International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 15-12-2022, Accepted: 01-01-2023, Published: 18-01-2023
Volume 8, Issue 1, 2023, Page No. 49-52

Antibacterial activity screening of an edible mushroom *Agaricus litoralis*

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Abstract
Agaricaceae is a family of edible mushrooms that have rich source of bioactive metabolies. The main objectives of this work were to check out the relative abundances of phytochemical constituents of dried fruiting bodies of *Agaricus litoralis*. The screening was accomplished for alkaloids, phenolic compounds, sterols, triterpenoids, coumarins and Carbohydrates. The color strength or the precipitate formation was used as analytical answers to these tests. The results of preliminary phytochemical screening showed that *A. litoralis* contained various metabolites: Alkaloids, flavonoids, tannins, sterols, triterpenoids, carbohydrates etc. Aqueous and organic extracts (*n*-hexane and CH_2Cl_2) of this mushroom were also exposed to preliminary antibacterial activity screening by disc diffusion method and found to be active against some selected bacterial and fungal strains. The organic extracts (*n*-hexane and CH_2Cl_2) of *A. litoralis* were exhibited highest antibacterial activity against both selected bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*). The aqueous organic extracts have high antifungal activity against one fungal specie (*Fusarium oxysporium*).

Keywords: phytochemicals, antibacterial, antifungal, fruiting bodies, *Agaricus litoralis*

Introduction
Edible mushrooms are the fleshy and edible fruit bodies of several species of macro-fungi that can be seen with the naked eye. They are traditionally consumed by people as comestibles for nutritional and medicinal characteristics (Chowdhury *et al*., 2015; Zatout *et al*., 2021a) [3, 187]. In addition, edible mushrooms especially the higher Basidiomycetes, containing the various ingredients suitable for human consumption and play a crucial role in food and medicine industry (Ho *et al*., 2020) [7]. However, approximately 16,000 species of edible mushrooms have been described in the world, only about 3000 are eaten by humans, and about 700 are considered to have certain medicinal value (Liu *et al*., 2022) [14]. Furthermore, the fruiting body of edible mushrooms are a good source of polysaccharides, amino acids, fber, vitamins and minerals. At the same time, they contain a number of secondary metabolites, such as terpenoids, steroids, polyphenol, polyketides, polyglucan, flavonoids, alkaloids which possess various properties benefcial for health (Li *et al*., 2021; Zhang *et al*., 2021) [9, 13]. Thus, several studies have shown some edible mushrooms to have antibacterial (*Lentinula edodes*), and anti-viral activities (*Agrocybe aegerita* and *Hypsizigus mamoreus*), anti-inflammatory (*Pleurotus ostreatus* and *Auricularia auricularia*), antioxidants (*Termitomyces spp.),* immune-modulating and antitumor properties (*Agaricus bisporus, Agaricus blazei, Cordyceps sinensis, Grifola frondosa, Ganoderma lucidum, Poria cocos* and *Trametes versicolor*) (Bita *et al*., 2022 [2]; Ganeshpurkar *et al*., 2021; Kumar *et al*., 2021[8]; Ndungutse *et al*., 2015 [11]; Xu *et al*., 2022) [14]. *Agaricus* L. is a genus of edible mushrooms within the Agaricaceae family including more than 500 species worldwide (Zatout *et al*., 2021b) [18]. Thus, some *Agaricus* species have great potential as a source for natural health products such as *A. bisporus, A. blazei, A. Brunnescen* (Bhushan, Kulshreshtha, 2018; Nivedita *et al*., 2021) [1, 12]. The main aims of this study were two. First, to identify the presence or absence of different phytochemicals of the aqueous and organics extracts of an edible mushroom (*Agaricus litoralis*) from the forests of Djebel el Ouahech of Constantine region of Algeria, and second, to determine their antimicrobial activity against two bacterial strains (*Pseudomonas aeruginosa* and *Bacillus subtilis*) and two fungal species (*Fusarium oxysporium* and *Aspergillus niger*) mostly pathogens.

Materials and Methods
Collection of samples
The fresh mushrooms *Agaricus litoralis* (MW165560) were collected from the forest of Djebel el Ouahch of Constantine region, Algeria. The fresh mushrooms were washed under running up water, and then dried out it under shadow. The dried mushrooms were grinded to fine powder using electrical blender and preserved in airtight bottles, then stored the powder for tests.

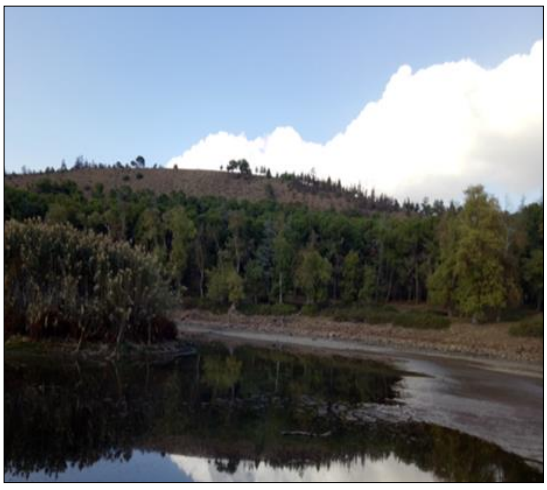

Fig 1: Forest of Djebel el Ouahch, Constantine, Algeria

Fig 2: *Agaricus litoralis*. Pictures by Zatout. R

Chemicals and reagents

Distilled water, methanol, ethanol, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), metal magnesium (Mg), ferric chloride ($FeCl_3$), Ammonium hydroxide (NH4OH), Mayer's reagent, wagner's reagent, acetic anhydride ($C_4H_6O_3$), petroleum ether (C_6H_{14}), chloroform ($CHCl_3$), Fehling's reagent A and B, dichloromethane (CH_2Cl_2), *n*-hexane (C_6H_4), dimethyl sulfoxide (DMSO).

Phytochemical screening

A phytochemical screening of primary and secondary metabolites such as phenolic compounds, alkaloids, Sterols, steroids, and carbohydrates were carried out according to the common phytochemical methods described, as described in Harborne (1973) [5], Trease and Evans (1983) [17], Sofowara (1993) [16], and Raaman (2006) [15], as follows:

Test for alkaloids

Alkaloids test

A powder of 5 g of *A. litoralis* was added into 25 ml of 10% sulfuric acid. After 24 h of soaking at room temperature, the macerated is filtered and washed with water to obtain 25 ml of filtrate.

a. **Mayer's Test:** 5 drops of Mayer's reagent were added into 1 ml of the collected filtrate and was allowed to wait 15 min. Formation of a yellow-colored precipitate indicates the presence of alkaloids.

b. **Wagner's Test:** 5 drops of Wagner reagent were added into 1 ml of the collected filtrate and was allowed to wait 15 min. Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for the phenolic compound

a. **Flavonoids**
   About 5 ml of the ethanol extract of *A. litoralis* was added to a concentrated hydrochloric acid (1 ml) and 0.5g of metal magnesium. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

b. **Tannins (catechic or gallic)**
   About 1 ml of the ethanol extract of *A. litoralis* was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution (1%) were added and green (catechic tannins) or a blue-black (gallic tannins) coloration.

c. **Coumarins**
   Evaporate 5 ml of ethanolic extract, dissolve the residue in 1 to 2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% Ammonium hydroxide. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

Test for sterols and triterpenoids

Liebermann*-Burchard *reaction

10 ml of the petroleum ether extract was placed in a test tube and evaporated to dryness on a water bath. The residue was dissolved in 1ml of acetic anhydride and 1ml of chloroform. A few drops of concentrated sulfuric acid were added. A violet ring was formed at the liquid, with the supernatant becoming violet this indicates the presence of sterols and triterpenoids.

Test for fehling test (carbohydrates)

About 1 ml of the ethanol extract was mixed with 1 ml Fehling solutions (A + B) and heated until boiling. The appearance of a brick red precipitate indicates the presence of carbohydrates (sugar)

Extraction of *Agaricus litoralis*

200 ml of MeOH: H_2O (1:1) were added to 50 g of mushroom powder of *A. litoralis*. The suspension was homogenized in a blender for a few minutes and left 24 h at room temperature, this maceration was repeated three times. The suspension was centrifuged for 20 min at 6000 rpm and the supernatant was concentrated under reduced pressure to eliminate methanol. The aqueous phase was extracted with *n*-hexane (3×50 ml) and then with CH_2Cl_2 (3×70 ml). The *n*-hexane and CH_2Cl_2 organic extracts were separately combined, dried by Na_2SO_4, and evaporated under reduced pressure, giving two different oily residues. Both the organic extract residues (*n*-hexane and CH_2Cl_2) and the remaining aqueous phases were used for antimicrobial test. The organic solvent in the extracts was evaporated until dry, under vacuum.

Antimicrobial activity

The antimicrobial activities of the aqueous and organic extracts (*n*-hexane, dichloromethane) of the edible mushroom *A. litoralis* on two bacterial strains: *Pseudomonas aeruginosa* and *Bacillus subtilis* and two fungal species: *Fusarium oxysporium* and *Aspergillus niger* were evaluated by the Agar Disk Diffusion method with a little modification, which determines minimum inhibitory concentration (MIC). The dried extracts were dissolved in DMSO to obtain the required concentrations which were evaluated for their antimicrobial activities. Young bacterial cultures (24 h) and young fungal mycelia (120 h) are tested. Suspensions of the tested microorganisms were spread over the surface of Petri plates using a distilled swab. 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. The agar plates were incubated. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around. All tests including controls are repeated 03 times under the same experimental conditions

Results are presented as mean± SD of three independent determinations.

Results and discussions

Phytochemical screening

The phytochemical screenings showed the presence or absence of certain phytochemicals in the *A. litoralis* mushroom. Phenolic compounds (flavonoids, tanins and coumarins), alkaloids, sterols, triterpenoids and Carbohydrates showed the positive results. The phytochemical analysis of this mushroom presented in Table

Table 1: Phytochemical screening of extract of *A. litoralis* The results have been classified according to: highly positive: +++; fairly positive: ++; weakly positive: +; negative test: -.

Métabolites		Expected results	Result
Phenolic compounds	Flavonoides	A pink or red coloration	+++
	Tanins	Green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration	++
	Coumarins	Intense fluorescence indicates the presence of coumarins	++
Alkaloids		*Mayer's test* Formation of a yellow-colored precipitate	+++
		Wagner's test Red precipitate – dark brick	+++
Carbohydrates		*Fehling's test* A brick red precipitate	+++
Sterols and triterpenoids		*Liebermann-Burchard* A violet ring was formed at the liquid, with the supernatant becoming violet	+++

Extraction of *A. litoralis* sample

The *A. litoralis* sample was extracted with MeOH: H_2O and this in succession with *n*-hexane (C_6H_4) and dichloromethane (CH_2Cl_2) as detailed in materials and methods. Yields of both organic extracts are reported in Table 1. These residues of the organic extracts and the remaining aqueous phases were assayed for their antimicrobial activity against two bacteria and two fungal strains isolates mostly pathogens.

Table 2: Yield of organic extracts obtained from dry *A. litoralis* sample (mg/50 g of dry fungi)

mushrooms	*n*-hexane extract (mg)	Dichloromethane extract (mg)
Agaricus litoralis	120	170

Antimicrobial activity

In *vitro* antimicrobial activity of the *A. litoralis* on two bacterial strains: *P. aeruginosa* and *B. subtilis* and two fungal species: *F. oxysporum and A. niger* was carried out on the of the aqueous and organic extracts (*n*-hexane, dichloromethane) of *A. litoralis*. The result of inhibition diameters of this mushroom extracts against four microbial strains is shown in Tables 2 and 3. The organic extracts (*n*-hexane and CH_2Cl_2) of *A. litoralis* were exhibited highest antibacterial activity against both selected bacteria (*P. aeruginosa* and *B. subtilis*) by the way the aqueous and *n*-hexane organic extracts have high antifungal activity against one fungal specie (*F. oxysporium*).

Table 3: antibacterial activity of fungal organic and aqueous extracts of *A. litoralis*

P. aerugenosa				***B. sibtilis***			
n-hexane	CH_2Cl_2	Aqueous	MeOH 5%	*n*-hexane	CH_2Cl_2	Aqueous	MeOH 5%
23 ± 1	22 ± 1.43	22 ± 1	ND	12 ± 1.6	11 ± 1.52	ND	ND

Inhibition zone (mm)

Table 4: Antifungal activity of fungal organic and aqueous extracts of *A. litoralis*

F. oxysporum				***A.niger***			
n-hexane	CH_2Cl_2	Aqueous	MeOH 5%	*n*-hexane	CH_2Cl_2	Aqueous	MeOH 5%
20 ± 1	ND	18 ± 1	ND	ND	ND	ND	ND

Inhibition zone (mm)

Conclusions and Future Perspectives

In conclusion, the *A. litoralis* mushroom was tested for phytochemical analysis it indicates the phenolic compounds, carbohydrats and steroids. The Zone of inhibition of the aqueous and organics extracts of *A. litoralis* show good antibacterial activity against tow pathogen like *P. aeruginos*a and *B. subtilis* and one fungal specie *F. oxysporium*. when concentration increases antibacterial activity also increases.

Acknowledgement

The authors of this study are appreciative to the Laboratory of Mycology, Biotechnology and Microbial Activity (LaMyBAM), Department of Applied Biology, University of the Mentouri Brothers, Constantine 25000, Algeria. The authors also thank Dr Radia Cherfia for laboratory support of chemical work.

References

1. Bhushan A, Kulshreshtha M. The medicinal mushroom Agaricus bisporus: review of phytopharmacology and potential role in the treatment of various diseases. Journal of Nature and Science of Medicine,2018:1(1):4.
2. Bita AM, Bibalou CND, Boula FEM, Okeli PO. Evaluation of the Drying Quality of Two Types of Edible Mushrooms (*Termitomyces* sp. and *Pleurotus* sp.) and Their Impact on the Antioxidant Content. *Open* Journal of Applied Sciences,2022:12(2):256-265.
3. Chowdhury MMH, Kubra K, Ahmed SR. Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. Annals of clinical microbiology and antimicrobials,2015:14(1):1-6.
4. Ganeshpurkar A, Pardhi P, Bhadoriya SS, Jain N, Rai G, Jain AP. Antioxidant potential of white oyster culinary-medicinal mushroom, *Pleurotus Florida* (higher basidiomycetes). International Journal of Medicinal Mushrooms, 2015, 17(5).

5. Harborne JB. Phytochemical methods, a guide to modern technique of plant analysis. London: Chapman and Hall, Ltd,1973, 49-188.
6. Hawksworth D. Global species numbers of fungi: Are tropical studies and molecular approaches contributing to a more robust estimate? Biodivers. Conserv,2012:21(9):2425-2433.
7. Ho LH, Zulkifli NA, Tan TC. Edible mushroom: nutritional properties, potential nutraceutical values, and its utilisation in food product development. *An introduction to mushroom*, 2020,10.
8. Kumar S, Kumari R. Traditional Uses, Phytochemistry and Biological Activities of Agaricus blazei murrill: A Comprehensive Review. *Am J Pharmacol Pharmacother*,2021:8(3):14.
9. Li H, Tian Y, Menolli NJr, Ye L, Karunarathna SC, Perez-Moreno J *et al*. Reviewing the world's edible mushroom species: A new evidence-based classification system. Compr. Rev. Food Sci. *Food Saf*,2021:20:1982-2014.
10. Liu S, Liu H, Li J, Wang Y. Research Progress on Elements of Wild Edible Mushrooms. Journal of Fungi,2022:8(9):964.
11. Ndungutse V, Mereddy R, Sultanbawa Y. Bioactive properties of mushroom (*Agaricus bisporus*) stipe extracts. Journal of Food Processing and Preservation,2015:39(6):2225-2233.
12. Nivedita PS, Joy HH, Torvi AI, Shettar AK. Applications of Polysaccharides in Cancer Treatment. Polysaccharides: Properties and Applications, 2021, 501-515.
13. Zhang Y, Wang D, Chen Y, Liu T, Zhang S, Fan H *et al*. Healthy function and high valued utilization of edible fungi. Food Science and Human Wellness,2021:10(4):408-420.
14. Xu J, Shen R, Jiao Z, Chen W, Peng D, Wang L, Liu B. Current Advancements in Antitumor Properties and Mechanisms of Medicinal Components in Edible Mushrooms, Nutrients,2022:4(13):2622.
15. Raaman N. Phytochemical Technique. *NIPA: Putampura*, New Delhi, 2006, 19-24.
16. Sofowara A. Recent trends in research into African medicinal plants. Journal of ethnopharmacology,1993:38(2-3):197-208.
17. Trease GE, Evans WC. Text book of Pharmacognosy. 12thed. UK, London: Bailliare Tindall, 1983.
18. Zatout R, Cimmino A, Cherfia R, Chaouche NK. Isolation of tyrosol the main phytotoxic metabolite produced by the edible fungus Agaricus litoralis. Egyptian Journal of Chemistry,2021:64(10):5741-5745.
19. Zatout R, Chaouche NK, Evidente A. Potential bio-control substances produced by fungi and plants of different Mediterranean Basin ecosystems (Doctoral dissertation, جامعة الإخوة منتوري قسنطينة(2021, b.