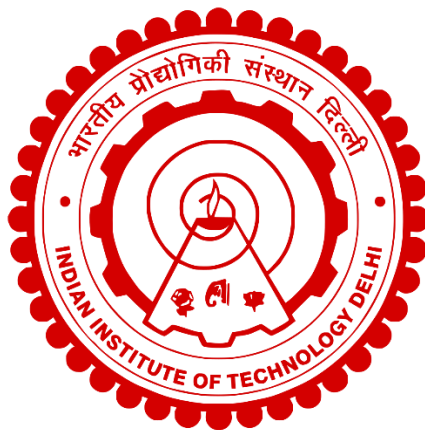


**DESIGN, DEVELOPMENT AND EVALUATION
OF FERMENTED HERBAL NUTRA-BEVERAGE
FROM SHATAVARI (*ASPARAGUS RACEMOSUS*)**

DIVYA



**CENTRE FOR RURAL DEVELOPMENT AND
TECHNOLOGY**

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2025

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by

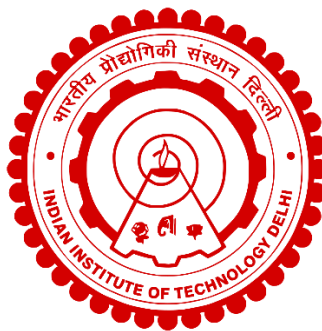
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Centre for Rural Development and Technology

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2025

CERTIFICATE

This is to certify that the thesis entitled, “**Design, Development and Evaluation of Fermented Herbal Nutra-beverage from Shatavari (*asparagus racemosus*)**” being submitted by **Ms. Divya** to the **Indian Institute of Technology Delhi** for the award of ‘**Doctor of Philosophy**’ is a record of bonafide research work carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis. To the best of my knowledge, the results contained in the 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

This research explores the nutraceutical potential of *Asparagus racemosus* (Shatavari), a medicinal plant indigenous to tropical and subtropical regions of India, emphasizing its application as a functional food and nutraceutical ingredient. The study utilized dried Shatavari roots sourced from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, which underwent comprehensive physicochemical and biochemical evaluations. The analysis revealed a moisture content of 9.82% (w/w), ash content of 7.06% (w/w), saponin content of 2.82% (w/w), and total starch content of 56.85% (w/w), highlighting the plant's nutritional and functional attributes.

Antioxidant activities were assessed using DPPH, ABTS, and FRAP assays for both methanol and aqueous extracts. Aqueous extracts consistently exhibited superior antioxidant potential. Quantification of total phenolic and flavonoid contents demonstrated that methanol extracts contained higher phenolic content (13.50 mg of GAE/g) compared to aqueous extracts (0.80 mg of GAE/g). High-performance liquid chromatography (HPLC) was employed to identify and quantify bioactive compounds, revealing significant levels of quercetin, rutin, ferulic acid, and gallic acid. These findings underscore Shatavari's rich phytochemical profile and its potential for health applications.

The study also involved the development of a Shatavari-based low-alcohol nutraceutical beverage (SLANB) through the fermentation of Shatavari root juice using *Saccharomyces cerevisiae* NCIM 2428. Process optimization was achieved through advanced methodologies, including artificial neural networks (ANN) and response surface methodology (RSM). Optimal fermentation conditions—temperature at 32°C, pH at 4.0, and inoculum concentration of 10% (v/v)—were established, significantly influencing the beverage's pH, total soluble solids, yeast count, and ethanol yield. The fermentation process resulted in an ethanol concentration of 3.21 g/L and an antioxidant activity of 421.47 µg/L. Sensory evaluation using a 9-point hedonic scale indicated high consumer acceptability, with sample 3 achieving the highest preference due to its balanced flavor, aroma, and overall appeal.

Toxicological evaluations were conducted to ensure the safety of SLANB. Acute and subacute toxicity studies on mice, administered doses of up to 1.5 mL/100g body weight

over 28 days, revealed no adverse effects on body weight, organ weight, food consumption, haematological parameters, or mortality. These findings confirmed the beverage's safety for consumption.

In conclusion, this study demonstrates the feasibility of incorporating Shatavari into nutraceutical applications through innovative biotechnological approaches. The research highlights the plant's rich bioactive compound profile, optimized fermentation methodologies, and its safety and acceptability as a functional beverage. These findings pave the way for further exploration and commercialization of Shatavari-based products in health-focused food and beverage sectors.

Keywords: *Shatavari; Starch; Bioreactor; Saccharomyces cerevisiae; Modelling; Product validation; Characterization; Sensory attributes; Toxicity*

सारांश

यह शोध भारत के उष्णकटिबंधीय और उपोष्णकटिबंधीय क्षेत्रों के लिए स्वदेशी औषधीय पौधे एस्पेरागस रेसमोसस (शताब्दी) की न्यूट्रास्युटिकल क्षमता की खोज करता है, जो एक कार्यात्मक खाद्य और न्यूट्रास्युटिकल घटक के रूप में इसके अनुप्रयोग पर जोर देता है। अध्ययन में केंद्रीय औषधीय और सुगंधित पौधों के संस्थान (सी. आई. एम. ए. पी.) लखनऊ से प्राप्त सूखे शताब्दी जड़ों का उपयोग किया गया, जिनका व्यापक भौतिक रासायनिक और जैव रासायनिक मूल्यांकन किया गया। विश्लेषण से पता चला कि 9.82% (w/w) राख सामग्री की 7.06% (w/w) सैपोनिन सामग्री 2.82% (w/w) और कुल स्टार्च सामग्री 56.85% (w/w) पौधे की पोषण और कार्यात्मक विशेषताओं को उजागर करती है।

मेथनॉल और जलीय अर्क दोनों के लिए डी. पी. पी. एच., ए. बी. टी. एस. और एफ. आर. ए. पी. परख का उपयोग करके एंटीऑक्सीडेंट गतिविधियों का आकलन किया गया। जलीय अर्क लगातार बेहतर एंटीऑक्सीडेंट क्षमता का प्रदर्शन करते हैं। कुल फेनोलिक और फ्लेवोनोइड सामग्री की मात्रा ने प्रदर्शित किया कि मेथनॉल अर्क में जलीय अर्क (0.80 मिलीग्राम जीई/जी) की तुलना में उच्च फेनोलिक सामग्री (13.50 मिलीग्राम जीई/जी) होती है। उच्च प्रदर्शन वाली तरल क्रोमैटोग्राफी (एच. पी. एल. सी.) को जैव सक्रिय यौगिकों की पहचान करने और उनकी मात्रा निर्धारित करने के लिए नियोजित किया गया था, जिससे क्लेरसेटिन, रुटीन, फेरुलिक एसिड और गैलिक एसिड के महत्वपूर्ण स्तर का पता चलता है। ये निष्कर्ष शतावरी के समृद्ध फाइटोकेमिकल प्रोफाइल और स्वास्थ्य अनुप्रयोगों के लिए इसकी क्षमता को रेखांकित करते हैं। इस अध्ययन में सैक्रोमाइसेस सेरेविसिया एनसीआईएम 2428 का उपयोग करके शताब्दी जड़ के रस के किण्वन के माध्यम से एक शताब्दी-आधारित कम अल्कोहल वाले न्यूट्रास्युटिकल पेय (एसएलएएनबी) का विकास भी शामिल था। प्रक्रिया अनुकूलन कृत्रिम तंत्रिका नेटवर्क (ए. एन. एन.) और प्रतिक्रिया सतह पद्धति (आर. एस. एम.) सहित उन्नत पद्धतियों के माध्यम से प्राप्त किया गया था। इष्टतम किण्वन की स्थिति- 32 डिग्री सेल्सियस पर तापमान, 4.0 पर पीएच, और 10% (वी/वी) की इनोकुलम सांद्रता-स्थापित की गई थी, जो पेय के पीएच, कुल घुलनशील ठोस, खमीर की गिनती और इथेनॉल उपज को महत्वपूर्ण रूप से प्रभावित करती है। किण्वन प्रक्रिया के परिणामस्वरूप 3.21 g/L की इथेनॉल सांद्रता और 421.47 µg/L की एंटीऑक्सीडेंट गतिविधि हुई। 9-बिंदु हेडोनिक पैमाने का उपयोग करके संवेदी मूल्यांकन ने उच्च उपभोक्ता

स्वीकार्यता का संकेत दिया, जिसमें नमूना 3 अपने संतुलित स्वाद, सुगंध और समग्र अपील के कारण उच्चतम वरीयता प्राप्त करता है। एस. एल. ए. एन. बी. की सुरक्षा सुनिश्चित करने के लिए विषाक्त मूल्यांकन किए गए। चूहों पर तीव्र और उप-तीव्र विषाक्तता अध्ययन, 28 दिनों में 1.5 एमएल/100 ग्राम शरीर के वजन तक की प्रशासित खुराक, शरीर के वजन, अंग के वजन, भोजन की खपत, हेमेटोलॉजिकल मापदंडों, या मृत्यु दर पर कोई प्रतिकूल प्रभाव नहीं दिखा। इन निष्कर्षों ने उपभोग के लिए पेय की सुरक्षा की पुष्टि की।

अंत में, यह अध्ययन नवीन जैव प्रौद्योगिकी दृष्टिकोण के माध्यम से शतवारी को न्यूट्रास्युटिकल अनुप्रयोगों में शामिल करने की व्यवहार्यता को दर्शाता है। शोध पौधे की समृद्ध जैव सक्रिय यौगिक रूपरेखा, अनुकूलित किण्वन पद्धतियों और एक कार्यात्मक पेय के रूप में इसकी सुरक्षा और स्वीकार्यता पर प्रकाश डालता है। ये निष्कर्ष स्वास्थ्य-केंद्रित खाद्य और पेय क्षेत्रों में शताब्दी-आधारित उत्पादों के आगे के अन्वेषण और व्यावसायीकरण का मार्ग प्रशस्त करते हैं।

Keyword: शतवारी; स्टार्च; बायोरिएक्टर; सैक्रोमाइसेस सेरेविसिया; मॉडलिंग; उत्पाद सत्यापन; लक्षण वर्णन; संवेदी विशेषताएँ; विषाक्तता

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
<	Less than
>	Greater than
≥	Greater than or equal to
AOAC	Association of Official Analytical Chemists
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
ANN	Artificial Neural Network
ATCC	American Type Culture Collection
A_w	Water activity
BET	BET Brunauer-Emmett-Teller
CFU	Colony Forming Unit
CFU.g ⁻¹	Colony Forming Unit per gram
CFU.mL ⁻¹	Colony Forming Unit per milliliter
°C	Degree Celsius
°C min ⁻¹	Degree Celsius per minute
cm	Centimeter
CDRI	Center of Drug Research Institute
CSIR-CIMAP	Centre Institute of Medicinal Aromatic Plant
DSC	Differential Scanning Calorimetry
DPPH	2,2-diphenyl-1-picrylhydrazyl
d.b.	Dry basis
E_a	Activation energy
eg.	Example
Eq.	Equation
FAO	Food and Agriculture Organization
Fig.	Figure
FTIR	Fourier Transform Infrared Spectroscopy
FSSAI	Food Safety and Standards Authority of India
FAO	Food and Agriculture Organization
G	Gram

g/L	gram per litre
GA	Genetic Algorithm
GC	Gas Chromatography
GAE	Gallic equivalent acid
h	Hour
HCL	Hydrochloric acid
HDL	High density lipoprotein
HPLC	High-Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
J	Joule
K	Kelvin
kg	Kilogram
kg.cm ⁻²	Kilogram per centimeter square
kg.m ⁻³	Kilogram per meter cube
kg s ⁻¹	Kilogram per second
KJ	Kilo Joule
KJ mol ⁻¹	Kilo Joule per Mole
kJ mol ⁻¹ K ⁻¹	Kilo Joule per Mole per Kelvin
log	logarithmic
LC-RID	Liquid Chromatography with Refractive Index Detection
Mm	Micrometer
μl	Microliter
Mg	Milligram
ml	Milliliter
mL min ⁻¹	milliliter per minute
Mv	Millivolt
Meq	milli equivalent
MPa	mega Pascal
Min	Minute
Mol	Mole
MSI	Moisture sorption isotherm
MS	Mass Spectroscopy
MSE	Mean Square Error

MLFF	Multi-Layer Feed Forward
NaOH	Sodium Hydroxide
NCIM	National Collection of Industrial Microorganisms
NCL	National Chemical Laboratory
no.	Number
O ₂	Oxygen
°C min ⁻¹	Degree Celsius per minute
rpm	rotation per minute
s	Second
SD	Standard deviation
SCADA	Supervisory Control and Data Acquisition
SLANB	Shatavari based Low Alcohol Nutrabeverage
SGPT	Serum Glutamic Pyruvic Transaminase
SGOT	Serum Glutamic Oxaloacetic Transaminase
TSS	Total Soluble Solids
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TIC	Total Ion Chromatogram
U/g	units per gram
v/v	volume/volume
WHO	World Health Organization
W	Weight
w.b.	wet basis
w/w	weight/weight
w/v	weight/volume