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Assessment of Polyphenol oxidase enzyme activity in *Auricularia auricula.*

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ABSTRACT

The *A. auricula* is used as both an edible and medicinal mushroom worldwide. It is the third most cultivated mushroom and is widely grown in East Asian countries. This paper highlights the polyphenol oxidase (PPO) activity in A. auricula. The PPO activity was determined using catechol extract at various concentrations. It was observed that as the concentration increased, the PPO activity also increased. The presence of the PPO enzyme in this mushroom suggests that *A. auricula* could be very useful in the food industry as a future resource.

Key Words - Mushroom, Polyphenol oxidase, *Auricularia auricula*, Edible

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INTRODUCTION

Auricularia auricula (A. auricula) is a large edible and medicinal fungus with a long history in China. Globally, *Auricularia auricula-judae* (AAJ) is the third most important cultivated edible fungus. It reproduces both asexually and sexually, undergoing a typical fungal haploid-diploid life cycle that includes spore production from its fruiting body, spore germination, and hyphal fusion forming a network of mycelium. It is a significant edible and medicinal fungus widely cultivated in China, Japan, and other countries. Recently, scientific data have emerged suggesting the potential use of AAJ in pharmaceuticals, cosmetics, and functional foods (Liu *et al.,* 2021; Q. Zhao *et al.,* 2016; Y. Zhao *et al.,* 2019).

In several nations, including Japan, Korea, China, the United States, and France, cultivated edible fungi are used as raw materials for producing medicinal and therapeutic compounds, as well as being consumed as food. The most widely cultivated among these in East Asian countries is *Auricularia auricula-judae*. This fungus is known as "tree jellyfish" in Japan, "wood ear" in China, and "black fungus" in Russia. In Asian countries, this fungus is valued for its flavour and medicinal properties. In Chinese traditional medicine, it is believed to contain antitumour compounds and is applied topically to sore throats, ophthalmia, tonsillitis, staphylococcus, and laryngocele (Kadnikova *et al.,* 2015; Wasser, 2002).

Due to its low cost, environmentally friendly characteristics, and gentle reaction conditions, enzyme-assisted extraction (EAE) is considered a vital extraction method for AAPs. In particular, the effluents produced can be less problematic than those from the acid hydrolysis process (Xiao *et al.,* 2022; R. Zhao *et al.,* 2019). In this paper, the study of the polyphenol oxidase enzyme in A. auricula is conducted using catechol as a substrate.

MATERIALS & METHODS

Sample collection and Extraction from mushrooms

The collection of *Auricularia auricula*, edible mushrooms from Girnar Wildlife Sanctuary, Junagadh, Gujarat, India, was gathered from a tree trunk. The fruiting bodies of the mushroom sample were cleaned and oven-dried for 3 days at 50 °C. The powdered dried mushrooms were then preserved in labelled polythene bags for further use. For extract preparation, three mushrooms were processed in deionised water using the Soxhlet extraction method. 10 g of mushroom powder was extracted with deionised water for 5-8 h, and the extract was filtered through Whatman No. 1 filter paper and evaporated at 40 °C. The extract was stored at 4 °C for the extracellular enzyme assay (Hao *et al.,* 2022; Klausen *et al.,* 2023). Figure 1 shows the collected *Auricularia auricula*.

Figure 1. *Auricularia auricula*

Determination of polyphenol oxidase activity

Determination of polyphenol oxidase activity was performed as described, with a few modifications. Polyphenol oxidase (PPO) activity is measured using a catechol substrate at various concentrations. The 0.1 ml enzyme extract was mixed with 3 ml of 0.1 mol/L phosphate buffer at pH. 6.0 Then, 1 ml of catechol solution was added. In the reaction, the mixture was incubated for 20 minutes at 35 °C and then rapidly cooled in ice water. It was then quickly placed in ice water. The PPO activity was measured at 410 nm.

RESULTS & DISCUSSION

During post-harvest processing and storage, mechanical and physical stressors cause enzymatic browning in fruits and vegetables. Polyphenol oxidase, an intracellular o-diphenol oxidase that is widely found in higher plants and fungi, is the primary catalyst of this process (Tinello & Lante, 2018). Determination of polyphenol oxidase (PPO) activity in the *A. auricula* mushroom using water extract.

Evaluate the PPO activity at various concentrations such as 0.01, 0.04, 0.08, 0.12, and 0.16 M, and observe measurements of 0.024, 0.027, 0.031, 0.036, and 0.040 U/ml, respectively. The highest PPO activity was observed at the maximum concentration, while the lowest was at the minimum concentration. The enzyme activity increased as the concentration increased. The PPO enzyme activity was found in three wild mushrooms*: Armillaria mellea*, *Lepista nuda*, and *Hypholoma fasciculare*, with levels of 0.187 U/mg protein, 0.067 U/mg protein, and 0.111 U/mg protein, respectively. (Colak *et al.,* 2007). *Lactarius piperatus* (L.) Pers. was used to purify polyphenol oxidase (PPO) using a Sepharose 4B-L-tyrosine-p-amino benzoic acid affinity column. Using catechol as a substrate, it was discovered that the ideal pH and temperature for pure PPOs of *L. piperatus* were 7.0 and 20 °C, respectively. The enzyme maintained its optimal pH level for 24 and 72 hours, as well as 100% of its initial activity at 4C. After four hours of incubation, *L. piperatus* PPO was likewise relatively stable at 20 °C. The values for Km and Vmax were

determined to be 1 mM and 25 U/mg protein, respectively (Öz *et al.,* 2013). Figure 2. Depicts the PPO enzyme activity with various concentration.

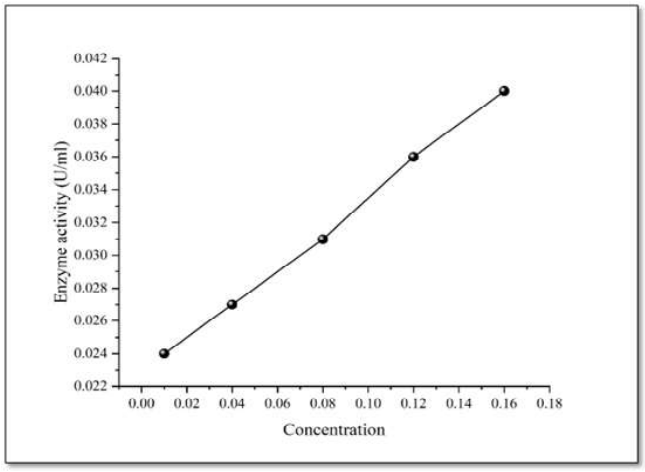

Figure 2. PPO enzyme activity with different concentration

CONCLUSION

The conclusion of this study is the evaluation of PPO enzyme activity in *A. auricula* with a significant amount. The enzyme activity was highest at a concentration of 0.040 U/ml and lowest at 0.024 U/ml. The enzyme activity increased as the concentration increased. The PPO enzyme is essential for edible mushrooms. The PPO enzyme is present in a significant amount in *A. auricula*, indicating that *A. auricula* could be a primary source for future food.

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