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Evaluation of Antioxidant for *Agaricus Bisporus* and Different Strains of *Pleurotus Ostreatus*

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Abstract

Four mushrooms were used in the present study, *Agaricus bisporus* (B62) strain (Lelion, Varrains , France) , *Pleurotus ostreatus* (Blue grey-BG) and *P. ostreatus* (White oyster-WH) from Mushroom Box Company, United Kingdom, while *P. ostreatus* (P2) was the local fungus isolated and identified in this study. Radical scavenging activity of alcoholic extracts from mushrooms was found to be higher than those of aqueous extracts at the same concentration tested in all results in the present study and alcoholic extract for *P. osteatus (*P2) gave the highest result in concentration (25) mg/ml (60.53±0.55 %) in DPPH test , reducing power (5.4±0.1), total phenolic component (11.46±0.05) mg/g, chelating activity (75.86±16.95%) and antioxidant activity (86±0.1%), if concentration decreased the antioxidant activity decreased also. In aqueous extract(57.40±2.16%) in DPPH test, reducingpower (3.63±0.05), total phenolic component (7.20±0.10) mg/g, chelating activity (73.66±1.15%) and antioxidant activity (70.53±0.37%) for the same fungus and same concentration.

Keywords: *Pleurotus ostreatus*,antioxidant

تقييم الفعاليه ضد الاكسدة للفطر الغذائي *Agaricus Bisporus* وسلالات مختلفه من الفطر *Pleurotus Ostreatus*

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الخلاصة

استخدمت في هذه الدراسة اربعة عراهين شملت الفطر *Agaricus bisporus* وسلالات مختلفة من الفطر*Pleurotus ostreatus* شملت السلالات *P.ostreatus* (Blue grey–BG) و *P. ostreatus* (White oyster–WH) مجهزة من شركة Mushroom Box الولايات المتحدة بينما تم عزل وتشخيص الفطر المحلي *P. ostreatus* (P2) واستعملت المستخلصات الكحولية والمائية لهذه الفطريات لتقييم الفعاليه ضد الاكسدة وكان المستخلص الكحولي للفطر المحلي *P. ostreatus* (P2) بتركيز (25) ملغرام لكل مليلتر اكفأ من باقي الفطريات بفعاليتة ضد الاكسدة في اختبار الداي فينيل بكريل هيدرازيل (60.53±0.55%) وبلغت قوة الاختزال (5.4±0.1) في حين بلغ المحتوى الفينولي (11.46±0.05) ملغم لكل غرام من حامض الكاليك والقابلية المخلبية كانت (75.86±16.95%) وكانت الفعالية المضادة للاكسدة (86±0.1%) وكلما قل تركيز المستخلص قلت فعاليتة ضد الاكسدة . اما فعالية المستخلص المائي المضادة للاكسدة فقد ابدى الفطر نفسه *P. ostreatus* (P2) اعلى فعالية ضد الاكسدة فقد بلغت في اختبار الداي فينل بكريل هيدرازيل (57.40 ± 2.16%) وبلغت قوة الاختزال(0.05 ± 3.63) والمحتوى الفينولي

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(7.20 ± 0.10) ملغم لكل غرام من حامض الكاليك فيما بلغت القابلية المخلبية (73.66 ± 1.15%) والفعالية ضد الاكسدة (70.53 ± 0.37%) لنفس الفطر وبنفس التركيز.

Introduction

Mushrooms possess high contents of qualitative protein, crude fiber, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, as antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory mushrooms [1]. [2] Found antioxidant properties of several ear mushrooms, many species of mushrooms have been found to be highly potent immune enhancers, potentiating animal and human immunity against cancer. Oxidation is essential for the production of energy to fuel biological processes in many living organisms, the recent changes in life style or environmental factors such as pollution, radiation, cigarette smoke and herbicides can generate free radicals among people worldwide. Thus, this can cause further oxidative stress responsible for damages in DNA, proteins and membranes in addition contribute to current non communicable diseases, many studies have found that some species of mushrooms especially from Basidiomycetes have therapeutic properties such as antioxidant, antimicrobial, anticancer and immuno stimulatory effects [3].

Material and methods

Mushrooms

Four mushrooms were used in the present study, *Agaricus bisporus* (B62) strain (Lelion, Varrains, France), *Pleurotus ostreatus* (Blue grey-BG) and *P. ostreatus* (White oyster-WH) from Mushroom Box Company, United Kingdom, while *P. ostreatus* (P2) was the local fungus isolated and identified in this study. All these strains were obtained from the Mushrooms Production Unit in the College of Agriculture, University of Tikrit. All fungal strains were routinely maintained on Malt Extract Agar (MEA) slants, then kept in refrigerated at (5) °C and sub culturing at least monthly.

Isolation and identification of *P. ostreatus* (P2)

The mushroom *P. ostreatus* (P2) was obtained from fruit body grown on peach tree in Salah Alddin province, the culture of this mushroom made by tissue culture method, briefly, a part (1×1) cm of fruitbody transferred to the center of MEA then incubated at (25) ^{o}C for (7-10) days. According to [4] this fungus classified as *P .ostreatus* depend on spore print, mycelium, spores, gills and dimensions of stalk and cap of its fruit body.

Preparation of alcohol extract

The mushroom extracts were prepared from dried mushrooms to evaluate the antioxidant property, the extractions were made using (95%) ethanol and water following the method of [5], the dried sample (10) g was extracted with (100) ml solvent at room temperature on a shaker at (150) rpm for (24) h and filtered through Whatman filter paper no. (4) , the residue was re-extracted twice and the filtrates were combined. The extract was evaporated almost to dryness in a rotary evaporator at (40) °C and then subjected to freeze drier, the dried extract was re-dissolved in the solvent to a concentration of (50) mg/ml, then prepared different concentrations (10,15,20,25) mg/ml for mushrooms and assay the antioxidant activity.

Preparation of aqueous extract

This method as (2-3) only replaced ethanol (95%) by distilled water (D.W.).

Estimation of antioxidants

DPPH (1, 1- dipheny l-2-picryl hydrazyl radical) assay

DPPH was used to determine the free radical scavenging activity of mushroom extract according to [6]. Mushroom extract at different concentrations (10, 15, 20, 25) mg/ml was added with an equal volume to ethanolic solution of DPPH (10) µM. After (15) min incubation at room temperature, the absorbance was read at (517) nm.

Reducing Power

The reducing power was determined according to the method of [7]. (2.5) ml for alcohol extract and water extract for mushrooms were mixed with (2.5) ml of (200) mM sodium phosphate buffer pH(6.6) and (2.5) ml of (1%) potassium ferricyanide and the mixture was incubated at (50) °C for (20) min, then (2.5) ml of (10%) trichloroacetic acid was added, the mixture was centrifuged for (10) min, the upper layer (5) ml was mixed with (5) ml of deionized water and (1) ml of (0.1%) ferric chloride

and the absorbance was measured at (700) nm. A solution with all reagents without the extracts was used as a blank; a higher absorbance indicates a higher reducing power.

Determination of Total Phenolic Compounds

Total amount of phenolic compounds was measured according to [8] A folin ciocalteure reagent was diluted with D.W. (1:10) and added (4) ml to (1) ml of ethanolic extract for mushroom and water extract, the color was developed by adding (5) ml of (7.5%) sodium carbonate solution in distilled water. UV-VIS spectrophotometer used to read the absorbance at (765) nm after (30) min. Gallic acid was used as a standard substance for calibration.

Chelating ability on ferrous ions

Chelating ability was determined according to the method of [9] included (1) ml for alcohol and water mushroom extract was completed by ethanol in alcohol extract or water in water extract to (3.7) ml then added (0.1) ml of (2) mM ferrous chloride. The reaction was initiated by the addition of (0.2) ml of (5) mM ferrozine. After (10) min at room temperature, the absorbance of the mixture was determined at (562) nm against a blank. Citric acid and ethylene diamino tetra acetic acid (EDTA) were used for comparison.

Antioxidant activity

The antioxidant activity was determined according [10] method. Mushroom extract was mixed with (2) ml of (10) mM linoleic acid emulsion in (0.2) M sodium phosphate buffer. Then (6.5) mM tween (20) was added to provide a stable emulsion and the mixture was incubated for (15) h in darkness, at (37) ^{o}C while shaken to accelerate oxidation. Then (0.2) ml of the antioxidant mixture was added to (6) ml absolute ethanol, the absorbance of the supernatant mixture was measured at (234) nm against a blank. Ascorbic acid and α -tocopherol were used as the positive control.

Statistical analysis

All analyses were performed in triplicate. Analysis of variance (ANOVA) was performed using Duncan's multiple range test to compare treatment means at ($P < 0.05$) using SPSS software version 16 (SPSS Inc., USA).

Result and discussion

Isolation and identification of *P. ostreatus* (P2)

The local isolate, *P. ostreatus* (P2) was identified depend on its macroscopic and microscopic features as follow: cap: (3-18) cm; convex, becoming flat or somewhat depressed; kidney-shaped to fan-shaped, or nearly circular if growing on the tops of substrate; pale brown to dark brown. Gills: Running down the stem; close; whitish. Stem: lateral about (1-3) cm in length and (0.5-1.5) cm in diameter stem sometimes absent when this mushroom is growing from the side of a substrate. When it grows on the tops of substrate develop a substantial and thick stem that is dry and slightly hairy near the base Flesh: Thick; white. Spores whitish to lilac, the dimensions of spores is (6-10 x 3-4) µ; smooth; cylindrical to narrowly kidney-shaped .Mycelium: white in color, sepetate and have clamp connection according to [4], this mushroom classified as *P. ostreatus* and coded as *P. ostreatus* (P2).

Evaluation of antioxidant of *A. bisporus*(B62) and different strains of *P. ostreatus*

Pleurotus ostreatus (P2) have highest scavenging activity for antioxidant ($p < 0.05$) than all other mushrooms due to their nutritional composition as well as their medicinal characteristics .The nutritional advantages include a low content of calories and a high content of proteins, minerals and dietary fiber .Alcoholic extract from all mushrooms have highest scavenging activity for antioxidant ($p<0.05$) than aqueous extract due to these bioactive compounds can soluble in alcoholic higher than water, most of bioactive compound have antioxidant activity [11].

Assay the antioxidant activity by different methods *In vitro*

The antioxidant activity of the samples was assessed through different methods because the response of antioxidants to different radical or oxidant sources may be different, therefore, no single assay accurately reflects the mechanism of action of all radical sources or all antioxidants in a complex system [12].

Scavenging Activity of DPPH Radical

In Figure-1 the alcoholic extract of *P. ostreatus* (P2) at (25) mg/ml showed the highest scavenging activity (60.53±0.55 %) minimum (60.00%) and maximum (61.10%). This result agree with [13] that observed the scavenging ability of alcoholic extract were similar to those of α-tocopherol, due to its physic-chemical characteristics of high solubility of phenolic compounds in organic solvents, these molecules are probably involved in the antioxidant found in alcoholic extracts, therefore, polar

solvents such as ethanol, ethyl acetate and acetone are widely used for the extraction of antioxidant components. However, extraction with ethanol often results in a higher recovery of total extractable compounds. This result not agree with [14] found the RSA of aqueous extracts (85%) at (20) mg/ml from *P.ostreatus* ($p < 0.05$) higher than of alcoholic extracts (30%) at the same concentration. In figure (1) also showed that the DPPH activity increases with increase the concentration, the DPPH activity of alcoholic extract for *P. ostreatus* (P2) (60.23±1.12%), (58.70±0.60%) and (55.80±5.05%) at (20), (15) and (10)mg/ml respectively, the absorbance decreases when concentration increases the decrease in absorbance is taken as a measure of the extent of radical scavenging, the method of scavenging DPPH, free radicals can be used to evaluate the antioxidant activity of extracts in a short time [15]. In Figure-2 aqueous extract of *P. ostreatus* (WH) ($p<0.05$) at (10) mg/ml was much lower than of all mushrooms in this study (35.13±0.32%) minimum (34.90%) and maximum (35.50%). [16] observed *P. ostreatus* to elicit antioxidant capacity using the DPPH method thereby expanding its nutraceutical value , DPPH a stable free radical with a characteristic absorption at (515) nm, was used to study the radical scavenging effects of extracts, as antioxidants donate protons to these radicals, free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity, the radical scavenging activity (RSA) of mushroom extracts was tested against the DPPH.

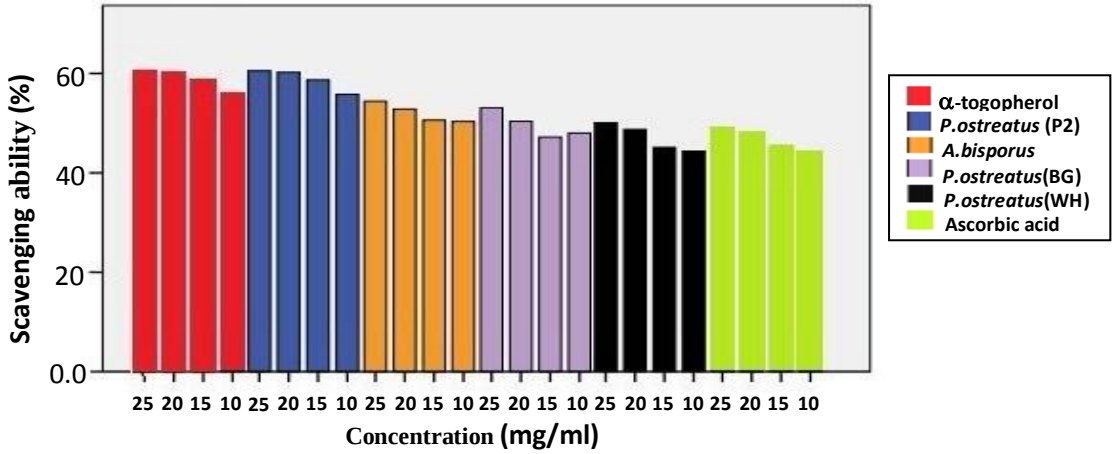

Figure 1- Scavenging activity of DPPH radical by mushroom alcoholic extracts mean ±standard deviation

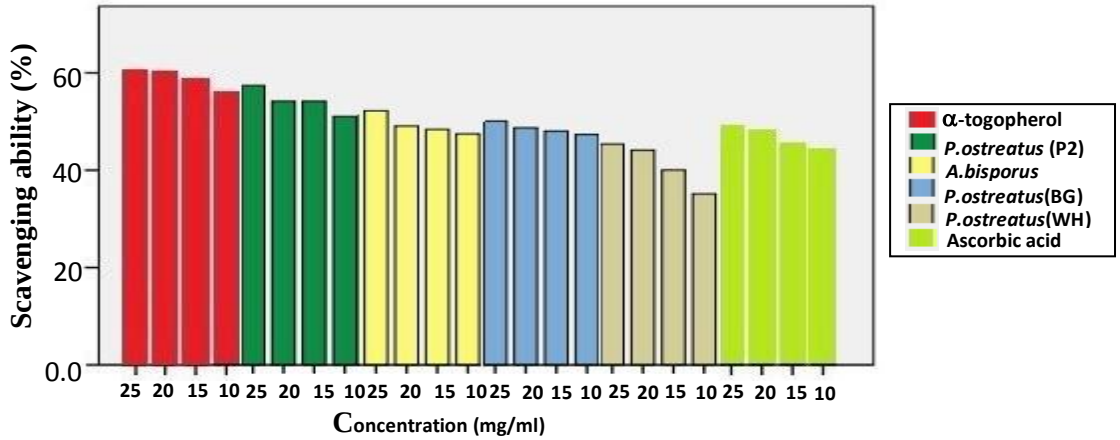

Figure 2- Scavenging activity of DPPH radical by mushroom aqueous extracts mean ±standard deviation

Reducing power

In Figure-3 the highest reducing power ($p < 0.05$) showed in *P. ostreatus* (P2) was (5.4±0.1) , minimum (5.3) and maximum (5.5) at (25) mg/ml in alcoholic extract, in aqueous extract (3.63±0.05), minimum (3.6) and maximum (3.7) at the same concentration .In Figure-4 the lowest reducing power ($p < 0.05$) showed in aqueous extract at (10) mg/ml of *P. ostreatus* (WH) was (0.8±0.05), minimum (0.8) and maximum (0.9) . In the present study, assay of reducing activity was based on the reduction of Fe 3+/ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the tested samples [7]. This result agreement with [17] were found that the reducing power increased as the

concentration of the tested samples increased (0.541 ± 0.03), (0.712 ± 0.05), (0.996 ±0.05) and (1.252±0.06) at (1,2,4,8,) mg/ml respectively, the increased reducing power observed may be due to the formation of reductants that could react with free radicals [18].This result agree also with [19] showed that the alcoholic extract exhibited the potent scavenging activity there by posses increased antioxidant.

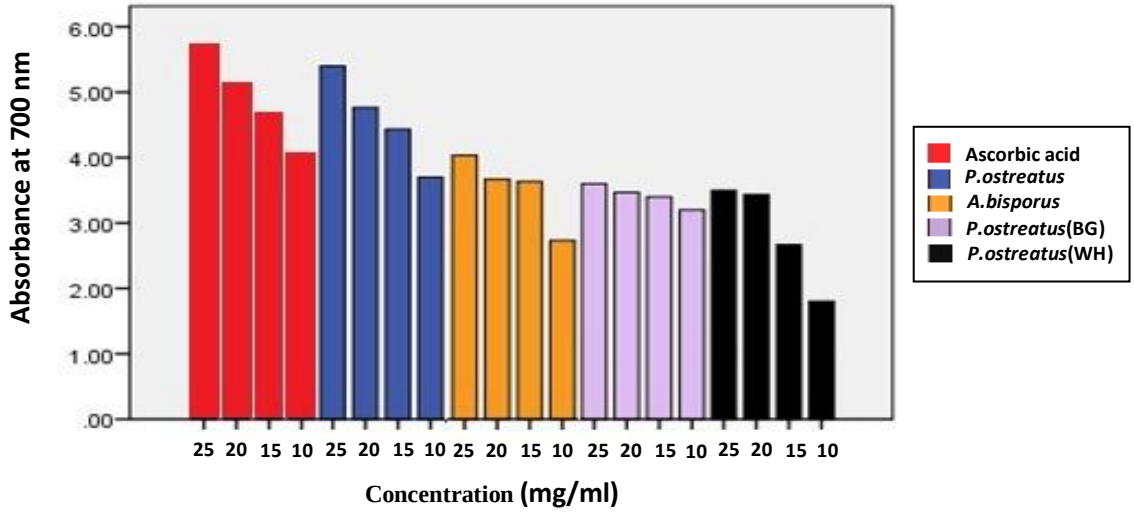

Figure 3- Reducing Power by alcoholic mushroom extracts mean ±standard deviation

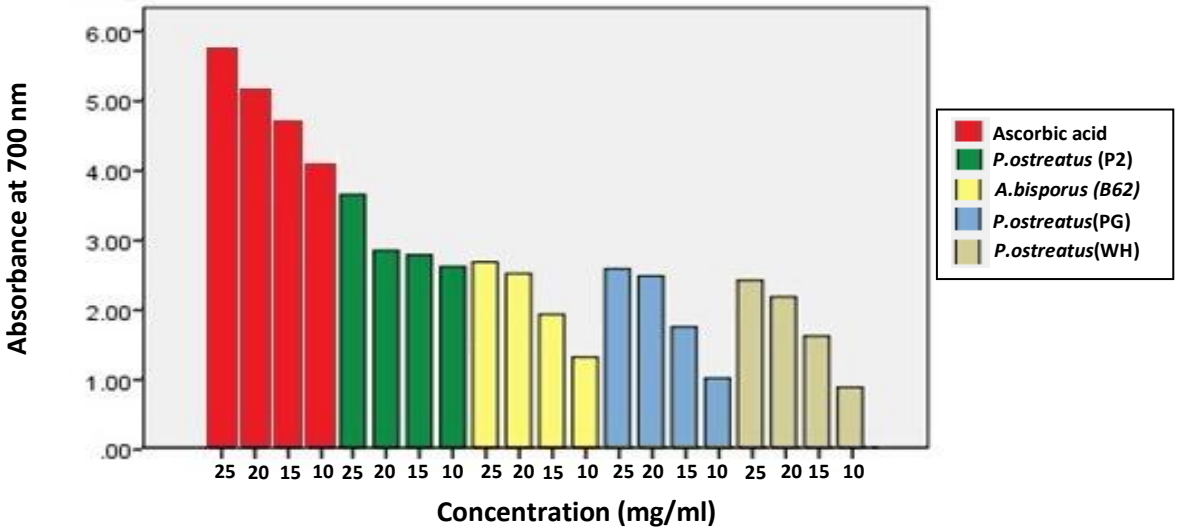

Figure 4- Reducing Power by aqueous mushroom extracts mean ±standard deviation

Total phenolic components

In Figure-5 showed highest phenol content ($p < 0.05$) in alcoholic extract of *P. ostreatus (*P2) was (11.46±0.05) mg/g at (25) mg/ml, minimum (11.4) mg/g and maximum (11.5) mg/g .In Figure-6 showed lowest phenol content was (1.8±0.1) mg/g in aqueous extract of *P. ostreatus* (WH) at (10) mg/ml, minimum (1.7) mg/g and maximum (1.9) mg/g .This results agree with [20] that reported alcoholic extract of *P. ostreatus (*P2) was better in reducing power, scavenging abilities and higher in the content of phenols, total amount of phenolic compounds in the ethanol extracts from all mushrooms were the highest comparative by aqueous extract, phenols are important mushroom constituents because of their scavenging ability due to their hydroxyl groups, total phenolic compounds may contribute directly to the antioxidative action, [21] reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation, [22] suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to (1.0) g was ingested daily from a diet rich in fruits and vegetable.[23] suggested that highest content of phenols in mushrooms might be the key components accounting for the better results found in antioxidant activity, reducing power, scavenging abilities as compared to other mushrooms. Numerous studies have conclusively showed

that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, because they act as antioxidants [24], therefore edible mushrooms may have potential as natural antioxidants in food. This result indicates that phenol may be the main antioxidant compounds found in mushrooms, in agreement with several authors [25].

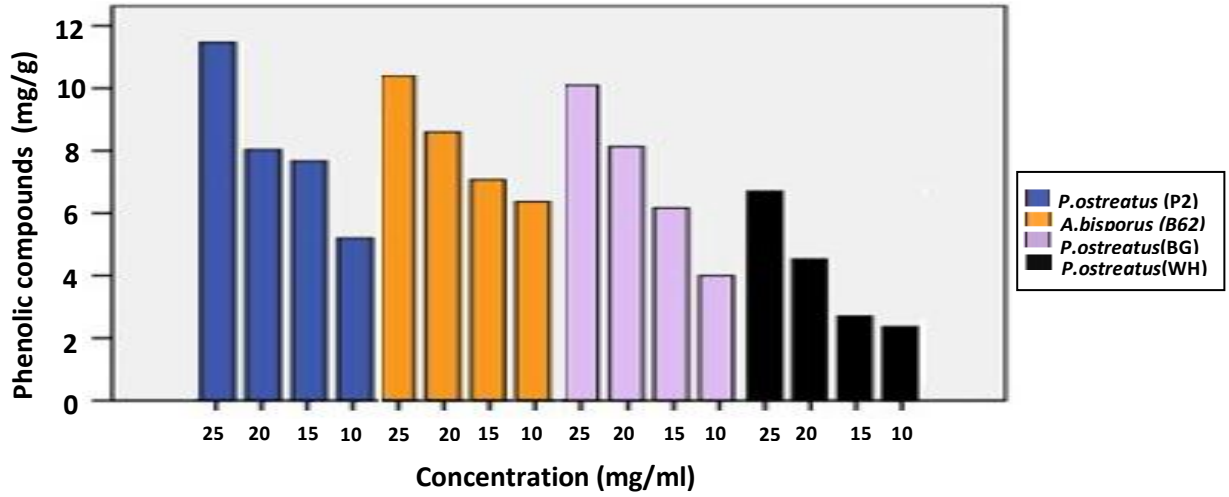

Figure 5- Total phenolic compounds by alcoholic mushroom extracts mean ±standard deviation

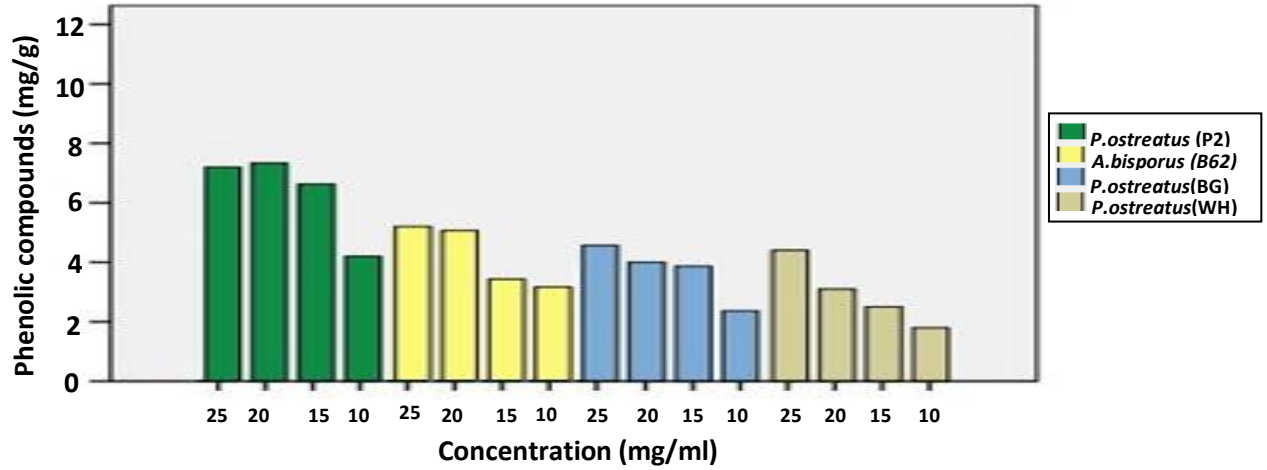

Figure 6- Total phenolic compounds by mushroom aqueous extracts mean ±standard deviation

Chelating ability

In our study Figure-7 showed the strongest chelating effect in alcoholic extract of *P. ostreatus (*P2) (75.86±16.95%) at (25) mg/ml, minimum (56.30%) and maximum (86.10%). This results agreement with (1) found that some phytochemicals are more soluble in alcohol than water due to the presence of major phytoconstituent. In Figure-8 Chelating ability in aqueous extract of *P. ostreatus* (P2) (68.66±1.15) at (25) mg/ml,(65.0±1.0) at (20) mg/ml, (65.0±0.0l) at (15) mg/ml and (60.26±0.15) at (10) mg/ ml. The chelating ability of a compound is defined as the formation of bonds between two or more separate binding sites within the same molecule and a single central atom [17].This result agree with [26] reported chelating ability increased as the concentration increases (88.2%) at (10) mg/ml and (65%) at (2) mg/ml. In Figure-8 also showed the lowest chelating effect was (26.10±0.10%) in aqueous extract of *P. ostreatus* (WH) at (10) mg/ml, minimum (40.00) and maximum (40.20).Chelating agents may serve as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. As ferrous ions are the most effective pro-oxidants in food systems, the high ferrous ion chelating ability of the various extracts from the fruiting bodies of *P. ostreatus* could be beneficial [15]. [22] Believed that transition metals are serving as the catalysts for the initial formation of radicals, chelating agents, on the other hand may stabilize transition metals in living systems and inhibit generation of free radicals, consequently reducing free radical mediated damage.

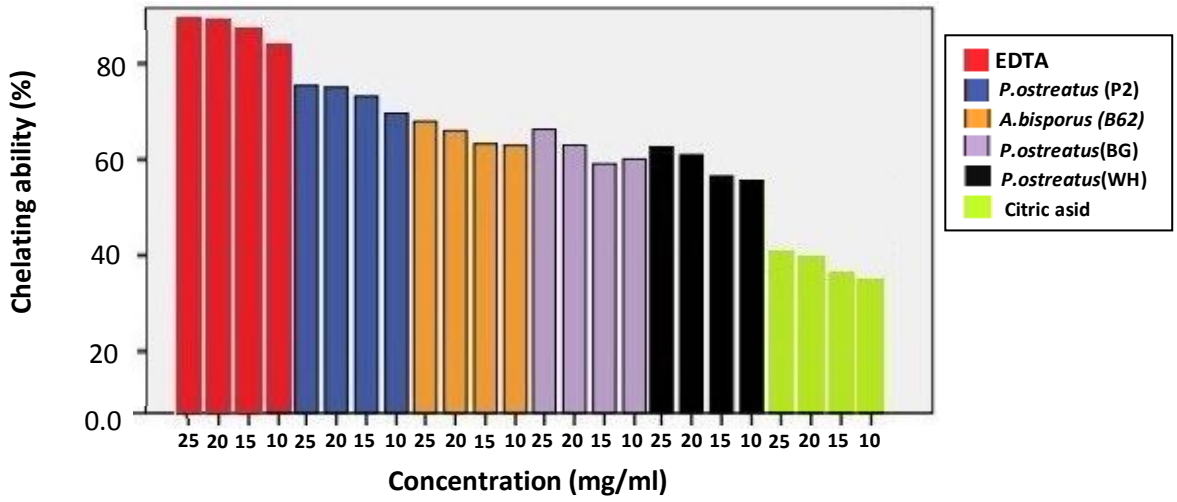

Figure 7- Chelating ability for mushroom alcoholic extracts mean ±standard deviation

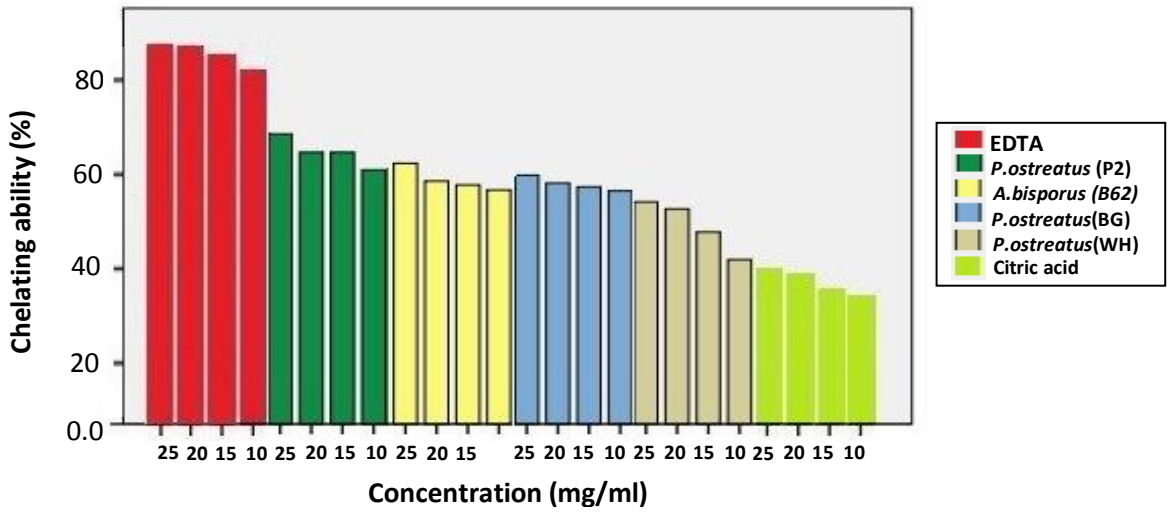

Figure 8- Chelating ability for mushroom aqueous extracts mean ±standard deviation.

Antioxidant activity

The highest antioxidant activity ($p < 0.05$) in alcoholic extract of *P. ostreatus (*P2) was (86±0.1%) at (25) mg/ml, minimum (85%) and maximum (87.1%).In While the lowest antioxidant activity was (47.5±0.5%) in aqueous extract of *P. ostreatus (*WH) at (10) mg/ml, minimum (45%) and maximum (50%). This result agree with [16] observed that antioxidant activity for *P. ostreatus* increased when concentration increases (32.56±0.05%) at (0.5) mg/ml and (92.41±0.08%) at (20) mg/ml .It is probable that the antioxidative components in the mushroom extracts can neutralizing the linoleate free radical [27].

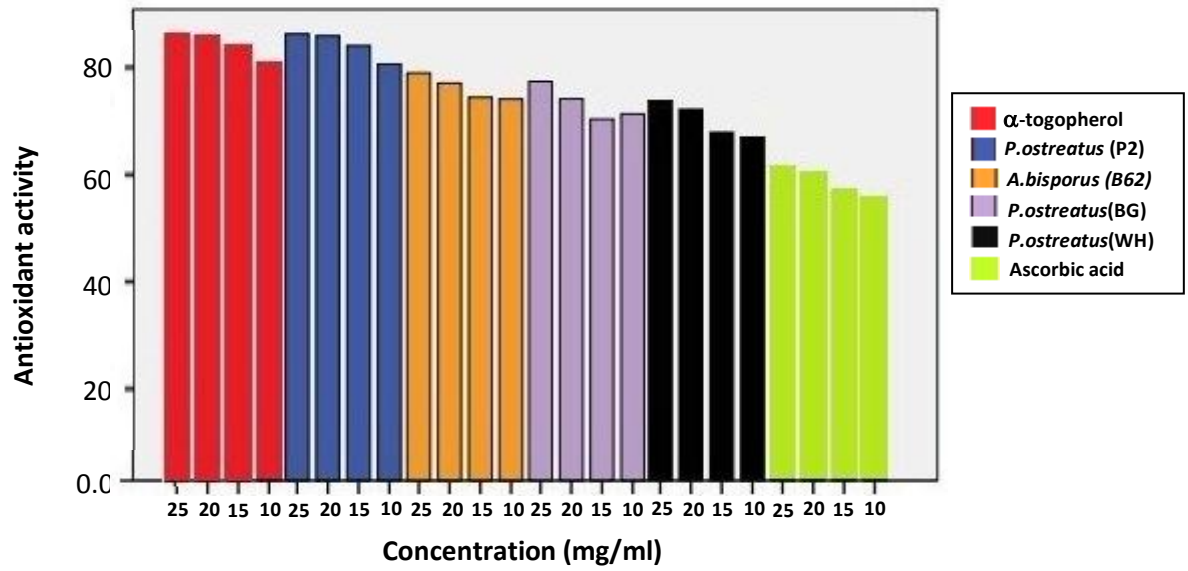

Figure 9- Antioxidant activity for mushroom alcoholic extracts

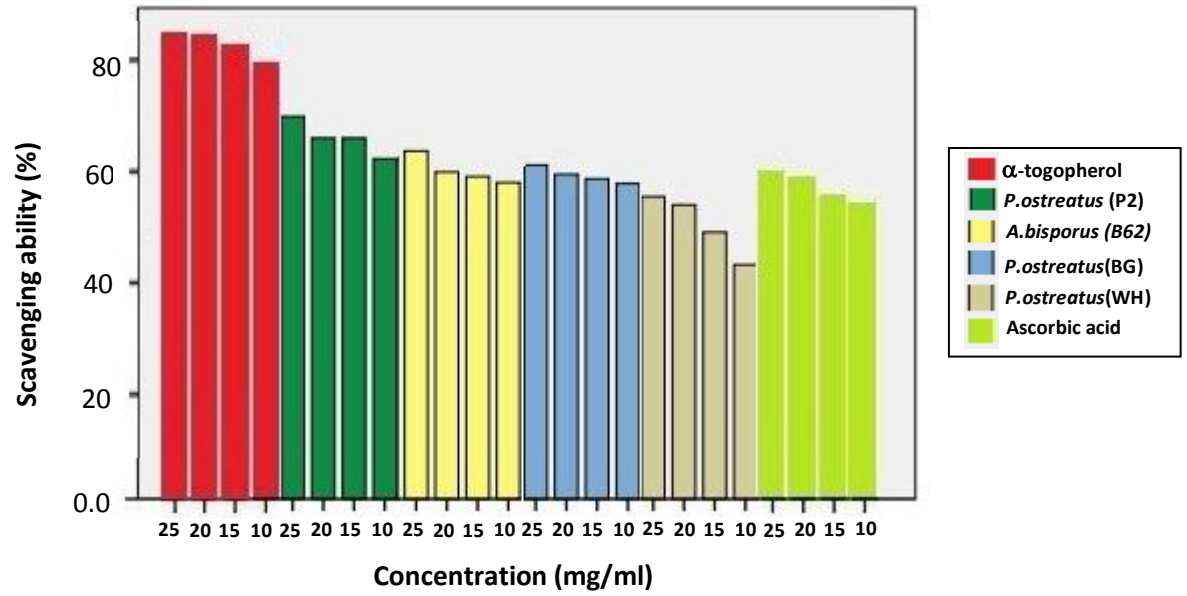

Figure 10- Antioxidant activity for mushroom aqueous extracts

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