**Metabolic profiling of the oyster spent mushroom substrate (SMS) extract against some soil borne fungal plant pathogens**

**ABSTRACT**

Mushroom farming is environment-friendly, utilizing agricultural, poultry, and brewery residues. Spent mushroom substrate (SMS) is a by-product after several mushroom cultivation cycles. SMS may contain beneficial microorganisms with potential antagonistic activity against phytopathogens. The use of oyster mushroom substrates has shown promising results in managing various plant diseases. This study explores the antimicrobial potential of water extracts from spent mushroom substrate (SMS) of various *Pleurotus* species, a byproduct of oyster mushroom cultivation. LCMS analysis identified several bioactive compounds with known antimicrobial properties: *Pleurotus sajor-caju* SMS contained 3-(o-chlorophenyl)-5-(ptolyloxymethyl)-2-oxazolidone; *Pleurotus sapidus* SMS revealed the presence of Cichoriin, Ethiin, and Cafestol palmitate; while *Pleurotus ostreatus* SMS showed Bronopol and Daphnetin-8-glucoside. These findings demonstrate the presence of natural antimicrobial agents in Oyster mushroom SMS, suggesting its potential as an eco-friendly, sustainable alternative for managing soil-borne fungal plant pathogens. This approach not only supports environmental sustainability but also adds value to agricultural waste through its application in plant disease management.

**Keywords:** *Pleurotus ostreatus, Pleurotus sajorcaju, Pleurotus sapidus,* Antimicrobial, Spent mushroom substrate

1. **Introduction**

Mushroom cultivation is an important branch of the agricultural industry, and global mushrooms production has increased more than sixfold in the last decade. This industry uses large amounts of agricultural, forestry, livestock, and industrial wastes and their by-products. However, it also generates millions of tons of spent mushroom compost which has emerged as a significant issue that hinders the growth of the mushroom business and impacts the environment. Many crop diseases, which cause significant economic losses, are introduced by soil-borne plant pathogens. Spreading spent mushroom compost to agricultural soils is a natural way to control plant diseases. Using organic waste material instead of chemicals, which is the most widely used method in agriculture today, is also a more environmentally responsible option. Spent mushroom compost can potentially be used to improve plant growth, crop yield, soil health, organic matter, and nutrient levels, as well as to suppress soil-borne diseases (Mwangi et al., 2024). Edible mushrooms, classified under the phylum Basidiomycota, naturally thrive on substrates like tree trunks, fallen leaves, roots, and decaying wood (Lindequist *et al*., 2005). Edible mushrooms are climate-smart, protein-rich food sources that can partially substitute meat, whose production has a significant climate impact. Furthermore, due to their high content in various health-promoting ingredients, e.g. β-glucans, peptides, proteins, and phenolic compounds, they possess immunomodulatory, antibacterial, cytostatic, antioxidant, and other properties, and for this reason, the term ‘medicinal mushrooms’ is also used when referring to them (Martín et al., 2023). For more than two thousand years, mushrooms have been consumed worldwide due to their remarkable nutritional and medicinal properties. They play a crucial role in human health by supplying digestible proteins, carbohydrates, dietary fiber, essential vitamins, minerals, and antioxidants (Acharya *et al*., 2017). Given their diverse benefits, advancing mushroom cultivation is essential to meet the growing global demand and support sustainable agricultural practices. Mushroom farming is environment friendly, utilizing agricultural, poultry, and brewery residues (Mahato *et al*., 2022). Currently, shiitake (*Lentinula edodes*), oyster (*Pleurotus* spp.), button (*Agaricus bisporus*), and milky mushrooms (*Calocybe indica*) account for 22%, 19%, 18%, and 15% of the world's total mushroom production, respectively (Manjit Singh *et al*., 2017). In India, five commercial mushroom varieties are cultivated: white button mushroom, oyster, paddy straw (*Volvariella volvacea*), milky, and shiitake mushroom. Among these, *Pleurotus* spp. is a unique group within the order *Agaricales* and the family *Tricholomataceae*, with various species (Raman *et al*., 2021). *Pleurotus* spp. are typically cultivated on lignocellulosic substrates. Agricultural byproducts like banana leaves, peanut hulls, corn leaves, mango fruits and seeds, sugarcane leaves, and wheat and rice straw serve as substrates for oyster mushroom production. *P. sajorcaju*, *P. sapidus*, *P. ostreatus*, and *P. florida* are grown using partially decomposed agricultural residues, including wheat straw, maize straw, pea straw, jowar straw, bajra straw, kauri straw, gram seed husk, groundnut seed husk, sugarcane bagasse, cotton waste, and waste paper.

Spent mushroom substrate (SMS) is a byproduct after several mushroom cultivation cycles. According to Ma *et al*. (2014) and Economou *et al.* (2017), each kilogram of mushrooms generates 5 kilograms of SMS. SMS contains carbon, nitrogen (0.4-13.7%), with a carbon-nitrogen ratio of 9:1 to 15:1, and essential cations (K+, Na+, Ca2+, Mg2+) and anions (Cl-, NO3-, SO42-) for plant growth. Additionally, SMS may contain beneficial microorganisms with potential antagonistic activity against phytopathogens. The use of oyster mushroom substrates has shown promising results in managing various plant diseases, including bacterial wilt (*Ralstonia solanacearum*) in potatoes, stem rot in onions, damping-off (*Phytophthora drechsleri*) in cucumbers, damping-off (*Rhizoctonia solani*) in tomatoes, and late blight (*Phytophthora capsici*) in peppers. Disease resistance in plants can be enhanced through exposure to water extracts from spent mushroom substrate (Kang *et al*., 2017). Furthermore, fungi isolated from spent oyster mushroom substrates have been effective in suppressing *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* (Verma *et al*., 2017). Bacterial isolates from these substrates have also demonstrated the ability to inhibit the *in vitro* growth of pathogens such as *Colletotrichum musae* and *Fusarium solani*.

Building upon the work of Bezbaruah *et al*. (2025), this study focuses on the metabolic profiling of oyster spent mushroom substrate (SMS) extract against major soil-borne fungal plant pathogens.

1. **Materials and Methods**

**2.1 Detection of secondary metabolites using LC-MS**

The aqueous extracts of the SMS of the three species (*Pleurotus sapidus, P. ostreatus* and *P. sajorcaju*) of mushroom (Bezbaruah *et al.,* 2025) were filtered through 0.22µm syringe filter and was sent to Mr. Biologist, Guwahati for the analysis of the secondary metabolites.

Each sample measuring 20µl was loaded into a UPLC system (Waters, USA). Samples were passed through C18 column with a gradient elution containing Acetronitrile (Solvent A), 0.1% Formic Acid 95:5 H2O:ACN (Solvent B) (Table 1).

**Table 1: Solvent gradient conditions for elution during LC-MS Analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.** | **Time(min)** | **Flow rate(ml/min)** | **%A** | **%B** |
| 1. | Initial | 0.250 | 5.0 | 95.0 |
| 2. | 1.00 | 0.250 | 5.0 | 95.0 |
| 3. | 6.00 | 0.250 | 30.0 | 70.0 |
| 4. | 12.00 | 0.250 | 60.0 | 40.0 |
| 5. | 16.00 | 0.250 | 60.0 | 40.0 |
| 6. | 20.00 | 0.250 | 80.0 | 20.0 |
| 7. | 24.00 | 0.250 | 80.0 | 20.0 |

The fractions were detected using an EST-QTOF-Mass Spectrometer connected to the UPLC System in a positive ionization mode.

1. **Results**

The bioactive compounds that may be present in the water extract of Oyster SMS showing highest inhibitory effect against the pathogens (*Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii*) were profiled using chromatographic technique (Table 2, Table 3 and Table 4).

**Table 2: Identified metabolites from aqueous extracts of spent mushroom substrate of *Pleurotus sajorcaju* using LCMS technique**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl no.** | **Name of the compound** | **Peaks** | **Chemical formula** |
| 1 | Valine | 117.1 | C5H11NO2 |
| 2 | Tranexamic acid | 157.1 | C8H15NO2 |
| 3 | 3-(o-chlorophenyl)-5-(p- tolyloxymethyl)-2- oxazolidone | 317.8 | C10H11Cl2NO |
| 4 | Sodium m- nitrobenzenesulfonate | 225.1 | C6H4NNaO5S |
| 5 | Esculin monohydrate | 340.1 | C15H18O |
| 6 | 3,4,5-tri pentadecanoate | 449.9 | C10H22 |
| 7 | Glycerol tripentadecanoate | 765.1 | C42H80O6 |
| 8 | Ethyl propionate | 102.1 | C5H10O2 |
| 9 | 2-bromo-5-chlorothiophene | 197.6 [M-H]- | C4H2BrCIS |
| 10 | Tocopheryl retinoate | 713.3 | C49H76O3 |

**Table 3: Identified metabolites from aqueous extracts of spent mushroom substrate of *Pleurotus sapidus* using LCMS technique**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl no.** | **Name of Compound** | **Peaks** | **Chemical formula** |
| 1. | Valine | 117.1 | C5H11NO2 |
| 2. | Tranexamic acid | 157.0 | C8H15NO2 |
| 3. | Ammonium oxalate | 107.2 | C2H10N2O5 |
| 4. | Diisobutyl maleate | 228.9 | C12H20O4 |
| 5. | Cichoriin | 340.0 | C15H16O9 |
| 6. | Ethiin | 165.9 | C9H22O4P2S4 |
| 7. | Sinapinic acid | 224.1 | C11H12O5 |
| 8. | Dimethyl malonate | 132.1 | C5H8O4 |
| 9. | Cadaverine | 102.4 | C5H14N2 |
| 10. | Adipic acid | 146.1 | (CH2)4(COOH)2 |
| 11. | Pentetic acid | 393.1 | C14H23N2O10 |
| 12.  | Cafestol palmitate | 568.8 | C36H58O4 |
| 13. | TEA-lauroylsarcosinate | 420.5 | C15H28NNaO3 |
| 14. | Adenine | 135 | C5H5N5 |

**Table 4: Identified metabolites from aqueous extracts of spent mushroom substrate of *Pleurotus ostreatus* using LCMS technique**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl no.** | **Name of Compound** |  **Peaks** | **Chemical formula** |
| 1. | Valine | 117.1 | C5H11NO2 |
| 2. | Tranexamic acid | 157.0 | C8H15NO2 |
| 3. | Bronopol | 200.9[M+H]+ | C3H6BrNO4 |
| 4. | Daphnetin-8-glucoside | 340.2 | C9H6O4 |
| 5. | Stigmasterol 3-stearate | 679.3 | C47H82O2 |
| 6. | Erythritol | 122 | C4H10O4 |
| 7. | Triphenyl phosphate | 326.1 | C18H15O4P |
| 8. | Ethyl propionate | 102.1 | C5H10O2 |
| 9. | Disodium fumarate | 160.7 | Na2C4H2O4 |
| 10. | 2-bromo-5-chlorothiophene | 197.5[M-H]- | C4H2BrCIS |
| 11. | Tocopheryl retinoate | 713.2 | C49H76O3 |
| 12.  | 4-bromophenacyl methyl succinate | 329.1 | C13H13BrO5 |
| 13. | Cytochalasin E | 494[M-H]- | C28H33NO7 |
| 14. | n-hexanoyl-d-glucosamine | 277.1 | C12H23NO6 |

1. **Discussion**

The metabolic profiling of water extract obtained from *P. sajorcaju* SMS revealed the presence of antimicrobial compounds like 3-(o-chlorophenyl)-5-(p- tolyloxymethyl)-2- oxazolidone, followed by water extract of *P. sapidus* (SMS)which showed antimicrobial compounds like Cichoriin, Ethiin, Cafestol palmitate and water extract of *P. ostreatus* which showed antimicrobial compounds like Bronopol, Daphnetin-8-glucoside. Oyster mushrooms enable to production of metabolism products in 48 h to inhibit the growth of pathogenic bacteria due to the production of polysaccharides, proteins, enzymes and triterpenoides of mycelia of *Pleurotus spp.*(Owaid et al., 2015).

Lately, metabolomics-based methodology has been increasingly applied in the study of edible and medicinal mushrooms to understand their chemical constituent and responses to certain environmental stimuli. While *Pleurotus* species have been extensively studied for their phytochemicals, little research exists on *P*. *columbinus* Quél, particularly regarding its phytochemistry, antioxidant properties, and antibacterial activities. This study employed ultra-performance liquid chromatography-mass spectrometry (UHPLC-QTOF) along with principal component analysis (PCA) to analyze the metabolome of *P. columbinus* and *P. sajorcaju*, aiming to fill this knowledge gap. This study was to examine how various agri-flod residues, used as growth substrates for *P. columbinus* cultivation, affect the bioactive chemical composition of the fruiting bodies.

Angelini *et al*. (2021) performed a quantitative analysis using high-performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD-MS) to investigate the phenolic and flavonoid chemicals present in *P. columbinus* extracts. This analysis aimed to provide a partial explanation for the reported biological effects associated with these extracts. Many isolated compounds from different species of *Pleurotus* genus possess nematicide compounds like trans-2-decenedioic acid, 2-hydroxy-(4'-methoxy)- propiophenone, s- coriolic acid, p- anisaldehyde , p- anisyl alcohol, which have shown their ability to suppress pests, diseases and weeds naturally (Bello *et al*., 2025). Mahato *et al*. 2024 have also found that SMS along with antogonistic (*Trichoderma asperellum*) microbial inoculants exhibit antifungal volatile compound 6- Pentyl-2- H-pyran-2-one (22.02%). Different SMS had high levels of trace elements such as Zn, Mn, B, Fe and Cu, which are known to have antifungal properties (Huber, 1980). High concentrations of Zn and Cu inhibit fungal growth by enhancing the production of antifungal compounds, such as 2, 4 diacetyl phloroglucinol (DAPG) that control fungal species such as *Fusarium*. It is reported that these compounds inhibit the biosynthesis of fusaric acid by *Fusarium sp*. in the plant cell infection process (Thind and Madan, 1977; Blevins and Lukazewski, 1998; Brown *et al*., 2002). These SMS also suppress the fungal growth of *Fusarium oxysporum* and *Rhizoctonia solani* (Nkosi, 2017). This analysis aimed to provide a partial explanation for the reported biological effects associated with these extracts. Although, proper application and methods are also crucial for maximizing their effectiveness.

1. **Conclusion**

The LCMS analysis of water extract from SMS of *P. sajorcaju*, *P. sapidus* and *P. ostreatus* showed different compounds with antimicrobial activity like having the presence of 3-(o-chlorophenyl)-5-(p-tolyloxymethyl)-2- oxazolidone, Cichoriin, Ethiin, Cafestol palmitate, Bronopol, Daphnetin-8-glucoside, etc. These compounds may be attributed to the antagonistic behaviour of the isolated fungal microflora against the tested pathogens. As the majority of SMSs exhibit efficacy as biological control agents and are applied directly as soil conditioners or SMS extracts, this study would be instrumental in that line of work. This study threw some light on the potentiality of aqueous extracts of SMS which may help to reduce the disease incidence by direct antagonistic activity to the pathogens involved or indirectly triggering pathways responsible for defence in host plants. Thus, more work needs to be undertaken in such kind of study in future.

**Disclaimer (Artificial intelligence)**

While preparing this work, we used Quillbot to improve the language. After using this tool/service, we reviewed and edited the content as needed and took full responsibility for the publication’s content.

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