

This research work was conducted by using locally available substrates for cultivation of *Pleurotus ostreatus*, commonly known as oyster mushroom. It has garnered significant attention for potent antimicrobial properties and ease of the cultivation. Study explored the cultivation technique and antimicrobial efficacy of the *Pleurotus ostreatus* to grown on agro-waste substrates, by utilizing the locally sourced substrates and optimal growth conditions were maintained to maximize the nutrient content and yield. Antimicrobial properties of *Pleurotus ostreatus* were evaluated amongst pathogenic fungi and multi-drug resistant bacteria by using standard assays. Results of this study revealed that *P.ostreatus* exhibited the substantial growth amongst all tested extracts samples, with varying concentrations. Mushroom extracts provided strong antioxidant activities; methanolic extracts showed the IC<sub>50</sub> value (57.59 µg/ml), ethanolic extracts showed (58.37 µg/ml), and aqueous extracts showed (60.35 µg/ml). However, highest phenolic content was observed in the methanolic extract (204.81 ± 0.91 mg of GAE/g) respectively. These findings highlights that *P.ostreatus* not only a sustainable food source but also a natural antimicrobial agent. This dual functionality of *P.ostreatus* underscores the value in pharmaceuticals and agricultural sectors, by offering a viable solution for antibiotic resistance challenges and food sources.