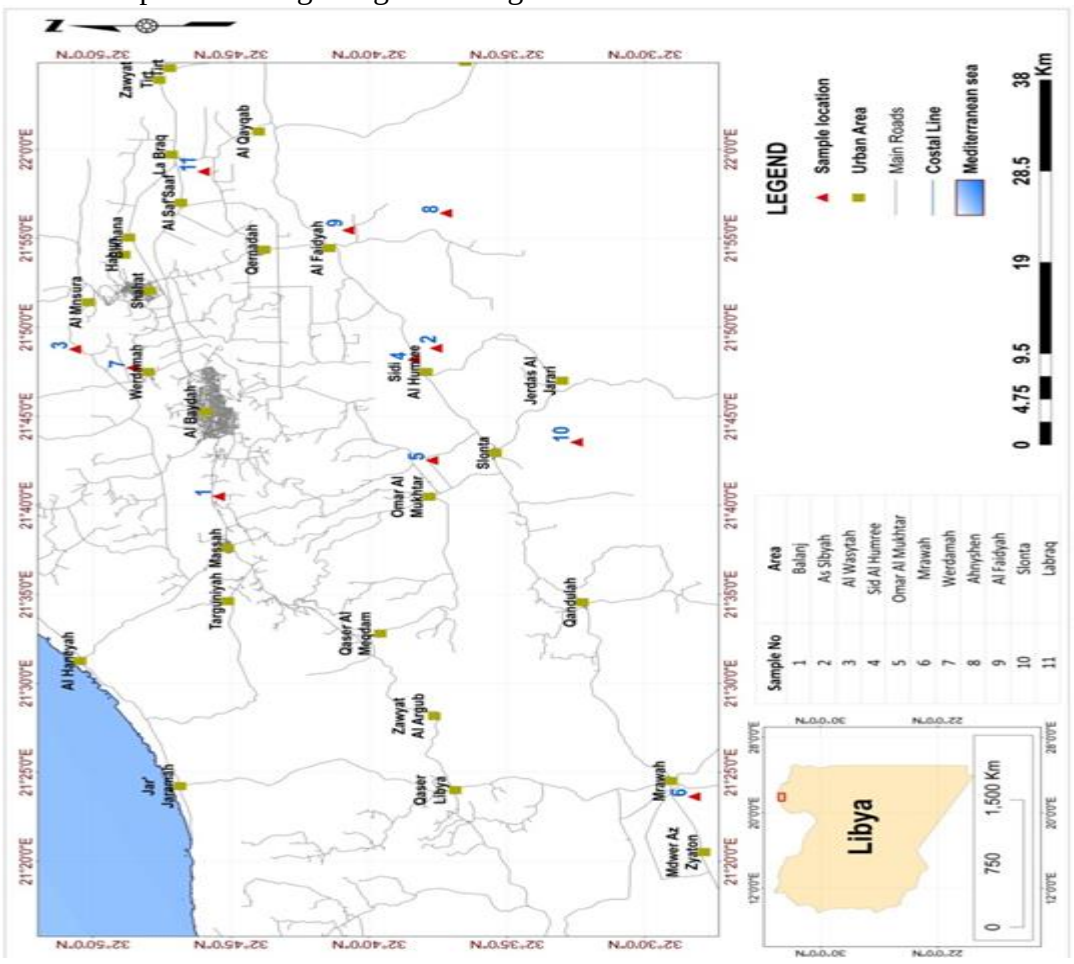

Figure 1: Study map.

Table (1): Scientific Classification.

Kingdom	Myceteae
Phylum	Basidiomycota
Class	Basidiomycetes
Subclass	Holobasidiomycetidae
Order	Agaricales
Family	Agaricaceae
Genus	***Agaricus***
Species	***A. bisporus***

2. Phenolic Concentration and Antioxidant Activities:

2.1. Reagents:

Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride (FeCl3•6H2O), aluminum trichloride ($AlCl_3.6H_2O$), sodium carbonate, gallic acid, , potassium peroxodisulfate, 6-hydroxy-2, 5,7,8-tetramethylchromane- 2-carboxylic acid (Trolox), 2,2′-azinobis-(3-ethylbenzo-thiazoline6-sulfonic acid) diammonium salt (ABTS), Butylated hydroxyanisole (BHA), (+)-catechin, Methanol HPLC grade were purchased from Sigma-Aldrich, Germany. Sodium nitrite, sodium hydroxide, potassium ferricyanide, trichloroacetic acid, was purchased from BDH Ltd. Pool England.

2.2. Preparation of Mushroom Extracts:

Six mushroom samples were selected at random from various regions (Alosita, Marawah, Werdama, Salantah, Sidihamri, and Faidiyah) in order to investigate their antioxidant, total flavonoid, and total phenolic content.

The caps of the fresh mushrooms were separated and freeze dried (Gamma 2-16 LSC, Christ, Germany), reduced to a fine dried powder, mixed to obtain homogenous samples, and stored at -20°C. A gram of the powdered freeze-dried mushroom was extracted using 80% methanol (50 ml) for over night at room temperature on orbital shaker at 120 rpm, and filtered using Whatman No. 1 filter paper.

2.3. Determination of Total Phenolics:

The total phenolic content of the mushroom extracts were determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965) with some modifications. 200 µl of the Folin–Ciocalteu reagent has been mixed with 100 µl of the mushroom extract. The mixture was incubated at room temperature for 5 minutes before 200 µl of 1N Na2CO3 and 1.5 ml of distilled water were added. The mixture was kept in the dark for 2 h, and the absorbance was then measured at 750 nm using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). Gallic acid with a concentration range of 40–120 µM was used as the standard for evaluating the phenolic content in tested samples. On a dry basis, the samples' total phenolic content was reported as milligrams of gallic acid equivalents (GAE) per gram of sample.

2.4. Determination of Total Flavonoids:

With minor modifications, the colorimetric method reported by Yoo et al. (2008) was used to measure the total flavonoid content. The 250 µl mushroom extract solution was combined with 75 µl of a 5% NaNO2 solution and 1.25 ml of deionized water. 150 µl of a 10% AlCl3.6H2O solution was added to the mixture after a period of six minutes. The Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England) was then used to measure the absorbance of the pink color at 510 nm in comparison to the blank after the mixture had been fully vortexed. The standard curve was obtained using (+)-catechin with a concentration range of 5 - 80 µg/ml, and the results were expressed as mg (+)-catechin equivalent (CAE) per gram of sample on a dry basis.

2.5. Reducing Power:

Reducing power of the mushroom extracts was determined according to the method described by (Vamanu & Nita, 2013). Using an Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England), absorbance was measured at 700 nm in comparison to a blank. The results were reported as milligrams of BHA equivalent

(BHAE) per gram of dry sample, with BHA at different concentrations used as the standard.

2.6. DPPH Radical Scavenging Activity:

The scavenging activity of the methanol extract on DPPH radicals was measured according to the method of (Sudha, *et al.*, 2012) with slight modification. An aliquot of 100 µl of extract was added to 1.9 mL of DPPH radical solution in methanol (0.1 mM), the mixture was shaken vigorously and left to stand for 60 minutes in the dark, and then using an Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England), the absorbance was recorded at 517 nm. Instead of using extract as a control, the extraction solvent (80% methanol) has been used as a blank. Using Trolox (0-400 µM) as a standard, the antioxidant activity was determined in µmole Trolox equivalents (µmol TE) per gram of dry sample.

2.7. ABTS Radical Cation Scavenging Activity:

With slight modifications, the Re et al. (1999) method was used to assess the spectrophotometric activity of ABTS radical scavenging. When 2.45 mM potassium persulfate and 7 mM ABTS in water reacted for 16 hours at room temperature in the dark, the result was the ABTS cation radical.

An absorbance of 0.800 ± 0.025 at 734 nm was measured after the ABTS solution was diluted with 80% methanol before to use. To measure the free radical scavenging activity, 100 µl of extract or Trolox standard (50–300 µM) was mixed with 1.9 ml of diluted ABTS solution. After 6 minutes, the absorbance was measured at 734 nm using 80% methanol as a blank rather than extract. The free radical scavenging activity of mushroom extracts was expressed as µmole Trolox equivalents per gram sample on dry weight basis.

3. Statistical Analyses:

Three replicates were used in the randomized complete design (RCD) experiment. Means ± standard error was used to express values, and one-way analysis of variance (ANOVA) was used to evaluate group differences at $P < 0.05$. Duncan's multiple range test with $\alpha = 0.05$ was used to separate the means. Microsoft Excel 2007 was the software used for all statistical analyses.

Results and discussion:

1. Total phenolics and Total Flavonoids Content:

Table (2) illustrates the total phenolics and total flavonoid content as well as the total flavonoid/total phenols ratio (TF/TP) of six studied mushroom samples. The total phenolic content of mushroom methanolic extracts ranged from 2.14 ± 0.02 to 4.37 ± 0.05 mg GAE/g dw, The Faidiyah region contained the lowest phenolic content, whereas the Alosita region contained the highest.

The differences in total phenolic content between mushroom samples were not always significant ($P \leq 0.05$).

A. bisporus (3.4 mg GAE/g dw) from Spain (Palacios et al., 2011) and A. bisporus (4.5 mg GAE/g dw) from Spain (Ramirez-Anguiano et al., 2007) had total phenolic content levels that are comparable to the results of the mushroom samples under evaluation.

According to Singha et al. (2012), the methanolic extract of A. bisporus from India (0.40 mg GAE/g dw) had a lower total phenolic concentration than in the present study, whereas lower than ethanolic extract of *A. bisporus* (8.0 mg GAE/g dw) from the United

States (Dubost, *et al.*, 2007) and ethanolic extract of *A. bisporus* (8.19 mg GAE/g dw) from India (Dhamodharan & Mirunalini, 2013), and much higher than that of water and ethanol extracts (42.47 and 30.93 mg GAE/g dw) of *P. ostreatus*, and (37.98 and 29.30 mg GAE/g dw) of *P. sajor-caju* respectively (Chirinang & Intarapichet, 2009).

Table (2): Total content of flavonoids and phenolics in mushrooms samples.

Region	Antioxidant Compounds		
	Total phenolic (mg GAE/g dw)*	Total flavonoid (mg CE/g dw)**	TP/TF Ratio (%)
Alosita	$4.37^{a} + 0.05$	$1.54^{a} + 0.01$	35.24%
Marawah	$3.65^{b} + 0.02$	$1.31^{c} + 0.02$	35.89%
Werdama	$2.71^{c} \pm 0.03$	$1.39^{b} \pm 0.01$	51.29%
Sidihamri	$2.67^{c} \pm 0.01$	$0.68^{e} \pm 0.02$	25.47%
Salantah	$2.67^{c} \pm 0.02$	$1.23^{d} \pm 0.02$	46.07%
Faidiyah	$2.14^{d} \pm 0.02$	$0.57^{f} \pm 0.02$	26.64%

a-e Different letters in a column indicate significantly different means ($P < 0.05$).

*Means (mg Gallic acid equivalent/g dry weight) ± standard error of triplicate

**Means (mg (+)-catechin equivalent/g dry weight) ± standard error of triplicate

The total flavonoid content of mushroom methanolic extracts ranged from 0.57 ± 0.02 for Faidiyah region to 1.54 ± 0.01 mg CAE/g dw for Alosita region (Table 4.2), the statistical analysis indicated that the differences among the samples were significant ($P \leq 0.05$). The regions with the highest and lowest flavonoid values, respectively, were Alosita and Faidiyah, which is similar to the results of total phenolic. For the studied mushroom samples, the TF/TP ratio varied from 25.47 for the Sidihamri region to 51.29% for the Werdama region. This indicates that the flavonoid concentration accounts for 25–51% of the total phenol content. The total flavonoids content in mushroom samples under study were higher than that of 0.41, 0.18, and 0.78 mg CAE/g dw for *L. edodes*, *H. erinaceus* and *A. aegerita* respectively, according to Mujic et al. (2010), which flavonoid content accounts for 16.96, 20.62, and 21.85% of the total phenol content. According to literature, because of their hydroxyl groups, phenolic compounds have the capacity to scavenge and are recognized to be potent antioxidants that break chains. The findings suggested that the high phenolic content of the ethanolic extract of A. bisporus may have contributed to its strong antioxidant activity both in vitro and in vivo (Liu et al., 2013a).

In fact, phenolics' ability to scavenge free radicals, inhibit lipoxygenase, and chelate metals may be associated to their bioactivity (Mallavadhani et al., 2006). According to Roedig-Penman and Gordon (1998), flavonoids have the ability to scavenge free radicals and inhibit the radical chain reactions that take place when triglycerides oxidize in food systems. Presence of phenolic compound in *A. bisporus* confirms its antioxidant activity and made this macrofungus a good candidate for formulating antioxidant products (Abah & Abah.G, 2010).

2. Antioxidant Activity:

2.1. Reducing Power Activity:

Several mechanisms have been proposed to explain the antioxidant activities of compounds, including preventing chain initiation, binding with transition metal ion catalysts, breaking down peroxides, stopping further hydrogen abstraction, reducing capacity, and radical scavenging ability (Chun-hui et al., 2007). One of these, the

decreasing a compound's capacity could be a significant indicator to its possible antioxidant properties.

Depending on the reducing power of each copound. the test solution's yellow color in this assay changes to different shades of green and blue. This method's Fe3+/ferricyanide complex is converted to the ferrous form when reducers are present. It is possible to determine the Fe2+ content by detecting the production of Perl's Prussian blue at 700 nm (Barros et al., 2008).

Table (3) represents the reducing power of the methanolic extract of the studied mushrooms, which ranged from 7.04 ± 0.02 to 3.40 ± 0.02 mg BHAE/g dw, in order of Alosita > Marawah > Faidiyah > Werdama = Sidihamri > Salantah. The difference between Werdama and Sidihamri was not significant ($P > 0.05$).

Interesting observation is that Faidiyah methanolic extract, which had the lowest phenolics and flavonoids content exhibited higher reducing power activity than that of Werdama, Sidihamri and Salantah methanolic extract. The reductive capacity is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action of phenolics (Nabavi, *et al.*, 2009). Due to its high phenolic and flavonoid content, Alosita extract demonstrated a stronger reductive capacity than other mushroom extracts.

2.2. DPPH Radical Scavenging Assay:

One often used model to evaluate free radical-scavenging ability is the DPPH radical scavenging assay (Naik et al., 2003). Side reactions like metal ion chelation and enzyme inhibition, which make tests like hydroxyl radical and superoxide anion more difficult, are avoided by the assay (Amarowicz et al., 2004). Antioxidants' hydrogen-donating properties are responsible for their capacity to scavenge DPPH (Liu et al., 2013b).

Table (3): Antioxidant activity of mushroom samples.

Region	Reducing power mg BHAE/g dw)*(	DPPH (µmol TE/g dw)**	ABTS (µmol TE/g dw)**
Alosita	$7.04^{a} + 0.02$	$24.26^{a} + 0.01$	$49.46^{b} + 0.24$
Marawah	$6.42^{b} \pm 0.01$	$23.68^{b} \pm 0.08$	$52.46^{a} \pm 0.29$
Werdama	$3.85^{d} \pm 0.04$	$12.96^{c} \pm 0.11$	$40.23^{e} \pm 0.10$
Sidihamri	$3.85^{d} \pm 0.02$	$11.21^{e} \pm 0.1$	$29.51^{f} \pm 0.15$
Salantah	$3.40^{e} \pm 0.02$	$12.47^{d} \pm 0.04$	$48.51^{c} \pm 0.15$
Faidiyah	$4.75^{c} + 0.01$	$9.83^{f} + 0.07$	$42.07^{d} + 0.51$

$^{a-e}$ Means within a column with different letter are significantly different ($P \leq 0.05$).

*Means (mg BHA equivalent/g dry weight) ± standard error of triplicate

**Means (micromole Trolox equivalent/g dry weight) ± standard error of triplicate

As shown in Table (3.2), Alosita region exhibited the highest DPPH radical scavenging activity (24.26± 0.01 µmol TE/g dw) on the other hand, Faidiyah extract showed the lowest radical scavenging activity (9.83± 0.07 µmol TE/g dw) and this compatible to lower its phenolics and flavonoids content. Furthermore, a significant difference ($P \leq 0.05$) in DPPH radical scavenging activity was observed among all extract.

Five *Agaricus* sp. mushrooms proved to have radical scavenging activity (Barros, *et al.*, 2008). According to (Reis et al., 2012b), the brown and white varieties of A. bisporus exhibited the highest DPPH radical scavenging activity among the different mushroom sp. The higher levels of total phenolics in the Alosita region's methanolic extract may be the main factors influencing the improved DPPH radical scavenging and reducing power results.

A methanolic extract of several edible mushrooms at a concentration of 6.4 mg/mL was used in research conducted by Menaga et al. (2013). Pleurotus geesteranus showed 81.8% scavenging potential against DPPH radicals, while Lentinus edodes, Flammulina velutipes in addition to oyster cap mushrooms recorded 43~70% scavenging activity against similar radicals.

Results from many studies different and this may be attribute to different environmental conditions, cultivation methods, stages of development when harvested, and genetic variation among the strains (Mwangi *et al.*, 2022).

2.3. ABTS Radical Scavenging Assay:

The assay on ABTS assesses the comparative capacity of antioxidants to scavenge the ABTS produced in the water phase when compared with a Trolox standard (water-soluble vitamin E). The ABTS is produced when a strong oxidant agent, for instance, potassium persulfate or potassium permanganate reacts with the ABTS-based salt (Mwangi *et al.*, 2022). The ABTS radical scavenging activity of methanolic mushroom extracts ranged from 52.46± 0.29 to 29.51 ± 0.15 µmol TE/g dw (Table 4-2). A significant difference ($P \leq 0.05$) was observed among all extract. Contrary to the total phenolics content and DPPH radical activity, Marawah region exhibited the highest ABTS radical scavenging activity while the lowest ABTS radical scavenging activity was traced by Sidihamri region.

The antioxidant activity values obtained using the ABTS assay were higher than those obtained using the DPPH assay for all extracts. Using the DPPH assay, the order of antioxidant efficiency was Alosita > Marawah > Werdama > Salantah > Sidihamri > Faidiyah, whereas, using the ABTS assay the order of antioxidant efficiency was Marawah > Alosita > Salantah > Faidiyah > Werdama > Sidihamri. The cause for these apparently discrepant results could be partly related to the fact that different methanolic extracts of wild mushroom samples may contain different types of polyphenolics with quite different reactivities. It should also be pointed out that the antioxidant activity of fungal extracts is not only given by phenolics. Several types of organic acids are also reactive in the various antioxidant methods (Carvajal, *et al.*, 2012). The high amounts of organic acids found in the mushroom extracts, especially citric acid, suggest that these compounds could be responsible, partly at least, for their high ABTS scavenging. Furthermore, possible synergistic effects involving phenolics and organic acids should not be ruled out and deserves future investigations (Carvajal, *et al.*, 2012).

Li *et al*. (2012) used different samples to investigate the antioxidant ability of mushroom extracts. They observed that the scavenging activity against ABTS+ radical rose consistently as the sample concentration was increased. The highest rate of radical scavenging of the mushroom species extracts ranged from 18.54% to 100% at a concentration level of 20 mg/mL. The Russula vinosa Lindblad acid extracts showed the highest antioxidant activity of all the extracts. In contrast to the alkaline and aqueous extracts, most of the acidic extracts exhibited higher scavenging activities (Panda et al., 2020).

Conclusion:

According to the findings of this investigation, extracts from wild mushrooms showed antioxidant activity in a variety of antioxidant studies. All wild A. bisporus extracts have been found to contain phenolic compounds, and all samples under investigation have the

ability to scavenge free radicals, reduce, and potentially function as major antioxidants. Alosita methanolic extract had the highest total phenolic, total flavonoid, and antioxidant activity among the studied mushroom samples, followed by Marawah methanolic extract in terms of phenolic contents and antioxidant capacities.

Wild edible mushrooms (A. bisporus) that grow in Al-Jabal Alakhdar have phenolic compounds, which indicate their antioxidant activity and make them a good choice for the production of antioxidant products.

The present study provides a thorough chemical analysis and assessment of the antioxidant properties of unreported wild mushroom species from Libya. Thus, this work intended to contribute to the increase of information concerning species of edible mushrooms as well as contribute to the conservation of these resources as sources of compounds of interest.

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