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PHYTOCHEMICAL PROFILES AND ANTIMICROBIAL ACTIVITIES OF *PHELLINUS* MUSHROOM: IMPLICATIONS FOR AGRICULTURAL HEALTH AND CROP PROTECTION

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Abstract

Mushrooms have long been recongnized for their capacity to produce a wide array of bioactive compounds with notable antimicrobial properties, rendering them valuable for both culinary and medicinal applications across diverse cultures. This study aims to evaluate the bioactive compounds and antimicrobial properties of the medicinal *Phellinus* mushroom. Phytochemical analysis was performed utilizing colorimetric assays and precipitate formation techniques, which identified the presence of carbohydrates, glycosides, alkaloids, sterols, terpenes, and tannins within the *Phellinus* species. Furthermore, antimicrobial screening of the ethyl acetate (EtOAc) extract demonstrated significant inhibitory activity against both *Escherichia coli* and *Staphylococcus aureus*. These results indicate that the antimicrobial effects of the *Phellinus* mushroom can be attributed to its bioactive metabolites, highlighting its potential as a natural source of antimicrobial agents. Additionally, the integration of *Phellinus* mushrooms into agricultural practices may provide innovative strategies for pest management and soil health, capitalizing on their bioactive properties to enhance crop protection and promote sustainability.

Keywords: Antibacterial, Antifungal, Mushroom, Phytochemical screening, *Phellinus* sp.

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Mushrooms represent a vital natural resource, historically consumed for their culinary and medicinal properties across various cultures (Chowdhury *et al.*, 2015; Zatout *et al.*, 2021a). Despite the identification of approximately 16,000 mushroom species globally, only a limited number have been extensively studied. Of these, about 10% are well-documented, with 3,000 classified as edible and 700 recognized for their medicinal value (Liu *et al.*, 2022; Wasser 2002; Zatout and Chaouche, 2023a).

Mushrooms are reservoirs of diverse phytochemical constituents, including minerals, amino acids, vitamins, crude fiber, proteins, carbohydrates, ergosterol, and various secondary metabolites such as terpenoids, steroids, polyphenols, flavonoids, and alkaloids (Li *et al.*, 2021; Zatout and Chaouche, 2023a). Notably, edible medicinal mushrooms exhibit a remarkable range of biological activities, encompassing antibacterial, antifungal, antiviral, antitumor, antioxidant, hepatoprotective, anti-diabetic, cholesterol-lowering, genoprotective, cardiovascular protective, anti-inflammatory, autoimmune, neuroprotective, and osteoprotective effects (Ahmad, 2018; Ganeshpurkar *et al.*, 2021; Kumar and Kumari, 2021; Xu *et al.*, 2022; Yang *et al.*, 2019; Zatout *et al.*, 2021a).

In agricultural contexts, mushroom substrate, known for its rich nutrient profile and organic matter content, serves as an excellent candidate for biofertilization and soil enhancement. This substrate also presents a sustainable alternative to conventional chemical fertilizers, addressing both energy consumption and environmental concerns (Leong *et al.*, 2022; Pérez-Chávez *et al.*, 2019). The genus *Phellinus*, belonging to the Hymenochaetaceae family within the kingdom Fungi, is predominantly found in tropical America, Africa, and East Asia (Ruggiero *et al.*, 2015; Yang *et al.*, 2016). *Phellinus* species are characterized by their wood-inhabiting, saprophytic, and parasitic nature, known for inducing white rot in both angiosperm and gymnosperm trees (Saha *et al.*, 2018; Bala *et al.*, 2019).

Traditionally, *Phellinus* mushrooms have been recognized for their medicinal properties, with research indicating the presence of triterpenoids, phenolics, lignins, ergosterol, and other bioactive compounds (Chen *et al.*, 2013; Li *et al.*, 2011; Sunthudlakhar *et al.*, 2022). However, studies on the phytochemical composition and biological activities of *Phellinus* mushrooms in Algeria remain limited (Kebaili *et al.*, 2021; Toumi *et al.*, 2020; Zatout *et al.*, 2021b; Zatout *et al.*, 2023b).

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Therefore, this study aims to investigate the presence of secondary metabolites in the aqueous and organic extracts of the edible medicinal mushroom *Phellinus* sp. and to assess their antimicrobial activity. These findings may provide innovative strategies for pest management and soil health in agricultural practices, leveraging the bioactive properties of *Phellinus* mushrooms to enhance crop protection and sustainability.

MATERIALS AND METHODS

Collection of samples

Fresh *Phellinus* mushrooms were collected from the Djebel El Ouahch forest in Constantine, Algeria (Fig. 1) during March 2018. The species was identified by Dr. Zatout at the Laboratory of Mycology, Biotechnology, and Microbial Activity at the University of Mentouri Constantine 1. Following collection, the mushrooms were washed with water, then dried and ground into a fine powder.

Extraction of samples

The extraction protocol was adapted from the methods described by Masi *et al.* (2019) and Zatout *et al.* (2021a). A total of 100 mL of a methanol-water mixture (1:1) was added to 20 g of powdered fungal material. The mixture was stirred with a magnetic stirrer for 20 minutes, after which it was filtered using filter paper under vacuum conditions. A second maceration was performed under the same conditions. The two filtrates were then combined, and the solvent was removed using a rotary evaporator under reduced pressure.

To obtain the organic extract, the combined filtrates were mixed with 100 mL of ethyl acetate (EtOAc) in a separatory funnel. The mixture was stirred to facilitate phase separation. The organic phase was subsequently dried with anhydrous sodium sulfate (Na_2SO_4) and evaporated under reduced pressure. Both the resulting organic extract residues and the aqueous phases were collected for antimicrobial testing.

Phytochemical screening

Phytochemical screening was performed using standard qualitative methods to assess various constituents in the *Phellinus* mushroom, including reducing compounds, cardiac glycosides, alkaloids, flavonoids, tannins, sterols, and terpenoids. These screening tests were conducted in accordance with the procedures outlined by Harborne (1973) and Zatout and Chaouche (2023a), as follows:

A. Aqueous extract

To prepare the aqueous extract, 1 g of fungal powder was added to 20 mL of boiling water and maintained at a boil for 15 minutes. The mixture was then filtered, and the residue was rinsed with a small amount of hot water to yield a total of 20 mL of filtrate.

B. Methanoic Extract

To prepare the methanolic extract, 1 g of fungal powder was introduced into 20 mL of methanol and macerated for 24 hours.

C. Screening

1. **Reducing compounds:** To test for reducing compounds, 2 mL of the aqueous extract was added to a test tube, followed by the addition of 2 mL of Fehling's solution. The mixture was then placed in a water bath for 8 minutes. The formation of a brick-red precipitate indicates the presence of reducing compounds.
2. **Cardiac glycosides:** To test for cardiac glycosides, 2 mL of chloroform ($CHCl_3$) was added to 1 mL of the methanolic extract. The addition of concentrated sulfuric acid (H_2SO_4) resulted in the development of a reddish-brown colour, indicating the presence of cardiac glycosides.
3. **Alkaloids:** To test for alkaloids, 10 g of *Phellinus* mushroom powder was added to 50 mL of 1:10





Fig. 1. A. The Jebel El Ouahch region (Kimour *et al*., 2023), B. The Jebel El Ouahch Forest Zatout, 2018



Fig. 2. *Phellinus* spp. Picture by Zatout, 2018

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diluted sulfuric acid (H_2SO_4) with distilled water. This mixture was stirred and macerated for 24 hours. Following maceration, 1 mL of the filtrate was treated with 5 drops of Dragendorff's reagent. The appearance of an orange precipitate indicates the presence of alkaloids.

4. **Tannins:** To test for the presence of tannins, 1 mL of the fungal extract was mixed with 1 mL of water, followed by the addition of 1 to 2 drops of a diluted ferric chloride solution (1%). The development of a dark green or blue-green colour indicates the presence of tannins.
5. **Flavonoids:** Flavonoids were assessed through a reaction with cyanidin. To do this, 2 mL of the fungal extract was evaporated, and the residue was reconstituted in 5 mL of 2:1 diluted hydrochloric alcohol. The addition of 2 to 3 magnesium shavings produced a heat release, resulting in a pink-orange or purplish colour. The addition of 3 drops of iso-amyl alcohol further intensified this coloration, confirming the presence of flavonoids.
6. **Sterols and triterpenes:** Sterols and triterpenes were investigated using the Liebermann reaction. In this procedure, 5 mL of the petroleum ether extract was placed in a test tube and evaporated to dryness on a water bath. The residue was then dissolved in 1 mL of acetic anhydride and 1 mL of chloroform. A few drops of concentrated sulfuric acid were added, resulting in the formation of a violet ring at the interface, with the supernatant turning violet. This coloration indicates the presence of sterols and triterpenoids.

Antimicrobial activity

The antimicrobial activities of the organic extract (ethyl acetate, EtOAc) and the aqueous extract of *Phellinus* mushroom were assessed against three bacterial strains—*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp.—as well as two fungal species—*Fusarium oxysporum* and *Aspergillus niger*. These activities were evaluated using the Agar Disk Diffusion method with minor modifications. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) to achieve the required concentrations. Young microbial cultures were tested, with bacterial strains incubated for 24 hours and fungal mycelia for 120 hours. Filter paper discs (Whatman No. 1; 6 mm in diameter) were impregnated with 10 µL of the sample and placed on the inoculated agar plates, which were then incubated at 37 °C. The zones of inhibition were measured by calculating the diameter of the inhibition zones surrounding the discs.

Statistical analysis

All tests, including controls, were carried out in triplicate and the experimental data were reported as means ± standard deviation. Statistical analysis was carried out using XLSTAT software (Addinsoft SARL, New York, NY, USA). Significant differences were determined at $p \leq 0.05$ by one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screenings were carried out using specific reactions based on precipitation or coloration phenomena induced by reagents characteristic of each compound family (Table 1). The results of the phytochemical analysis of the *Phellinus* mushroom align with previous reports regarding the presence of certain chemical families (Azeem *et al.*, 2016; Azeem *et al.*, 2018; Benedict and Brady, 1992;

Table 1. Phytochemical screening of *Phellinus* sp. extract

Carbohydrates	Cardiac Glycosides	Alkaloids	Flavonoides	Tanins	Sterols/ triterpenes
+++*	++	+++	-	+	++

*Highly positive: +++, Fairly positive: ++, Weakly positive: +, Negative test: -

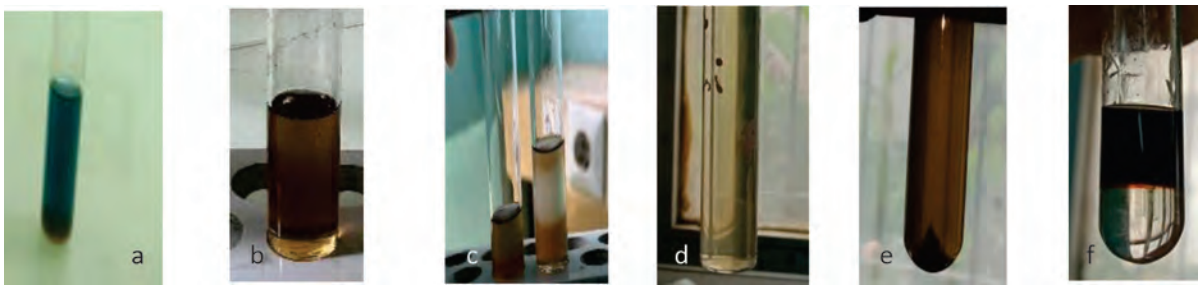


Plate I. Reaction colours of different metabolites
a. Carbohydrates b. Cardiac glycosides c. Alkaloids d. Flavonoids e. Tanins f. Sterols/ triterpenes

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Zhang *et al.*, 2022).

Antimicrobial activity

The ethyl acetate (EtOAc) extract of *Phellinus* sp. was tested *in vitro* to evaluate its antibacterial and antifungal activity. Antimicrobial efficacy was assessed based on the presence or absence of zones of inhibition compared to the control. The results showed significant inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* with inhibition zones of 22 ± 3 mm and 22 ± 3.5 mm, respectively, indicating that these bacterial strains are sensitive to the ethyl acetate extract. Statisticaly, there is no significant difference ($p \leq 0.05$) between the inhibition zones of *E. coli* and *S. aureus*, based on a one-way ANOVA followed by Tukey's HSD test. However, no zones of inhibition were observed for *Salmonella* species. Additionally, the antifungal activity of the ethyl acetate extract showed no inhibitory effect on the growth of *Aspergillus niger* and *Fusarium* sp. using the disk diffusion method.

Hande and Athar (2022) reported that *Phellinus pomoceus* exhibited the maximum zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*. Additionally, *Phellinus linteus* demonstrated measurable antimicrobial activities against *Propionibacterium acnes* and *Staphylococcus epidermidis* (Azeem *et al.*, 2016; Azeem *et al.*, 2018; Benedict and Brady, 1992; Zhang *et al.*, 2022). Furthermore, various extracts of *Phellinus* mushrooms have been documented to display antimicrobial activity against Gram-positive bacteria (Alves *et al.*, 2012).

The findings from our study on *Phellinus* mushrooms highlight their potential as a valuable resource in agricultural applications, particularly due to their rich content of bioactive compounds. These compounds, while largely underexplored, have significant implications for enhancing plant health, pest management, and overall agricultural productivity. The ability of *Phellinus* extracts to inhibit pathogens such as *Fusarium oxysporum* and *Aspergillus niger*, which are significant threats to field crops, further highlights their potential in disease management. *Fusarium oxysporum* is known for causing wilt diseases in a variety of crops, leading to substantial yield losses. Similarly, *Aspergillus niger* can affect crop quality and storage, resulting in economic impacts. The antimicrobial activity exhibited by *Phellinus* extracts against these pathogens suggests that they could play a crucial role in developing integrated disease management strategies, reducing reliance on synthetic fungicides.

Additionally, various by-products of mushroom production—including fruiting bodies, mycelium, and fermentation broth—are rich in bioactive compounds. The ability to extract and characterize these compounds opens avenues for their utilization in agriculture. Among these, polysaccharides have garnered particular attention due to their nutritional benefits and potential applications in soil health and crop protection. For instance, polysaccharides can enhance soil structure, improve moisture retention, and stimulate beneficial microbial activity, thereby fostering a healthier growing environment for crops (Zia *et al.*, 2020). Furthermore,

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Table 2. Antimicrobial activity of fungal organic and aqueous extracts

Bacterial strains						Fungal strains			
Escherichia coli		***Staphylococcus aureus***		***Salmonella* sp.**		***Fusarium oxysporium***		***Aspergillus niger***	
EtOAc	**Aqueous**	**EtOAc**	**Aqueous**	**EtOAc**	**Aqueous**	**EtOAc**	**Aqueous**	**EtOAc**	**Aqueous**
22±3[a]	ND	22±3.5[a]	ND	ND	ND	ND	ND	ND	ND

*ND: Not detected
Within a row values followed by same alphabet (a) did not differ significantly at $p \leq 0.05$ according to one way ANOVA followed by Tukey's HSD test

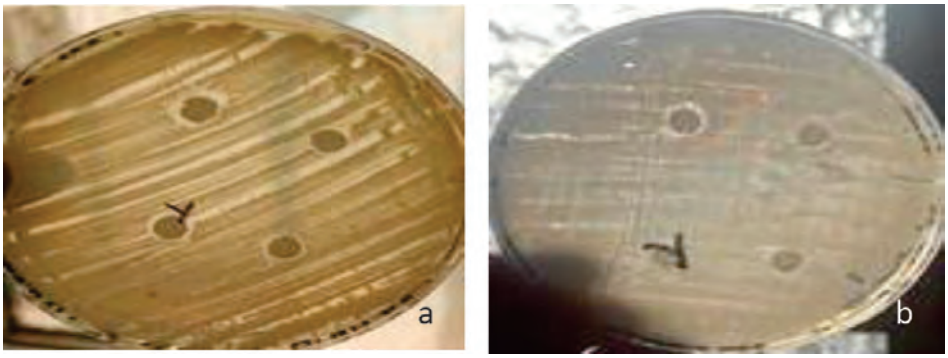


Plate II. a. *Escherichia coli*, b. *Staphylococcus aureus*

the emerging field of bio-based materials presents additional opportunities for leveraging *Phellinus* mushrooms in agriculture. Spent mushroom substrate and mycelium can be transformed into innovative biomaterials that not only contribute to waste reduction but also enhance sustainability in agricultural practices. These biomaterials align with the principles of a circular economy, offering eco-friendly solutions that can replace conventional materials in various agricultural applications, such as biodegradable plant pots, soil amendments, and pest barriers.

The integration of *Phellinus* mushroom extracts and by-products into agricultural systems can thus provide a multi-faceted approach to enhancing sustainability. By tapping into the antimicrobial properties and nutritional benefits of these bioactive compounds, alongside the development of bio-based materials, we can address critical challenges in agriculture, including soil degradation and the environmental impacts of synthetic inputs. The potential applications of *Phellinus* mushrooms in agriculture are vast and warrant further investigation. Future research should focus on optimizing extraction processes, evaluating the effectiveness of these compounds in field conditions, and exploring their roles in integrated pest management and soil health enhancement. By doing so, we can pave the way for innovative, sustainable agricultural practices that harness the power of nature's resources, ultimately contributing to more resilient agricultural systems.

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CONCLUSION

This study highlights the significant potential of *Phellinus* mushrooms as a source of bioactive compounds with antimicrobial properties, particularly against key pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Fusarium oxysporum*, and *Aspergillus niger*. The findings suggest that extracts from *Phellinus* mushrooms can serve as effective natural alternatives to synthetic pesticides and fungicides, contributing to sustainable agricultural practices and integrated disease management strategies. Furthermore, the exploration of various by-products from mushroom production, such as fruiting bodies and mycelium, opens avenues for the development of bio-based materials that align with the principles of a circular economy. These innovations not only address waste management issues but also enhance soil health and crop protection.

Overall, the diverse applications of *Phellinus* mushrooms in agriculture underline the need for further research to fully harness their benefits. By optimizing extraction methods and evaluating the effectiveness of these compounds in field conditions, we can foster more resilient agricultural systems that prioritize sustainability and environmental health. The integration of these natural resources into agricultural practices promises to advance our efforts toward sustainable food production while reducing reliance on chemical inputs.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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