

**Potential Utilization of Mahua  
(*Madhuca longifolia*) Flowers for the  
Development of Value-Added Products**

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**Centre for Rural Development and Technology**

**Indian Institute of Technology Delhi**

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**Potential Utilization of Mahua  
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Development of Value-Added Products**

by

**Achala Gupta**

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## CERTIFICATE

This is to certify that the thesis entitled, “**Potential Utilization of Mahua (*Madhuca longifolia*) Flowers for the Development of Value-Added Products**” being submitted by **Ms. Achala Gupta** 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 our guidance and supervision and has fulfilled the requirements for the submission of the thesis. To the best of our 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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I did it!!!

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## ABSTRACT

Minor forest produce (MFP) offers a green social security to approximately 275 million people accounting almost 70% of the tribal communities in India. Nevertheless, the systematic collection, processing, and utilization of NTFPs remain significantly underdeveloped. Therefore, effective utilization of MFP for the development of sustainable value-added products is imperative. *Madhuca longifolia* (mahua) flower is one of the high value commercial forest produce widely available in the Central and Northern regions of India, particularly in the states of Madhya Pradesh, Chhattisgarh, Odisha and Uttar Pradesh. The collection of mahua flowers accounts for around 85000 tons, out of a potential of 4,90,000 tons. About 17.55% of tribal people are employed around mahua flower collection of total workforces of India. Despite being readily available and nutrient-rich, mahua flowers remains largely underutilised. Therefore, it is essential to explore and investigate the broader and prospective applications of mahua flowers to fully harness their social and economic potential. This thesis encompasses a detailed study on understanding the potential of mahua flowers for the development of value-added products. The study focused on underscoring the multi-dimensional potential of mahua flowers, emphasizing its application in fermented fortified beverage, essential oil extraction, nutrient-rich feed and bioenergy source.

Mahua flowers are a rich source of fermentable sugars i.e. fructose, glucose and sucrose and provide an ideal substrate for fermentation to produce vitamin B12. In this thesis, a microbial consortium mediated co-fermentation technique was utilized to fortify vitamin B12 in mahua flower juice. Mono-cultured fermentation with *S. cerevisiae* revealed effective utilisation of sugars in the first 12 h i.e. 98.7%, 94.8%, and 92.6% for glucose, fructose and sucrose, respectively, resulting in a fermented mahua beverage lying in the category of '**Fruit Wine Other than Grape Wine**' under Food Safety and Standards Authority of India (FSSAI) guidelines. However, to cater to the non-alcoholic target group, additional microorganisms were screened to develop a low-alcohol beverage. Therefore, GRAS microorganisms exhibiting synergistic interactions i.e., *Lactobacillus fermentum* (LF), *Lactobacillus plantarum* (LP), *Propionibacterium freudenreichii* (PB), *Lactobacillus acidophilus* (LA) and *Saccharomyces cerevisiae* (SC) were identified through cross-streak and microbial interaction assays. Among them, LA demonstrated antagonistic behaviour toward the other strains. Further experimentations revealed that LP and PB exhibited good potential of production of vitamin B12 in mono and co-cultured system. Fermenter-based growth kinetics indicated that co-fermentation of mahua juice with LP and PB at pH 7 and a 1:1 inoculum ratio resulted in a

highly sensorily acceptable beverage with a vitamin B12 yield of 10.77 mg/L and an alcohol content of 1.97% by the 10th day, thereby, lying in the category of ‘**Low Alcoholic Beverage**’ under the FSSAI guidelines. The results highlighted microbial consortia as an effective method for vitamin B12 biofortification in plant-based beverage, however, fermentation masks the original aroma and flavour of the fortified beverage. In order to address this challenge, the fortified beverage was incorporated with the essential oil (EOs) extracted from fresh mahua flowers via conventional (hydrodistillation- HD, enzyme-assisted hydrodistillation- HDE and Soxhlet extraction- SOX) and emerging (ultrasound assisted extraction- UAE, supercritical CO<sub>2</sub> extraction- SCFE) techniques. The study illustrated extraction of highest quality EOs with better terpenoid concentration i.e. 2-acetyl-1-pyrroline,  $\gamma$ -terpinene, myrcene,  $\alpha$ -terpineol, limonene, L-linalool and the maximum yield (2.074%) by SCFE technique. The components of EOs were effectively detected by both E-nose and GC-MS, with 78.76% of similar compounds. SCFE-derived EOs exhibited significantly higher ( $p < 0.05$ ) inhibition activities against  $\beta$ -glucosidase (37.16%) and  $\alpha$ -amylase (43%) respectively and higher carotenoid content. Results demonstrated that SCFE is a superior technique for obtaining high-quality EOs from low-lipid plant sources like mahua flowers, outperforming conventional methods such as HD, HDE and SOX. However, the incorporation of extracted EOs into the fortified fermented beverage did not result in significant improvement in aroma and taste due to the strong presence of phenolic compounds developed during fermentation.

After the extraction of juice from mahua flowers and its utilisation for the production of fortified beverage and extraction of essential oil, approximately 45-48% of the spent biomass (MFSB) is generated from mahua flowers. This biomass is directly discarded for the degradation thereby affecting the environment. Therefore, to address the increasing challenge of dumped biomass the MFSB was explored to understand its potential for production of non-food value added products. Experimental and theoretical data revealed good potential of MFSB to be used as animal feed (cattle, poultry and fishery feed), compost, bioethanol and other bio-based products.

The findings of this thesis advocate for the integrated utilization of mahua flowers in diverse industries mainly supporting the livelihood of primary collectors and traditional users of mahua flowers in local tribal communities. This research promotes value addition and diversifies the applications of mahua flowers and will help shift mahua flower collectors/suppliers to entrepreneurs.

**Keywords:** Mahua flowers; Fortification; Vitamin B12; Microbial consortia; Co-fermentation; Essential oil; Waste biomass; Capacity building; Survey; Technology transfer

## सारांश

लघु वन उत्पाद (एमएफपी) लगभग 275 मिलियन लोगों को एक हरित सामाजिक सुरक्षा प्रदान करता है, जो भारत की जनजातीय आबादी का लगभग 70% है। फिर भी, गैर-काष्ठ वन उत्पादों (एनटीएफपी) का प्रणालीबद्ध संग्रह, प्रसंस्करण और उपयोग अभी भी काफी अविकसित है। इसलिए, टिकाऊ मूल्य संवर्धित उत्पादों के विकास के लिए एमएफपी का प्रभावी उपयोग अत्यावश्यक है। मधुका लोंगिफोलिया (महुआ) का फूल एक उच्च मूल्य वाला वाणिज्यिक वन उत्पाद है जो भारत के मध्य और उत्तरी क्षेत्रों, विशेष रूप से मध्य प्रदेश, छत्तीसगढ़, ओडिशा और उत्तर प्रदेश राज्यों में व्यापक रूप से उपलब्ध है। महुआ फूलों का संग्रह लगभग 85,000 टन है, जबकि इसकी संभावित उपलब्धता 4,90,000 टन है। भारत की कुल कार्यबल का लगभग 17.55% जनजातीय लोग महुआ फूल संग्रह से जुड़े हुए हैं। हालांकि महुआ फूल आसानी से उपलब्ध और पोषक तत्वों से भरपूर हैं, फिर भी इनका उपयोग बहुत कम किया जाता है। इसलिए, महुआ फूलों के व्यापक और संभावित उपयोगों की खोज करना और उनका अध्ययन करना आवश्यक है ताकि इनके सामाजिक और आर्थिक संभावनाओं का पूरा उपयोग किया जा सके। यह शोध-प्रबंध महुआ फूलों के मूल्य संवर्धित उत्पादों के विकास की संभावना को समझने पर केंद्रित एक विस्तृत अध्ययन प्रस्तुत करता है। अध्ययन ने महुआ फूलों की बहुआयामी संभावनाओं को रेखांकित किया, विशेष रूप से इसके किण्वित पोषक पेय, आवश्यक तेल निष्कर्षण, पोषक पशु आहार और जैव ऊर्जा स्रोत के रूप में उपयोग पर बल दिया।

महुआ फूल किण्वनीय शर्कराओं जैसे फ्रक्टोज़, ग्लूकोज़ और सुक्रोज का समृद्ध स्रोत हैं और विटामिन B12 उत्पादन हेतु किण्वन के लिए एक आदर्श सबस्ट्रेट प्रदान करते हैं। इस शोध-प्रबंध में एक सूक्ष्मजीव संघटित सह-किण्वन तकनीक का उपयोग करके महुआ फूल रस में विटामिन B12 की दृढ़ता (फोर्टिफिकेशन) की गई। *S. cerevisiae* के साथ एकल-संस्कृति किण्वन से पहले 12 घंटों में शर्कराओं की प्रभावी उपयोगिता पाई गई – ग्लूकोज़, फ्रक्टोज़ और सुक्रोज के लिए क्रमशः 98.7%, 94.8% और 92.6%। इसके परिणामस्वरूप तैयार हुआ किण्वित महुआ पेय भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण (FSSAI) के दिशा-निर्देशों के अंतर्गत 'अंगूर रहित फलों की वाइन' की श्रेणी में आता है। हालांकि, गैर-मद्यपान करने वाले लक्षित समूह को ध्यान में रखते हुए, कम-अल्कोहल पेय विकसित करने हेतु अतिरिक्त सूक्ष्मजीवों की स्क्रीनिंग की गई। इसलिए, ऐसे GRAS (सामान्यतः सुरक्षित माने जाने वाले) सूक्ष्मजीवों की पहचान की गई, जो आपसी सहक्रियात्मक क्रियाएं प्रदर्शित करते हैं, जैसे कि *Lactobacillus fermentum* (LF), *Lactobacillus plantarum* (LP), *Propionibacterium freudenreichii* (PB), *Lactobacillus acidophilus* (LA) और *Saccharomyces cerevisiae* (SC)। क्रॉस-स्ट्रिक और सूक्ष्मजीवीय परस्पर क्रिया परीक्षणों के माध्यम से पाया गया कि LA अन्य उपभेदों के प्रति

प्रतिकूल व्यवहार प्रदर्शित करता है। आगे के प्रयोगों में यह पता चला कि LP और PB ने एकल एवं सह-संस्कृति प्रणाली में विटामिन B12 उत्पादन की अच्छी क्षमता दिखाई। फर्मेंटर-आधारित वृद्धि गतिकी से संकेत मिला कि pH 7 और 1:1 इनोकुलम अनुपात पर LP और PB के साथ महुआ रस का सह-किण्वन 10वें दिन तक 10.77 मिलीग्राम/लीटर विटामिन B12 और 1.97% अल्कोहल सामग्री के साथ एक अत्यधिक इंद्रिय रूप से स्वीकार्य पेय प्रदान करता है, जो FSSAI दिशानिर्देशों के अंतर्गत 'निम्न-अल्कोहल पेय' की श्रेणी में आता है। परिणामों ने पौध-आधारित पेय में विटामिन B12 की जैव-दृढ़ता हेतु सूक्ष्मजीव संघटन को एक प्रभावी विधि के रूप में उजागर किया। हालाँकि, किण्वन के दौरान विकसित फेनोलिक यौगिकों की उपस्थिति के कारण, किण्वित पेय की मूल सुगंध और स्वाद में कमी पाई गई। इस चुनौती को दूर करने हेतु, किण्वित पेय में ताजे महुआ फूलों से निकाले गए आवश्यक तेलों (EOs) को सम्मिलित किया गया। इन आवश्यक तेलों को पारंपरिक (हाइड्रोडिस्टिलेशन-HD, एंजाइम-सहायता प्राप्त हाइड्रोडिस्टिलेशन-HDE और सॉक्सलेट निष्कर्षण-SOX) और उभरती (अल्ट्रासोनिक सहायक निष्कर्षण-UAE, सुपरक्रिटिकल CO<sub>2</sub> निष्कर्षण-SCFE) तकनीकों के माध्यम से निकाला गया। अध्ययन में SCFE तकनीक द्वारा उच्च गुणवत्ता वाले आवश्यक तेलों का निष्कर्षण प्राप्त हुआ, जिसमें बेहतर टरपीनॉयड सांद्रता थी, जैसे कि 2-acetyl-1-pyrroline,  $\gamma$ -terpinene, myrcene,  $\alpha$ -terpineol, limonene, L-linalool, और अधिकतम तेल निष्कर्षण 2.074% पाया गया। EOs के घटकों का सफलतापूर्वक पता लगाने के लिए E-nose और GC-MS का उपयोग किया गया, जिनमें 78.76% यौगिक समान पाए गए। SCFE-उत्पन्न EOs ने  $\beta$ -glucosidase (37.16%) और  $\alpha$ -amylase (43%) के विरुद्ध उल्लेखनीय अवरोधन गतिविधियाँ ( $p < 0.05$  पर महत्वपूर्ण) और उच्च कैरोटीनॉयड सामग्री प्रदर्शित की। परिणामों ने दिखाया कि SCFE कम-वसा वाले पौध स्रोतों जैसे महुआ फूलों से उच्च गुणवत्ता वाले आवश्यक तेल प्राप्त करने की श्रेष्ठ तकनीक है, जो HD, HDE और SOX जैसी पारंपरिक विधियों से बेहतर है। हालाँकि, निकाले गए आवश्यक तेलों को किण्वित पेय में सम्मिलित करने पर भी स्वाद और सुगंध में कोई उल्लेखनीय सुधार नहीं पाया गया, जिसका कारण किण्वन के दौरान बने प्रबल फेनोलिक यौगिक थे।

महुआ फूलों से रस निकालने, उसका उपयोग फोर्टिफाइड पेय उत्पादन में करने और आवश्यक तेल निकालने के बाद, लगभग 45-48% बचा हुआ बायोमास (MFSB) उत्पन्न होता है। यह बायोमास सीधे सड़ने के लिए फेंक दिया जाता है, जिससे पर्यावरण पर नकारात्मक प्रभाव पड़ता है। इसलिए, फेंके गए बायोमास की समस्या को हल करने के लिए MFSB के संभावित उपयोग को गैर-खाद्य मूल्य संवर्धित उत्पादों के लिए खोजा गया। प्रयोगात्मक और सैद्धांतिक डेटा ने दर्शाया कि MFSB का उपयोग पशु आहार (गाय-बकरी, मुर्गी पालन और मत्स्य पालन आहार), खाद, जैवइथेनॉल और अन्य जैव-आधारित उत्पादों के रूप में किया जा सकता है।

इस शोध-प्रबंध के निष्कर्ष महुआ फूलों के एकीकृत उपयोग को विविध उद्योगों में बढ़ावा देते हैं, विशेष रूप से महुआ फूलों के प्राथमिक संग्राहकों और पारंपरिक उपयोगकर्ताओं की आजीविका को समर्थन प्रदान करते हैं। यह अनुसंधान महुआ फूलों के मूल्य संवर्धन को बढ़ावा देता है और इसके उपयोगों में विविधता लाता है, जिससे महुआ फूल संग्राहक/आपूर्तिकर्ता उद्यमियों में परिवर्तित हो सकते हैं।

**कीवर्ड्स:** महुआ फूल; फोर्टिफिकेशन; विटामिन B12; सूक्ष्मजीव संघटन; सह-किण्वन; आवश्यक तेल; अपशिष्ट बायोमास; क्षमता निर्माण; सर्वेक्षण; प्रौद्योगिकी स्थानांतरण।

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## LIST OF SYMBOLS AND ABBREVIATIONS

%: Percent

<: Less than

>: Greater than

≥: Greater than or equal to

°C: Degree Celsius

Eq.: Equation

g: gram

g.L<sup>-1</sup>: gram per litre

h: hour

kg: kilogram

μm: micrometer

μL: micro Litre

mm: milimeter

min: minute

meq: mili equivalent

mL: mili Litre

mg: miligram

PSI: pound per square inch

R<sup>2</sup>: Co-efficient of determination

rad: radian

s: second

SD: Standard deviation

v: volume

w: weight