

**STUDIES ON MUSHROOM BASED
NUTRACEUTICALS PROBIOTIC
PRODUCTS**

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**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY
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NUTRACEUTICALS PROBIOTIC
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by

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

Submitted

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to the



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*Dedicated to my
Grand PARENTS*

CERTIFICATE

This is to certify that the thesis entitled "**Studies on Mushroom-based Nutraceuticals Probiotic Products**", being submitted by **Mr. Abhay Tiwari** to the **Indian Institute of Technology Delhi** for the award of "**Doctor of Philosophy**" is a record of bonafide research work carried out by him. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge, the results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any degree or diploma.

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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 D₂ content along with other nutraceutical properties (β -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 D₂ (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 β -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_{pro} and PL_{pro} 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-D₂ 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.

सारांश

न्यूट्रास्युटिकल से भरपूर खाद्य पदार्थ और स्वास्थ्य को बढ़ावा देने वाले वाले प्राकृतिक खाद्य पदार्थों ने लोगों का ध्यान आकर्षित किया है। न्यूट्रास्युटिकल्स जैविक तत्व या प्राकृतिक खाद्य पदार्थ हैं जो बुनियादी तौर पर पोषण संबंधी आवश्यकताओं के अलावा, स्वास्थ्य लाभ प्रदान करते हैं। वे तेजी से लोकप्रिय हो रहे हैं क्योंकि लोग पारंपरिक चिकित्सा के लिए प्राकृतिक विकल्प तलाश रहे हैं। लैटिनुला एडोइस मशरूम औषधीय गुणों की विस्तृत श्रृंखला के लिए अच्छी तरह से स्वीकार किए जाते हैं और इन्हें अत्यधिक पौष्टिक खाद्य स्रोत माना जाता है।

इस संदर्भ में, अनुसंधान, "मशरूम-आधारित न्यूट्रास्युटिकल और प्रोबायोटिक उत्पादों पर अध्ययन" नामक वर्तमान शोध मुख्य रूप से प्रोबायोटिक किण्वन के माध्यम से एल. एडोइस मशरूम के न्यूट्रास्युटिकल मूल्य को बढ़ाने के लिए एक व्यापक रणनीति स्थापित करने पर केंद्रित है। अनुसंधान में मशरूम के न्यूट्रास्युटिकल और पोषण संबंधी गुणों की गहन जांच, इन-सिलिको और इन-विवो दृष्टिकोणों को नियोजित करना, कार्यात्मक खाद्य उत्पादों को विकसित करना और उनके व्यापक रूप से अपनाने और स्वीकृति को प्रोत्साहित करने के लिए जन जागरूकता बढ़ाना शामिल है।

अध्ययन पराबैंगनी बी (यूवीबी) विकिरण का उपयोग करके सूखे मशरूम लैटिनुला एडोइस (एल. एडोइस) पाउडर में अन्य न्यूट्रास्युटिकल गुणों (β -ग्लूकेन्स, एंटीऑक्सिडेंट्स, कुल फिनोल, फ्लेवोनोइड्स, और अमीनो एसिड) के साथ-साथ विटामिन डी₂ बढ़ाने के साथ शुरू हुआ। सूखे मशरूम पाउडर को अलग-अलग समय अवधि (0.5, 1.0, 1.5, 2.0 और 2.5 घंटे) के लिए विकिरणित किया गया था। एचपीएलसी अध्ययनों ने 2 घंटे के विकिरणित नमूने में विटामिन डी₂ (24.94-49.31 $\mu\text{g/g}$) में सराहनीय ($p < 0.05$) वृद्धि की। बेहतर एंटीऑक्सिडेंट (DPPH, FRAP) के साथ फिनोलस (6.57-8.84 mg GAE/g), फ्लेवोनोइड्स (1.55-2.89 mgQE/g), और β -ग्लूकन (41.20-47.74%) का उंचा स्तर 2 घंटे यूवीबी उपचारित नमूना भी देखा गया।

जीसीएमएस प्रोफाइलिंग से औषधीय क्षमता वाले बायोएक्टिव्स की उपस्थिति का पता चला। इन-सिलिको अध्ययनों से पता चला है कि खाद्य मशरूम के प्री- और प्रो-विटामिन डी में कोविड-19 के एमप्रो और पीएलप्रो प्रोटीएज को रोकने की काफी क्षमता है।

अध्ययन में न्यूट्रास्यूटिकल-समृद्ध मशरूम पाउडर पर प्रोबायोटिक-मध्यस्थ किण्वन के प्रभाव का मूल्यांकन किया गया। परिणामों से पता चला कि किण्वित मशरूम पाउडर में अकिण्वित पाउडर की तुलना में कुल फेनोलिक, फ्लेवोनोइड, DPPH और FRAP काफी अधिक थे। किण्वित नमूनों में फायदेमंद गुणों के साथ लैक्टिक एसिड, शॉर्ट-चेन फैटी एसिड और बायोएक्टिव यौगिकों के बढ़े हुए स्तर भी दिखाई दिए। इसके अलावा, CatK डोमेन1 और डोमेन2 के खिलाफ 152 मशरूम मेटाबोलाइट्स लाइब्रेरी की सिलिको स्क्रीनिंग में CatK को रोकने के लिए कुशल अणुओं की खोज के लिए एक मजबूत आधार मिला, जो निर्णायक रूप से ऑस्टियोपोरोसिस में खाद्य मशरूम की प्रभावशीलता को स्थापित करता है।

ऑस्टियोपोरोसिस को रोकने में प्रीबायोटिक्स (मशरूम पाउडर) और प्रोबायोटिक्स (एल,ए) के संयुक्त प्रभाव का मूल्यांकन करने के लिए इन-विवो अध्ययन भी किए गए। सिनबायोटिक उपचार ने हड्डियों के नुकसान को रोका और Th17 सेल प्रतिशत को कम करते हुए Treg सेल प्रतिशत में वृद्धि की। सीरम साइटोकिन विश्लेषण ने प्रिनफ्लेमेटरी साइटोकिन्स में कमी और सिनबायोटिक-उपचारित समूहों में एंटी-इंफ्लेमेटरी साइटोकिन्स में वृद्धि दिखाई। अध्ययन में पाया गया कि Treg-Th17 अक्ष और ऑस्टियोक्लेस्टोजेनेसिस को संशोधित करके सिनबायोटिक्स ऑस्टियोपोरोसिस के लिए एक आशाजनक निवारक रणनीति हो सकती है।

अध्ययन के एक अन्य भाग में, प्रोबायोटिक-आधारित मशरूम अचार और फ्रोज़ेन डेसर्ट विकसित करना शामिल है, जिसका पोषण, संवेदी और न्यूट्रास्यूटिकल गुणों के लिए मूल्यांकन किया गया है। दो तरीकों और तेलों का उपयोग करके तैयार किए गए अचार, उच्च संवेदी स्कोर प्राप्त किया, और भंडारण के दौरान प्रोबायोटिक गिनती काफी काफी बढ़ गई। 2% विटामिन-

डी2 से भरपूर मशरूम पाउडर और 2% कोको पाउडर से भरपूर फ्रोजन डेसर्ट को भी अनुकूल संवेदी प्रतिक्रिया मिली। अध्ययन में ग्रामीण क्षेत्रों में जागरूकता कार्यक्रम भी शामिल हैं।

यह तकनीक कुपोषण से निपटने और वंचित समुदायों की स्वास्थ्य स्थिति में सुधार के लिए एक व्यावहारिक और कुशल दृष्टिकोण प्रदान करती है। खाद्य उत्पाद बनाने में यूवीबी विकिरण या सूर्य के प्रकाश के संपर्क के माध्यम से उत्पादित न्यूट्रास्यूटिकल-समृद्ध मशरूम का उपयोग उन लोगों को महत्वपूर्ण विटामिन और खनिज प्रदान कर सकता है जिनके पास पौष्टिक भोजन का विकल्प नहीं है। उत्तर प्रदेश के, हापुड़ जिले के ग्रामीण गांवों जैसे अल्पसेवित क्षेत्रों में कुपोषण को संबोधित करने में इस तकनीक की प्रदर्शित प्रभावशीलता, इसे दुनिया भर के समान क्षेत्रों के लिए एक आशाजनक समाधान बनाती है।

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ABBREVIATIONS

FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
UNDP	United Nations Development Programme
AIIMS	All India Institute of Medical Sciences
SDGs	Sustainable Development Goal
NK	Natural Killer
UV	Ultra-violet
BMI	Body Mass Index
CCD	Central composite design
RSM	Response surface methodology
P-UVL	Pulsed-UV light
PSK	Polysaccharide-protein complex
PSP	Polysaccharide-peptide complex
OFAT	One factor at a time
SFE	Supercritical fluid extraction
7-DHC	7-dehydrocholesterol or provitamin D ₃
CYPs	Membrane-associated proteins
EFSA	European Food Safety Authority
ICAR	Indian Council of Agricultural Research
BETi	Biotech Era Transforming India
VDR	Vitamin D receptor
BMD	Bone mineral density
IOF	International Osteoporosis Foundation
GIT	Gastrointestinal Track
LA	<i>Lactobacillus acidophilus</i>
LP	<i>Lactobacillus plantarum</i>
SCFAs	Short-Chain Fatty Acids
LS-BMD	Lumbar spine bone mineral density
CFU	Colony forming unit
GAE	Gallic acid equivalents
QE	Quercetin
MTCC	Microbial type culture collection
Fig.	Figure
GCMS	Gas chromatography mass spectrophometer
HPLC	High performance Liquid Chromatography
DMRT	Duncan's new multiple range test

ANOVA	Analysis of variance
SD	Standard deviation
IU	International Unit
Kg	Kilogram
FTIR	Fourier transform infrared spectroscopy
DW	Dry weight
fw	Fresh weight
h or hr	Hour
min	Minutes
mg	Milligram
s or sec	seconds
nm	Nanometers
L	Liters
cm	Centimetre
mm	Millimetre
µL	Microlitre
RPM	Revolutions per minute
µg	Microgram
°C	Degree centigrade
µM	Micro molar
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	ferric reducing antioxidant power
FE-SEM	Field Emission Scanning Electron Microscopy
3D	Three dimensional
AFM	Atomic Force Microscopy
kpa	kilopascal
RMSD	Root-mean-square deviation
MD	Molecular Dynamics
MRS	deMan Rogosa Sharpe
LAM	<i>Lactobacillus acidophilus</i> + Non irradiated mushroom
LAIM	<i>Lactobacillus acidophilus</i> + Irradiated mushroom
tb.th	Trabecular-thickness
Ovx	Ovariectomized
tb.Sp	Trabecular separation
BV/TV	Bone volume/Tissue volume
µ-CT	Micro-computed tomography
ELISA	Enzyme-linked immunosorbent assay
TIB	Tibisl
FRN	Femoral bone
PLN	Mesenteric lymph nodes
PP	Peyer's patches
DMO+LA	Dry Mushroom Mustard Oil pickle

DOO+LA	Dry Mushroom Olive oil pickle
FMO+LA	Fresh Mushroom Mustard Oil pickle
FOO+LA	Fresh Mushroom Olive oil pickle