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Effect of Boiling on the Antibacterial Activity of Agaricus bisporus and Pleurotus ostreatus Extracts Against Some Pathogenic Bacteria: An In Vitro Study

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Abstract

Agaricus bisporus and *Pleurotus ostreatus* extracts before and after boiling, along with their detailed chemical composition. The chemical analysis of dried mushroom extracts of *A. bisporus* and *P. ostreatus*, carried out before and after boiling, revealed notable variations in their biochemical constituents. The unboiled extract of *A. bisporus* contained protein (27.11%), vitamin C (33.62 mg/100 g), total phenols (8.90 mg/100 g), glutamine (187.2 mg/100 g), lysine (82.7 mg/100 g), β-glucan (6.33 mg/100 g), and glucose (0.677%), respectively. In contrast, the corresponding unboiled *P. ostreatus* extract contained protein (21.70%), vitamin C (55.03 mg/100 g), total phenols (14.97 mg/100 g), glutamine (101.9 mg/100 g), lysine (100.5 mg/100 g), β-glucan (8.17 mg/100 g), and glucose (0.800%), respectively. After boiling, the *A. bisporus* extract exhibited decreased levels of protein (15.29%), vitamin C (11.09 mg/100 g), total phenols (4.55 mg/100 g), glutamine (156.8 mg/100 g), lysine (77.6 mg/100 g), β-glucan (9.21 mg/100 g), and glucose (2.2%), while the *P. ostreatus* extract showed protein (12.88%), vitamin C (17.33 mg/100 g), total phenols (8.32 mg/100 g), glutamine (98.1 mg/100 g), lysine (95.0 mg/100 g), β-glucan (11.09 mg/100 g), and glucose (1.86%), respectively. The antimicrobial assay demonstrated that the *A. bisporus* extract before boiling exhibited potent *in vitro* antibacterial activity against all tested pathogens, showing inhibition zones of 11 mm against *Pseudomonas aeruginosa*, 15.2 mm against *Staphylococcus aureus*, and 14.7 mm against *Escherichia coli*. Similarly, *P. ostreatus* extract showed activity against all tested bacteria, with inhibition zone diameters ranging from 13.2 to 17.6 mm, indicating moderate inhibitory efficacy (average diameter 14.4 mm).However, all boiled ethanol extracts of *A. bisporus* and *P. ostreatus* exhibited reduced antibacterial

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effectiveness against *P. aeruginosa*, *S. aureus*, and *E. coli*, with inhibition zones ranging between 3.4 and 8.6 mm. These findings suggest that thermal processing (boiling) significantly affects both the chemical composition and antimicrobial potential of mushroom extracts, mainly due to the degradation of heat-sensitive bioactive compounds such as vitamin C and phenolic constituents.

Keywords: *Agaricus bisporus*, boiling, antimicrobial, *Pleurotus ostreatus*.

Introduction

Mushrooms are members of the Division Basidiomycota. Just 10% of approximately 14,000 species of mushrooms are well-known [1]. Antiviral, anticancer, and antitumor compounds were discovered in mushroom, [2]. Additionally, mushrooms have anti-diabetic, anti-inflammatory, cardiovascular, which has been traditionally employed in various regions worldwide for centuries as a therapeutic agent against numerous human diseases such as liver disease, fatigue and obesity. Anti-fibrotic, antioxidant, liver-protective, and anti-microbial properties [3]. With the use of hot water extraction techniques, *A.cylindracea* was discovered to contain high quantities regarding antioxidant compounds, including phenols, ascorbic acid, and tocopherols [4]. Numerous cultivated species, like *P. pulmonary*, *P. ostreatus*, *P. eryngii*, *P. cornucopia*, and *P. cystidiosus*, are members of genus Pleurotus [5]. There are about 70 known Pleurotus species. A large number of such species have antimicrobial characteristics. Anti-microbial activity against Gram-positive bacteria is present in mushroom extracts. *Agaricus bisporus* methanolic extract demonstrated activity towards *Bacillus cereus*, *Micrococcus flavus*, *Micrococcus luteus*, *S. epidermidis*, and *Staphylococcus aureus* [6] and MIC = 5µg/mL against *Bacillus subtilis* [7]. Methanolic extracts of *Agaricus bitorquis* demonstrated inhibitory effects on all tested Gram-positive bacteria, while methanolic extracts of *Agaricus silvicola* demonstrated anti-microbial properties against *Bacillus cereus* (MIC = 5.0µg/mL), *Staphylococcus aureus* (MIC = 5µg/mL), and *Bacillus subtilis* (MIC = 50µg/mL) [8]. Broad-spectrum anti-microbial activity has been demonstrated by *Pleurotus ostreatus*. Ethanolic extracts regarding *Pleurotus ostreatus* against *Sarcina lutea* demonstrated the maximum effect [9]. The two most widely cultivated mushrooms, *P. ostreatus* and *A. bisporus*, make up 38% of all cultivated mushrooms worldwide. Therefore, the present investigation primarily focused on evaluating the effect of boiling on the antibacterial activity of *Pleurotus ostreatus* and *Agaricus bisporus* extracts against several clinical bacterial isolates.

Materials and Methods

Mushroom samples collection:

The mushroom *Pleurotus ostreatus* and *Agaricus bisporus* were obtained from Mycology Laboratory, College of Education, Al-Qadisiyah University and stored in sterile Polyethylene bags at a 4°C temperature until used. After being transported to the laboratory, the material was kept at room temperature.

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Clinical Pathogenic Bacteria:

Two Gram-negative bacteria, *E. coli* and *P. aeruginosa*, and one Gram-positive bacteria, *S. aureus*, had been obtained from the Microbiology Laboratory at the College of Biotechnology at Al-Nahrain University and used in the presented study (table 1).

Table (1). The pathogenic Bacteria used for testing the antimicrobial activities.

Strains	Source
Staphylococcus aureus	Al-Nahrain university / College of Biotechnology
Escherichia coli	
Pseudomonas aeruginosa	

Preparation of Mushroom Extracts:

A total of 100 g of *P. ostreatus* and *A. bisporus* samples have been split to two parts; the first part was left raw, and the second has been cooked through boiling it for seven minutes at 100 Celsius. A total of 30 ml of methanol have been added to 10 g. of cooked and uncooked (separately) mushrooms, and the mix was after that put on a vial [10]. Total vitamin. nutrient, amino acid, total phenol, and sugar contents were then assessed in the cooked and uncooked (fresh) mushroom ethanol extracts in accordance with [11-15].

Antibacterial efficacy of boiled and unboiled mushroom ethanol extracts:

The fruiting bodies of both boiled and unboiled mushrooms were first dried. A total of 30 g of the dried material was then ground into a fine powder using a laboratory blender. The powdered samples were separately placed in extraction flasks and steeped in 200 mL of ethanol. Each flask was covered with aluminum foil and allowed to stand at room temperature for 48 hours to facilitate the extraction of bioactive compounds. A rotary evaporator was used for extracts at a temperature of 40 Celsius after they had been filtered through Whatman filter paper No. 1 [16, 17]. A stock solution with a concentration of 15mg/ml has been made after collecting the extracts. The positive control well just contained 60 µl of pure ethanol per well. The plates were kept in the incubator for 24 hours at a temperature of 37 Celsius. Following the incubation period, a ruler was utilized in order to quantify the inhibition zones in millimeters (mm) sterile swabs were used to spread 100 µL of each bacterial suspension uniformly onto Mueller-Hinton agar plates. Three wells, each with a diameter of 6 mm, were prepared in the agar using a sterile cork borer. Subsequently, 60 µL of each prepared ethanol extract from boiled and unboiled mushrooms (*Pleurotus ostreatus and Agaricus bisporus*) was carefully dispensed into the wells on each plats

Results and Discussion

Results of chemical Analysis of dried mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* extracts before and after boiling are shown in table (2). It has been revealed that the contents of Protein, Vitamin C, Total phenol, Glutamine, Lysine, β- glucan, Glucose before boiling were observed in extract of *A. bisporus* (27.11 %, 33.62mg/100g, 8.90mg/100g, 187.2mg/100, 82.7mg/100, 6. 33 mg/100g, 0.677 %)

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respectively. While in *P. ostreatus* extract were (21.70 %, 55. 03mg/100g, 14.97mg/100g, 101.9mg/100g, 100.5mg/100g, 8.17 mg/100g, 0.800 %) respectively.

The result of this study that presented in table2, showed that the contents of Protein, Vitamin C, Total phenol, Glutamine, Lysine, β- glucan, Glucose after boiling were observed in extract of *A. bisporus* were (15.29 %, 11.09mg/100g, 4.55mg/100g, 156.8mg/100g, 77.6mg/100g, 9.21 mg/100g, 2.2%) respectively, compared with the extract of *P. ostreatus* after boiling were contents of Protein, Vitamin C, Total phenol, Glutamine, Lysine, β- glucan, Glucose (12.88 %, 17. 33mg/100g, 8.32mg/100g, 98.1mg/100g, 95.0mg/100g, 11.09mg/100g, 1.86%) respectively.

Table (2). Chemical Analysis of dried mushrooms *Agaricus bisporus* and *Pleurotus ostreatus* extracts before and after boiling. (g /100 g)

Ingredients (g/100 g)	Ingredients contents before boiling		Ingredients contents after boiling	
	A. bisporus	*P. ostreatus*	*A. bisporus*	*P. ostreatus*
Protein (%)	27.11 %	21.70 %	15.29 %	12.88 %
Vitamin C (mg/100g)	33.62	55. 03	11.09	17. 33
Total phenol (mg/100g)	8.90	14.97	4.55	8.32
Glutamine (mg/100g)	187.2	101.9	156.8	98.1
Lysine (mg/100g)	82.7	100.5	77.6	95.0
β- glucan (mg/100g)	6. 33	8.17	9.21	11.09
Glucose	0.677 %	0.800 %	2.2%	1.86%

A nutrient-dense food, mushrooms are high in vitamins, protein, and minerals, they're also known to have substances that lower cholesterol and blood pressure, combat infectious diseases, and strengthen the immune system. One of the biggest genera regarding macro fungi is *Agaricus,* which has a number of edible species with excellent nutritional and medicinal value [18]. *A. bisporus* has nutritionally valuable bioactive compounds that support human health. Because it includes specific ingredients that improve facial beauty through regulating a variety of skin issues, such mushroom has also been used in cosmetics business, according to several research [19].

Endogenous amino acids, primarily arginine, alanine, histidine, glycine, aspartic acid, glutamic acid, serine, and proline are present in significant proportions in mushroom protein [20-22]. Protein in fresh *P. ostreatus* is 74% assailable, based on [23], although dried *P. ostreatus* and *A. bisporus* had assimilability rates of 84% and 77%, respectively, according to [19]. *A. bisporus* and *P. ostreatus* mushrooms have comparatively

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high levels of vitamin C, A, and β-carotene, which have beneficial effects due to their anti-oxidant qualities, according to [22].

Proline and glycine were the least prevalent endogenous amino acids in both species, but glutamine and asparagine were the most prevalent. In *A. bisporus*, the equivalent numbers were 12%, 19%, 5%, and 5% and 20%, 32%, 8%, and 8%. In *P. ostreatus*, these amino acids made up 10%, 12%, 5%, and 5% of total amino acids and 20%, 23%, 10%, and 9% of total endogenous amino acids, respectively [23]. It is commonly recognized that heat treatment alters the quality regarding the protein composition of food by promoting denaturation or degradation of proteins [24]. According to [24], boiling has a major impact on the availability and retention regarding phenolic compounds. The unprocessed *Lentinus edodes* (shiitake) mushroom has a total phenolic content of 6.37–8.68 mg/g. The sample of *L. edodes* (shiitake) mushrooms had the highest total phenolic following microwaving as well as the lowest following boiling and frying. This could be because the shiitake mushrooms come into direct contact with oil or water, which causes phenolic substances to be lost into the heating medium. Following frying and then boiling, the *L. edodes* mushroom's total phenolic content decreased by 26–36%.

It's possible that essential amino acids (EAAs). Though higher temperatures may cause negative structural changes that lessen proteins' vulnerability to enzymatic hydrolysis, FAAs are produced by inducing positive modifications in the protein structure under diverse boiling procedures [25]. However, according to [26], cellular breakdown as well as the oxidative and hydrolytic enzymes cause more bound phenolics to be released as the temperature rises. These might account for the research's findings that the overall phenolic content regarding mushrooms dropped as the temperature rose. According to [18], Pleurotus species are abundant in lysine.

Antibacterial activity of ethanol extracts from unboiled mushrooms

Prior to boiling, the *Pleurotus ostreatus* and *Agaricus bisporus* extracts have been screened using Agar-Well Diffusion method, and the growth inhibition zones against certain bacterial pathogens were quantified in millimeters. The findings are displayed in table3. *In vitro* anti-bacterial activity of *Agaricus bisporus* extract against all tested pathogens has been demonstrated. The extract from *A. bisporus* exhibited the highest activity against *S. aureus* (15.2mm), *P. aeruginosa* (11mm), and *E. coli* (14.7mm). Additionally, *P. ostreatus* extract has demonstrated activity against all bacterial pathogens, as evidenced by inhibition zone sizes ranging from 13.2 to 17.6 mm. With inhibition zone diameters of 14.4 mm, *P. ostreatus* extract has demonstrated a moderate inhibitory efficacy against pathogenic bacteria.

Table (3). Effect activity of *Pleurotus ostreatus* and *Agaricus bisporus* extracts against some pathogenic bacteria before boiling.

Mushroom species	Inhibition zone before boiling (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
Agaricus bisporus	15.2	14.7	11.0
Pleurotus ostreatus	14.4	17.6	13.2

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P. ostreatus exhibits a wide range of anti-microbial activity, based on similar research [26]. The antimicrobial activity regarding *Terfezia boudieri, Agaricus bisporus, P. sajor-caju, P. ostreatus, P. eryngii var. eryngii, P. eryngii var. ferulae,* and *Pleurotus eryngii var. eryngii* was examined in the work by Akyuz et al. (2010) [27]. The disk diffusion method was used to assess anti-microbial activity of methyl alcohol extract of *Pleurotus spp., A. bisporus, and T. boudieri* with the use of *S. aureus, E. coli, Bacillus megaterium, Candida albicans, K. pneumoniae, Trichophyton spp., C. glabrata, and Epidermophyton spp*. At the conclusion of the experiments, it has been demonstrated that methyl alcohol extracts regarding *Pleurotus* species, *T. boudieri*, and *A. bisporus* inhibited the microorganism's growth to varying degrees (7.50mm-15.50mm). Additionally, the extract from mushrooms has less antimicrobial activity than a comparison antibiotic (13mm–18mm).

Traditional medicine has made considerable use of mushrooms as antiviral, antimicrobial, and antitumor medicines. Because of widespread antimicrobial resistance, infectious diseases continue to pose a serious risk to human health. The search for effective and novel anti-microbial compounds has increased as a result.[28] utilized the agar disc diffusion approach for examining the anti-microbial activity of 10 mushroom species (*Amanita sp., Amanita zambiana, Cantharellus miomboensis, Boletus edulis, Cantharellus heinemannianus, Ganoderma lucidum, Coprinus sp., Cantharellus symoensii, Trametes strumosa, and Lactarius kabansus)* in both aqueous (hot and cold) and organic solvents (ethanol, methanol, and acetone). For every bacterium examined, the mushrooms' crude extracts shown antibacterial qualities. In tests against bacteria, ethanol extracts have been the most efficient (36.50%), followed by acetone (30.80%) and methanol (30.80%). Despite containing the extract with the highest inhibitory activity (14mm), aqueous extracts had the least impact on the inhibition regarding bacterial growth (1.9%). The second-highest inhibitory value was 11.5mm for acetone extract of *Cantharellus symoensii*, followed by 11.0, 10.67, and 10.0 mm for methanol extract of *Cantharellus miomboensis* and ethanol extracts of *Ganoderma lucidum* and *C. symoensii*, respectively. As demonstrated by various extracts exhibiting high inhibitory characteristics ranging between 8mm and 14mm (15.40% (8) each), followed by *G. lucidum* (13.50% (7)), *Cantharellus heinemannianus* and *C. symoensii* had maximum effect on bacterial inhibition, whereas *Coprinus sp., Boletus edulis, and Trametes strumosa* had the lowest (5.80% (3) each). The development of antimicrobial medicines and additional phytochemical research against common fungal and bacterial infections in humans are based on the favorable results regarding screening local mushrooms for anti-bacterial activity. According to certain research, *Pleurotus ostreatus* as well as *Agaricus bisporus* have anti-inflammatory qualities. Comparable results from Iraqi strains were reported by Risan *et al.* (2017) [29] in their assessment of *Pleurotus ostreatus* and *Agaricus bisporus* activity against two clinical isolates, *S. aureus* and *E. coli*. *Agaricus bisporus* and *Pleurotus ostreatus* cold and hot water extracts, respectively, demonstrated the highest inhibition against *S. aureus* and *E. coli* at 75% concentration. The maximum concentration (75%) of chloroform, ethanol, and acetone mushroom extracts regarding *Agaricus bisporus* as well as *Pleurotus ostreatus* resulted in the greatest growth inhibition for *S. aureus* and *E. coli*. The impact of crude extract on the development and growth of both gram-negative and gram-positive bacteria was investigated by Soltanian et al. (2016) [30]. Inhibitory effects on gram-positive bacteria were just seen in crude extract from wild as well as cultivated A. bisporus.

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P. sajor-caju, P. ostreatus, Agaricus bisporus, Terfezia boudieri, and *Pleurotus eryngii var. eryngii* were all examined for their anti-microbial activity. The disk diffusion method was used to assess anti-microbial activity of methyl alcohol extract of *Pleurotus spp., A. bisporus*, and *T. boudieri* with the use of *S. aureus, K. pneumoniae, Bacillus megaterium, E. coli, Candida albicans, C. glabrata, Trichophyton spp., and Epidermophyton spp*. At the conclusion of the experiments, it has been demonstrated that methyl alcohol extracts of *Pleurotus* species, *T. boudieri*, and *A. bisporus* inhibited the growth regarding microorganisms to varying degrees (7.50-15.50mm). Additionally, the extract from mushrooms has less antimicrobial activity than a comparison antibiotic (13mm–18mm). [26]

Antibacterial activity of ethanol extract from boiled mushroom

The findings of the present study demonstrated that the ethanol extracts from boiled *Pleurotus ostreatus* and *Agaricus bisporus* mushrooms exhibited lower inhibitory activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* compared to the unboiled extracts.

(Table4). The inhibition zone measured between 3.4 and 8.6 mm.

Table (4). Effect activity of *Agaricus bisporus* and *Pleurotus ostreatus* extracts against some pathogenic bacteria after boiling.

Mushroom species	Inhibition zone after boiling (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
Agaricus bisporus	6.9	3.7	5.4
Pleurotus ostreatus	7.3	8.6	3.4

The antimicrobial activity of *Agaricus bisporus* ethanol extracts, both before and after boiling, was evaluated against three Gram-negative bacterial species, two Gram-positive bacterial species, and one yeast strain. The *A. bisporus* extract exhibited a significant inhibitory effect, particularly against the tested Gram-positive bacteria. Like *B. subtilis*, while having antibacterial range against Gram-negative bacteria. The range of maximal inhibition zones was 12–22 mm. With a diameter of 18 mm for boiled extract and 22 mm for the raw extract, *S. aureus* was the most vulnerable bacteria. Both *A. bisporus* extracts demonstrated antibacterial activity against *P. aeruginosa*, although the activity range was smaller in boiled extract (12) mm in comparison with raw mushroom extract (16) mm. The boiled ethanol extract of *A. bisporus* exhibited no antibacterial activity against *K. pneumoniae*, yet a clear zone of 15 mm diameter was seen in the raw extract. This could be because of the way that temperature affected the compound that causes the activity. [27].

Conclusions

The antimicrobial activity of *Agaricus bisporus* and *Pleurolus ostreatus* extracts before and after boiling were tested against gram negative and gram positive species. The pre-boiling extracts exhibited higher inhibitory activity, indicating the presence of bioactive compounds such as protein, vitamin C, glutamine, lysine and phenolic compounds. In contrast, after boiling extracts showed a marked decrease in

antimicrobial activity. Therefore both *Agaricus bisporus* and *Pleurolus ostreatus* possess promising antimicrobial properties.

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Author's Declaration

-Extracts of *Agaricus bisporus* and *Pleurolus ostreatus* have demonstrated *in vitro* antimicrobial activity against various gram negative and positive bacteria.

- More research is needed to confirm safety, stability and real –world application.

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