

## ABSTRACT

Nutraceutical-enriched foods and natural food items with health-promoting properties have gained public attention. Nutraceuticals are bioactive compounds or natural food substances that offer health benefits beyond basic nutritional requirements. They are becoming increasingly popular as people seek natural alternatives to conventional medicine. *Lentinula edodes* are well-acknowledged for their wide range of medicinal qualities and considered as highly nutritious food source.

Contemplating this evidence, the present research entitled "*Studies on Mushroom-based Nutraceutical and Probiotic Products*" is mainly centered on establishing a comprehensive strategy to enhance the nutraceutical value of *L. edodes* mushrooms through probiotic fermentation. The research includes thorough examinations of the nutraceutical and nutritional properties of the mushrooms, employing *in-silico* and *in-vivo* approaches, developing functional food products, and raising public awareness to encourage their widespread adoption and acceptance.

The study started with optimizing the parameters for increasing vitamin D<sub>2</sub> content along with other nutraceutical properties ( $\beta$ -glucans, antioxidants, total phenols, flavonoids, and amino acid contents) in dried mushroom *Lentinula edodes* (*L. edodes*) powder using ultraviolet B (UVB) irradiation. The dried mushroom powder was irradiated for different time durations (0.5, 1.0, 1.5, 2.0, and 2.5 h). HPLC studies indicated a significant ( $p < 0.05$ ) increase in vitamin D<sub>2</sub> (24.94–49.31  $\mu\text{g/g}$ ) content in 2h irradiated sample. An elevated level of total phenols (6.57–8.84 mg GAE/g), total flavonoids (1.55–2.89 mgQE/g), and  $\beta$ -glucan content (41.20–47.74%) along with improved antioxidant activity (DPPH, FRAP) in the 2h UVB treated sample was also observed. GCMS profiling revealed the presence of bioactives reported to have medicinal potential. *In-silico* studies revealed that pre- and pro-vitamin Ds of edible mushrooms have enormous potential in inhibiting M<sub>pro</sub> and PL<sub>pro</sub> proteases of COVID-19.

Further, the study evaluated the effect of probiotic-mediated fermentation on nutraceutical-enriched mushroom powder. The results showed that fermented mushroom powder had significantly higher values of total flavonoid content, total phenolic content, DPPH, and FRAP than unfermented powder. The fermented samples also showed increased levels of lactic acid, short-chain fatty acids, and bioactive compounds with beneficial properties. Also, *In-silico* screening of 152, mushroom metabolites library against CatK domain1 and domain2 put

forward a strong base towards the search for efficient lead molecules to inhibit CatK, which conclusively establishes the effectiveness of edible mushrooms in osteoporosis.

Next, *In-vivo* studies were also performed to evaluate the combinatorial effect of prebiotics (mushroom powder) and probiotics (LA) in preventing osteoporosis. The synbiotic treatment prevented bone loss and increased Treg cell percentages while decreasing Th17 cell percentages. Serum cytokine analysis showed a decrease in proinflammatory cytokines and an increase in anti-inflammatory cytokines in synbiotic-treated groups. The findings suggest that synbiotics could be a promising preventative strategy for osteoporosis by modulating the Treg-Th17 axis and osteoclastogenesis.

Furthermore, the study involves developing probiotic-based mushroom pickles and frozen desserts, evaluated for nutritional, sensory, and nutraceutical properties. The pickles, prepared using two methods and oils, receive high sensory scores, and the probiotic count significantly increases during storage. The frozen dessert fortified with 2% vitamin-D<sub>2</sub> enriched mushroom powder and 2% cocoa powder also receives favorable sensory feedback. The study also includes awareness programs in rural areas to promote shiitake-based products as an enterprise.

The successful demonstration of this technology offers hope for its potential adoption in other regions facing similar challenges and can provide a sustainable and effective solution to address malnutrition and promote better health outcomes in underserved communities.