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Extraction and Identification of Aziridine derivatives in VOCs from *Pleurotus ostreatus*: Impact on Plant Pathogens†

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Abstract: *Pleurotus ostreatus* has potent antimicrobial properties. In this study, bioactive compounds were extracted from *P. ostreatus* and screened against bacterial and fungal phytopathogens. In terms of antibacterial activity, n-hexane extract of *P. ostreatuse* exhibited a significant inhibition zone of 88.55 mm against *Xanthomonas axonopodis*, while highest antifungal activity of 83% was against *Fusarium oxysporum*. It was observed that highest level of concentrations i.e., 25 mg ml^{-1} caused 76, 82, 82, 83 and 60 % decrease in fungal biomass over control against the fungal strains i.e., *A. alternata*, *A. flavus*, *D. australiensis*, *F. oxysporum* and *M. phaseolina*, respectively. GC-MS analysis was performed on n-hexane extract depicting the presence of 26 compounds. A compound identified as Toluene (Molecular weight= 92) exhibited peak area as 91 % followed by another compound named as Cyclopentane, methyl- (Molecular weight= 84) showing peak area 56%. A well know antimicrobial compound Aziridine (Mol. Weight = 99) was identified and show maximum hit of 84% showing peak area 56%. *P. ostreatus* could be a potent biocontrol antagonist against the plant pathogens.

Keywords: *Pleurotus ostreatus;* Aziridine; Antibacterial; Antifungal;

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1. Introduction

Bacteria and fungi are major phyto-pathogens that affect crop yields worldwide [1]. With a growing global population, a well-organized disease management and control program is essential for ensuring food security and safety [2,3]. Using agrochemicals to control crop diseases impacts the environment and humans [4]. Biocontrol is an eco-friendly solution to this problem [5]. The number of tested biocontrol agents (BCAs) and commercial products are not positively connected [3]. Identification of novel BCAs is essential to commercial biocontrol product development and needs a reliable screening procedure [6]. According to research, fungi produce various bioactive volatile organic compounds (VOCs) [7]. Specifically, mushroom-derived compounds exhibit promising potential for biological activity [8,9]. By considering the aforementioned characteristics of macro-fungus. Our study investigated the antimicrobial efficacy of *P. ostreatus* against phytopathogens.

2. Experimental

2.1. Pathogens and antagonist

P. ostreatus, was purchased from BioTech, Sahiwal, Pakistan. Bacterial and fungal pathogens were purchased from Culture bank of Pakistan, Institute of Agricultural Sciences Punjab University, main campus Lahore. The bacterial plant pathogens, *Erwinia carotovora*, *Pseudomonas syringae*, *Ralstonia solanacearum*, and *Xanthomonas axonopodis,* were subcultured on malt extract agar, while fungal pathogens, *Alternaria alternata*, *Aspergillus flavus*, *Drechslera australiensis*, *Fusarium oxysporum* and *Macrophomina phaseolina* on PDA medium. Then these grown cultures were stored in a refrigerator at 4° C.

2.2. Extraction of the bioactive compounds

P. ostreatus bioactive components were extracted using [10] with minor changes. Piston mortal crushed sun-dried fruiting bodies into powder. 500g of dry *P. ostreatus* powder was steeped in 1000 mL methanol for 5 days. After filtering using Whattman paper, the extract was evaporated in a rotary evaporator at 45 ºC for one hour. Dry methanolic extract was remeasured and re-suspended in 200 mL distilled water to yield 9 g. Store it aseptically for future use.

Additional solvent extraction was done using n-hexane. Add 200 mL of re-suspend extract to a 500 mL separating funnel and add 200 mL of n-hexane organic solvent at 1:1 ratio. Let it sit overnight. The flask had clear layers, which were carefully transferred to an electrically pre-weighted beaker for evaporation. This is done three times to remove everything. Re-evaporated by rotary evaporator to pure fractions.

2.3. Gas Chromatography Mass Spectrometry Analysis

The biochemical components of *P. ostreatus* n hexane extract were evaluated using GC/MS with column number HP-5MS (30 m×250 μm ×0.25 μm). GC/MS analysis was conducted on a Thermo GC Trace ultra-version 5.0, Thermo MS DSQ II, using a fused silica column and Elite ZB 5 MS capillary standard nonpolar column (30 m, 0.25 mm, 0.25 μm). Components were separated using helium at 1 mL/min. Thermo MS DSQ II identified the 1 μL sample extract supplied into the instrument. The oven was set at 260°C for 38.50 min (mass analyzer). After 40 min of MS detection, the relative amounts of all components were computed.

2.4. Antibacterial Assays

The disk diffusion technique was employed for antibacterial trials. The treatment included dissolving 50 mg of mushroom extract in n-hexane solution and 140 μL of DMSO and adding distilled water to reach 500 μL. To generate the negative control solution, combine 140 μL DMSO with 360 μL of distilled water. For the positive control, dissolve 50 mg of *Penicillin* in 140 μL of DMSO and add distilled water to achieve a volume of 500 μL. All bacteria were cultured on Lysogeny agar.

2.5. Antifungal Assays

Antifungal screening of the isolated compounds will be carried out by dilution technique. Prepared Potato Dextrose Broth (PDB) in a conical flask and autoclaved at 121ºC for 20 min. Mix 300 mg mushroom extract in n-hexane solution, then dissolve in 333 μL of DMSO and add distilled water to reach 1000 μL for stock solutions. Control solution with DMSO was made similarly. To promote fungal development, mycelial spores of each species were introduced to growth media and incubated at 27 ºC for 6 days. Following filtering using pre-weighted filter paper, the filtered material was oven-dried for 2 days at 65 ºC to extract dry fungal biomass.

2.6. Statistical Analysis

In statistical analysis, the two way ANOVA is applied using the Fisher's Least Significant Difference (LSD) test to elucidate the treatment means. In ANOVA two variable

factors were analyzed i.e., Extracts over Control treatments. This analysis is performed utilizing the Minitab-17 statistical software.

3. Results and Discussion

The results showed that, *P. ostreatus* has potent antimicrobial properties against bacterial and fungal phytopathogens. This indicated that, *P. ostreatus* could be an efficient antagonistic agent. Various species of *basidiomycetes*, including the edible mushroom *P. ostreatus*, have the ability to inhibit the growth of plant pathogens [11].

3.1. Antibacterial Activity of n-hexane of P. ostreatus Extract

Results showed that n-Hexane extract of *P. ostreatus*, showed the highest zone of inhibition of 88.55 mm against the *X. axonopodis*. However *E. carotovora*, was the only pathogenic strain that was not inhibited by this extracts (Figure 1). Even the control showed less bioactivity against *E. carotovora*. This extracts also exhibited a significant bioactivity against other pathogenic bacteria such as *P. syringae*, and *R. solanacearum*, with an inhibition zones of 69.18 mm, and 59.79 mm (Figure 1).

The positive control (Penicillin) formed significant inhibition zones in all bacterial strains. In *E. carotovora* 58.02 mm, in *P. syringae* 99.87 mm, in *R. solanacearum* 78.34 mm and in *X. axonopodis* 98.61 mm zone of inhibition was formed. Penicillin is a synthetic antibacterial capsule which contain pure compound that's why it showed maximum activity against all bacterial strains.

The negative control didn't show any antibacterial activity against any bacterial strains. This confirmed that DMSO didn't harm any plant pathogenic bacteria. According to this experimental result this extract was further analyzed by GCMS and its chemical composition was observed. Heleno et al., [12] studied the antibacterial properties of *P. ostreatus* acidic extract against *Pseudomonas aeruginosa* and found similar results to our experiment. Other information against bacterial strains are not available and firstly reported in our experiment. n-hexane was significantly active against bacteria. The broad spectrum antibacterial activity of Aziridine, 1-(2-aminoethyl)-, was optimized by a one-variable-at-a-time system coupled with response surface methodology, which led to a 45% enhancement of the antibacterial activity [13].

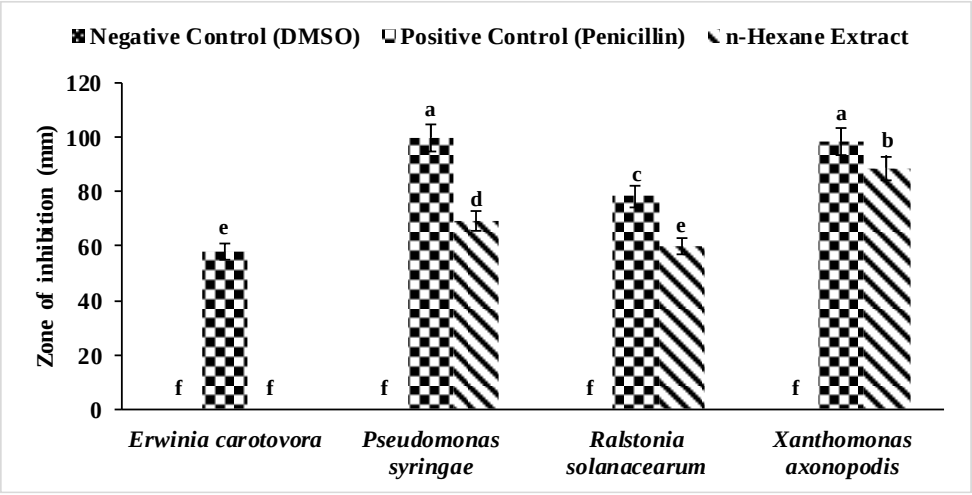

Figure 1. Effect of n-hexane extract of P. ostreatus against E. carotovora, P. syringae, R. solanacearum and X. axonopodis. Vertical bars show standard error of means of three replicates. Values with different letters show significant difference ($P \leq 0.05$) as determined by ANOVA followed by Fisher's LSD Test using 17.

3.2. Antifungal activity

The antifungal activity of n-hexane Extract of P. ostreatus against Alternaria alternata, Aspergillus flavus, Drechslera australiensis, Fusarium oxysporum and Macrophomina phaseolina. It was observed that organic extracts of n-hexane and its different concentrations increases from 5 to 25 mg ml^{-1} the fungal growth was inhibited which results in the decrease of fungal biomass (Figure. 2). The maximum growth reduction was occurred in all extracts of 25 mg ml^{-1} concentration. In these bioassays, n-hexane extracts showed pronounced inhibitory effects on test fungal pathogen. The maximum growth was seen in control in which no extract is added as the organic extracts were added the fungal biomass was decreases. All the extracts were prepared in DMSO, so it was used as the control. DMSO have no activity against any plant pathogenic fungal strains.

The maximum antifungal activity was observed in n-hexane extracts of 25 mg ml^{-1} concentration on fungal pathogen *A. flavus* which decreases fungal biomass 82% and 81% over control respectively. Among all the fungal strains examined in this experiment the growth of *F. oxysporum* was maximum reduced which confirmed that these extracts were very effective against this strain. In a previous investigation, antifungal component, obtained from *Pleurotus sajorcaju*, which showed bioactivity against *Fusarium* and *Mycosphaerella* species [14]. The isolation of antifungal constituents from *Pleurotus* spp. with activity upon these fungi can be fruitful from industrial point of view [15].

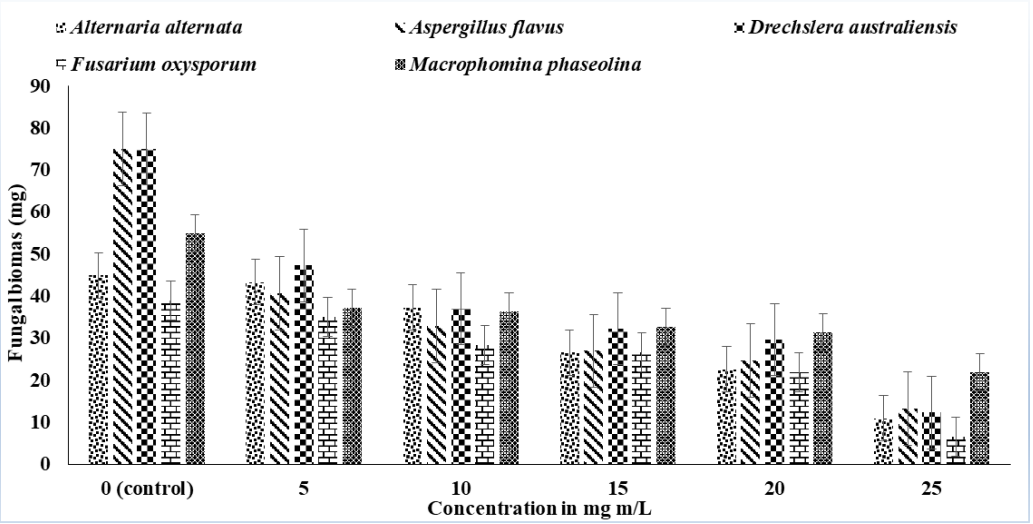

Figure 2. Effect of n-hexane extract of P. ostreatus against Alternaria alternata, Aspergillus flavus, Drechslera australiensis, Fusarium oxysporum and Macrophomina phaseolina. Vertical bars show standard error of means of three replicates. Values with different letters show significant difference ($P \leq 0.05$) As Determined by ANOVA followed by Fisher's LSD test using Minitab 17.

3.3. GC/MS Analysis of n-hexane Extract of P. ostreatus

A total of 26 compounds were identified in the n-hexane extract of *P. ostreatus* mushroom, as presented in Table S1. Among the 26 compounds, 7 compounds exhibited a higher frequency of occurrence and demonstrated a greater number of successful outcomes. Aziridine, 2-methyl-3-(1-methylethyl)-, trans- Aziridine, 2-isopropyl-3-methyl-, trans 2-Isopropyl-3-Methyl (Mol. Weight = 99) was identified only one time and show maximum hit of 84%. The potential structure is depicted in (Figure.3A,B). Santra et al., [13] first reported the Aziridine, 1-(2-aminoethyl)-, from any endophytic source. *Cochliobolus* sp. APS1 possesses industrial importance for the production of bioactive alkaloids with broad spectrum bactericidal action. Kowalczyk et al., [16] reported that Aziridines were widely used as building blocks in multi-step syntheses of more complex molecules; however, due to the presence of the aziridine ring, their derivatives exhibit significant biological activity. Toluene (Mol. Weight = 92) show maximum hit of 91%, and Hexane (Mol. Weight = 86) show maximum hit of 51%. The other prominent compounds were,

pentane, specifically the isomer 3-methylpentane, with a molecular weight of 86, exhibits a maximum yield of 57%. in the same way, the compounds pentane, 2-methyl-Isohexane, 2-methylpentane, and methyl pentane (CH3)2CH(CH2)2CH3, with a molecular weight of 86, are all identified. *P. ostreatus* contain bioactive compounds such as polysaccharides (glucans and chitin) as well as secondary metabolites (phenolic compounds, terpenoids, and lectins). These compounds have been found to possess antibacterial, antiviral, and antifungal properties [17].

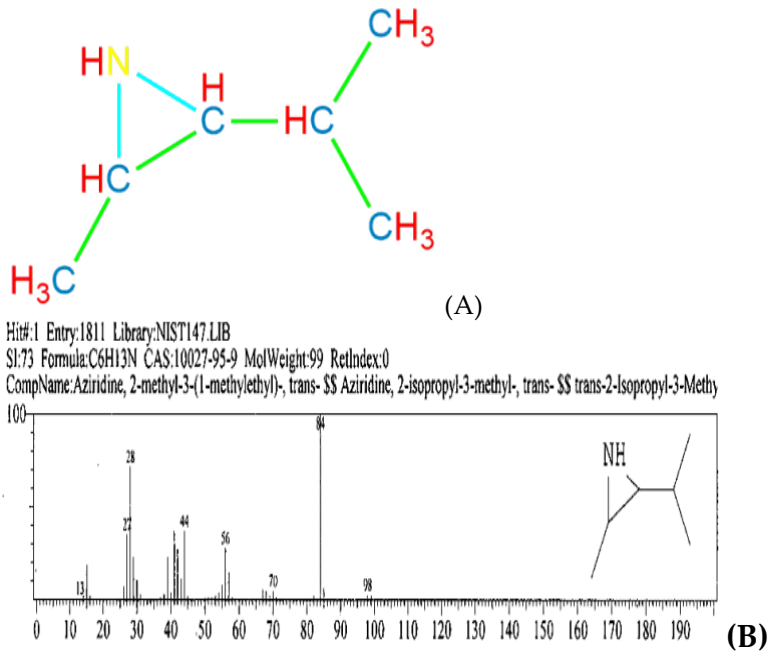

Figure 4. A typical chemical structure of Aziridine derivatives (A). Chromatogram of Aziridine derivatives (B). .

Conclusions

This study revealed that the extract derived from *P. ostreatus* exhibits significant antibacterial and antifungal activities against crop pathogens. The GC-MS analysis revealed the presence of aziridine and its derivatives in the extract of *P. ostreatus*. This study could be useful to develop an eco-friendly and sustainable biocontrol agent for crop disease management.

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Ethical approval: No data was used in this article which needs approval.

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Supplement file.

Table 1. GC/MS Analysis of n-hexane extract of P*leurotus ostreatus.*

Sr. #.	R.Time.	Name Of Compound.	Molecular Formula.	Molecular Weight.	Peak Area...
1.	1.610	Pentane, 2- Methyl	C_6H_{14}	86	0.43
2.	1.633	Pentane	C_5H_{12}	72	0.10
3.	2.440	Butane, 2,3-dimethyl-	C_6H_{14}	86	0.10

4.	2.921	Pentane, 3-methyl	C_6H_{14}	86	0.20
5.	2.985	(Methylpentane) 3-Methyl Pentane	$(C_2H_5)_2CHCH_3$	86	0.43
6.	3.173	Hexane, 2,2,3-trimethyl-	C_9H_{20}	128	0.35
7.	3.554	1-Butanol, 2-methyl- (Butyl-carbinol)	C_5H_{120}	88	0.35
8.	3.208	Hexane	C_4H_{14}	86	0.48
9.	3.587	Butane, 2,2,3-trimethyl-	C_7H_{16}	100	0.72
10.	3.662	Pentane, 2,4-dimethyl-	C_7H_{16}	100	0.69
11.	3.876	Cyclopentane, methyl (Methyl-cyclopentane)	C_6H_{12}	84	0.55
12.	4.251	Cyclopentane, methyl-	C_6H_{12}	84	0.58
13.	4.174	1-Pentene, 2-methyl-	C_6H_{12}	84	1.24
14.	4.891	4-Methyl-4-pentene	$C_2H_5CH_2C(CH3)=CH2$	84	0.61
15.	5.491	Aziridine, 2-methyl-3-(1-methylethyl)-, trans- Aziridine, 2-isopropyl-3-methyl-	$C_6H_{13}N$	99	0.51
16.	5.874	Triazine, 2,4,6-tris(cyanomethoxy)- 2, 2′, 2″-[1,3,5-Triazine-2,4,6-triyltris(oxy)] triacetonitrile-	$C_9H_6N_6O_3$	246	0.50
17.	6.494	Cyclopentane, methyl- Methyl-cyclopentane	C_6H_{12}	84	0.52
18.	6.329	2-Butenedioic acid, 2-methyl-, Citraconic acid, Methylmaleic acid, cis-Methylbutenedioic acid	$C_5H_6O_4$	130	7.21
19.	6.459	Cyclopentane, methyl-	C_6H_{12}	84	31.2
20.	6.601	Toluene	C_7H_8	92	5.87
21.	6.775	Toluene-Benzene, methyl- Methacide, Methylbenzol-Phenylmethane-Antisal	C_7H_8	92	2.55
22.	6.947	cis-5,8,11,14,17-Eicosapentaenoic Acid	$C_{20}H_{30}O_2$	302	1.47
23.	7.107	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	2.06
24.	7.195	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	0.29
25.	7.305	trans-8-Isopropylbicyclo [4.3.0]non-3-en	$C_{12}H_{20}$	164	0.34
26.	7.657	Cholesta-4,6-dien-3-ol, (3.beta.)-	$C_{29}H_{46}O_2$	426	0.59