

**INTEGRATED PROCESSING FOR VALUE CHAIN
DEVELOPMENT OF INDIAN TRANS HIMALAYAN
SEA BUCKTHORN (*HIPPOPHAE SP.*)**

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**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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by

NIKITA SANWAL

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CERTIFICATE

This is to certify that the thesis entitled, “**Integrated Processing for Value Chain Development of Indian Trans Himalayan Sea buckthorn (*Hippophae sp.*)**” being submitted by **Ms Nikita Sanwal** 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 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.

(Prof Jatindra K Sahu)

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ABSTRACT

The Trans Himalayan region of the Indian subcontinent being rich in traditional medicinal plants offers numerous socio-economic benefits. Referred to as ‘God sent medicine’ or ‘Liquid Gold’ in the Tibetan, Chinese, and Mongolian cultures, sea buckthorn (*Hippophae sp.*) is cultivated in various countries around the world, but it is particularly prominent in certain regions. There are significant opportunities for producing high value products using various novel-processing techniques while retaining their medicinal and aromatic properties. Furthermore, the declining livelihood opportunities for communities in the remote Himalayan regions necessitate the scientific exploitation of underutilized wild bioresources like sea buckthorn, involving local communities to enhance nutritional and livelihood security. Various parts of sea buckthorn plant, including seeds, pulp, fruit, juice, and leaves, contain a wide range of bioactive compounds such as fatty acids, lipids, organic acids, amino acids, vitamins, flavonoids, phenols, terpenes, tannins, and minerals. This research is an attempt for creating an integrated processing system for sea buckthorn, encompassing its various parts such as leaves, berries, and seeds, sourced from the Indian trans-Himalayan regions (IHR). Within this scope, the processing of the sea buckthorn leaves involved two primary objectives: (i) analyzing gender-specific phytoconstituents and its bioactivity potentials, and (ii) utilizing *in-situ* fermentation with kombucha pellicle to develop a functional beverage. Similarly, the seeds were processed to examine the impact of ultrasound-assisted extraction (UAE) on sea buckthorn seed oil, while for the processing/ value addition of berries, the study aimed to evaluate the changes in phenolic compounds, antioxidant activity, sugars, and minerals and flavor profile during the *in-situ* kombucha fermentation of sea buckthorn berry juice.

A comparative bioactive functionality of male and female leaves of two sea buckthorn species i.e., *Hippophae rhamnoides* and *Hippophae salicifolia* was analyzed to comprehend the selection of sea buckthorn (*Hippophae sp.*) leaves (SBL) as a potential substrate for tea and/or nutraceutical formulations based on gender and species. Gallic acid, epigallocatechin, sesamin, epigallocatechin-gallate, rutin and quercetin were observed to be present significantly in male leaves, unlike female. Consequently, male leaves exhibited better antioxidant activity as the ranges of IC₅₀ and ferrous reducing antioxidant power (FRAP) values of male and female leaves were 1.21–3.86 and 1.32–5.09 µg/mL, 303.45–407.33 and 233.45–356.92 mg BHT/g dry weight respectively. However, aroma-producing components like linalool and geraniol were only detected in female leaves. Moreover, the mineral concentrations including Mg, K, Ca, Cr, Fe, Cu, and Zn varied in two species and between the genders. The relationships between phytoconstituents and the factors (gender, species and extraction methods), a principal component analysis (PCA) based on correlation coefficients established a significant difference ($p < 0.05$) between the species, gender and extraction methods.

A study on enhancing the functional potential of SBL-infusion via *in-situ* kombucha fermentation utilizing a symbiotic culture of bacteria and yeast (SCOBY) pellicle was carried out. To develop a unique functional beverage SCOBY was employed to ferment SBL at 30±2°C. The dynamics of phytoconstituents during the fermentation was monitored on the 3rd, 7th, 10th, and 14th days. The PCA differentiated the two species of SBL, *H. rhamnoides* and *H. salicifolia* suitable for kombucha fermentation, as well as the natural variation of biochemical composition during the fermentation period

from 0th day to 14th day. Some of the polyphenolic components like epigallocatechin, kaempferol, hamamelitannin, rutin, and quercetin were significantly ($p < 0.05$) increased during the fermentation. Cu and Zn content decreased in fermented kombucha (Log2 change of -0.51 and -0.18 respectively), while Mg, K, Ca, and Ni increased (Log2 change of 0.201, 0.15, 0.44, 0.41 respectively). Fe content was augmented from 0 to 70.51 ppb as a result of kombucha cultivation in the SBL infusion. The developed organic and phenolic acids resulted in increasing titratable acidity and decreasing pH. 3-methylmannoside, xanthosine, and diisopropyl silyl isopropyl ether were the prominent flavor compounds detected in kombucha.

Effects of UAE conditions viz., treatment time, temperature, solvent-to-sample ratio, and ultrasound power on antioxidant activity, stability, and extraction efficiency of oil from sea buckthorn seeds were studied. The treatment time, temperature, and power showed a significant effect ($p < 0.05$) on the extraction yield and quality of sea buckthorn seed oil (SBSO). The UAE process achieved a maximum oil recovery of 6.87% and was significantly higher than that by the solvent extraction technique. The optimum values of UAE conditions attained were at 50°C for 8.28 min at 552 W ultrasound power with a solvent-to-sample ratio 10:1 (mL:g). The SBSO samples revealed higher carotenoid content (237.04 ± 3.86 mg per 100 g β -carotene equivalent), potent free radical scavenging ability (1.42 ± 0.01 IC50 μ g per mL), and oxidative stability (totox value= 8.04 ± 0.02) upon the UAE treatment. GC-MS profiling of the oil sample at optimum UAE conditions exhibited better retention of fatty acids.

To create a unique functional beverage, sea buckthorn berry juice was fermented using a kombucha pellicle for 14 days at 25°C. The resulting kombucha beverage was extensively examined and compared to the original juice, revealing increased antioxidant activity (23.49%), phenolic from 28.45 mg GAE/g to 35.89 mg GAE/g, and flavonoid content from 49.66 to 58.08 mg CE/g, and essential elements (Mg, K, Fe, Cr, Cu, and Zn) for human health. Analysis of sugar content revealed that mannose was the dominant component in the berry kombucha, accounting for 57.37% of the total, while glucose constituted 42.63% of the total sugar content. The fermentation process completely converted the initial sucrose in the berry juice (which accounted for 14.1%) into glucose, rendering sucrose negligible in the final berry kombucha. The fermentation process also transformed the flavor profile, resulting in a highly desirable sensory experience.

To sensitize and empower different aligned stakeholders in sea buckthorn processing, several interactive workshops, seminars and stakeholders' meeting were conducted in Lahaul & Spiti district of Himachal Pradesh, India. The idea was to encompass enhanced production, establishing marketing connections, improving processing methods, disseminating information, and promoting in local community.

This research work would serve as an all-encompassing rural action plan that leverages transformative rural technologies, enhancing sea buckthorn processing, generating local livelihood opportunities, conserving resources, and promoting sustainable development in the Himalayan regions of Himachal Pradesh.

Keywords: Sea buckthorn; Novel processing technologies; Process optimization; Process dynamics; Bioactive potentials; Functional foods; Metabolomics; Principal Component Analysis; Value chain development

सारांश

भारतीय उपमहाद्वीप का ट्रांस हिमालयी क्षेत्र पारंपरिक औषधीय पौधों से समृद्ध होने के कारण कई सामाजिक-आर्थिक लाभ प्रदान करता है। तिब्बती, चीनी और मंगोलियाई संस्कृतियों में 'ईश्वर द्वारा भेजी गई दवा' या 'लिक्रिड गोल्ड' के रूप में संदर्भित, सी बकथॉर्न (हिप्पोफे) की खेती दुनिया भर के विभिन्न देशों में की जाती है, लेकिन यह कुछ क्षेत्रों में विशेष रूप से प्रमुख है। उनके औषधीय और सुगंधित गुणों को बनाए रखते हुए विभिन्न प्रसंस्करण तकनीकों का उपयोग करके उच्च मूल्य वाले उत्पादों के उत्पादन के लिए महत्वपूर्ण अवसर हैं। इसके अलावा, दूरदराज के हिमालयी क्षेत्रों में समुदायों के लिए आजीविका के घटते अवसरों के लिए सी बकथॉर्न जैसे कम उपयोग किए गए जंगली जैव संसाधनों के वैज्ञानिक दोहन की आवश्यकता है, जिसमें स्थानीय समुदायों को पोषण और आजीविका सुरक्षा बढ़ाने के लिए शामिल किया गया है। बीज, लुगदी, फल, जूस और पत्तियों सहित सी बकथॉर्न के पौधे के विभिन्न भागों में फैटी एसिड, लिपिड, कार्बनिक अम्ल, अमीनो एसिड, विटामिन, फ्लेवोनोइड्स, फिनोल, टेरपेन, टैनिन, खनिज और बायोएक्टिव यौगिकों की एक विस्तृत श्रृंखला होती है। यह शोध सी बकथॉर्न के लिए एक एकीकृत प्रसंस्करण प्रणाली बनाने का एक प्रयास है, जिसमें भारतीय ट्रांस-हिमालयी क्षेत्रों (IHR) से प्राप्त पत्तियों, जामुन और बीजों जैसे इसके विभिन्न भागों को शामिल किया गया है। इस दायरे के भीतर, सी बकथॉर्न के पत्तों के प्रसंस्करण में दो प्राथमिक उद्देश्य शामिल थे: (i) लिंग-विशिष्ट पादप घटकों और इसकी जैवसक्रियता क्षमता का विश्लेषण, और (ii) एक कार्यात्मक पेय विकसित करने के लिए कोम्बुचा पेलिकल के साथ इन-सीटू किण्वन का उपयोग करना। इसी तरह, सी बकथॉर्न सीड ऑयल पर अल्ट्रासाउंड-असिस्टेड एक्सट्रैक्शन (यूआई) के प्रभाव की जांच करने के लिए बीजों को संसाधित किया गया था, जबकि बेरीज के प्रसंस्करण/मूल्यवर्धन के लिए अध्ययन का उद्देश्य सी बकथॉर्न बेरी रस के इन-सीटू कोम्बुचा किण्वन के दौरान, फेनोलिक यौगिकों, एंटीऑक्सीडेंट गतिविधि, शर्करा, खनिज और स्वाद प्रोफ़ाइल में परिवर्तन का मूल्यांकन करना था।

न्यूट्रास्युटिकल और/या चाय के लिए संभावित सबस्ट्रेट के रूप में सी बकथॉर्न (हिप्पोफे एसपी.) पत्तियों (एसबीएल) के चयन को समझने के लिए दो सी बकथॉर्न प्रजातियों यानी, हिप्पोफे रम्नोइड्स और हिप्पोफे सैलिसिफोलिया की नर और मादा पत्तियों की तुलनात्मक जैव सक्रिय कार्यक्षमता का विश्लेषण किया गया। लिंग और प्रजातियों के आधार पर, मादा के विपरीत, नर पत्तियों में गैलिक एसिड, एपिगैलोकैटेचिन, सेसमिन, एपिगैलोकैटेचिन-गैलेट, रूटिन और केरसेटिन महत्वपूर्ण रूप से मौजूद पाए गए। नतीजतन, नर पत्तियों ने बेहतर एंटीऑक्सीडेंट गतिविधि प्रदर्शित की क्योंकि IC50 की रेंज और नर और मादा पत्तियों के फेरस रिड्यूसिंग एंटीऑक्सीडेंट पावर (FRAP) मान 1.21–3.86 और 1.32–5.09 $\mu\text{g/mL}$,

303.45–407.33 और 233.45–356.92 mg BHT/g थे। हालाँकि, सुगंध पैदा करने वाले घटक जैसे लिनालूल और गेरानियोल केवल मादा पत्तियों में पाए गए थे। इसके अलावा, Mg, K, Ca, Cr, Fe, Cu, और Zn सहित खनिज सांद्रता दो प्रजातियों और लिंगों के बीच भिन्न है। पादप घटकों और कारकों (लिंग, प्रजाति और निष्कर्षण विधियों) के बीच संबंध, सहसंबंध गुणांक पर आधारित एक प्रमुख घटक विश्लेषण (पीसीए) ने प्रजातियों, लिंग और निष्कर्षण विधियों के बीच एक महत्वपूर्ण अंतर ($p < 0.05$) स्थापित किया।

SCOBY (बैक्टीरिया और खमीर की सहजीवी संस्कृति) पेलिकल का उपयोग करके इन-सीटू कोम्बुचा किण्वन के माध्यम से एसबीएल-इन्फ्यूजन की कार्यात्मक क्षमता को बढ़ाने पर एक अध्ययन किया गया। एक अद्वितीय कार्यात्मक पेय विकसित करने के लिए SCOBY और SBL को $30 \pm 2^\circ\text{C}$ पर किण्वित करने के लिए नियोजित किया गया। किण्वन के दौरान पादप घटकों की गतिशीलता की निगरानी 3, 7, 10, 14 और 14वें दिन की गई। PCA ने कोम्बुचा किण्वन के लिए उपयुक्त SBL, *H. rhamnoides* और *H. salicipholia* की दो प्रजातियों के साथ-साथ 0 वें दिन से 14 वें दिन तक किण्वन अवधि के दौरान जैव रासायनिक संरचना की प्राकृतिक भिन्नता को अलग किया। किण्वन के दौरान एपिगैलोकैटेचिन, कैम्फेरोल, हैममेलिटैनिन, रूटिन और केरसेटिन जैसे कुछ पॉलीफेनोलिक घटकों में काफी वृद्धि हुई ($p < 0.05$)। Cu और Zn की मात्रा किण्वित कोम्बुचा (क्रमशः -0.51 और -0.18 का $\log_2$ परिवर्तन) में कम आई, जबकि Mg, K, Ca, और Ni में वृद्धि हुई (क्रमशः 0.201, 0.15, 0.44, 0.41 का $\log_2$ परिवर्तन)। एसबीएल में कोम्बुचा किण्वन के परिणामस्वरूप Fe मात्रा को 0 से 70.51 ppb तक बढ़ाया गया। विकसित कार्बनिक और फेनोलिक एसिड के परिणामस्वरूप टिट्रेटबल अम्लता और घटते pH में वृद्धि हुई है। 3-मिथाइलमैनोसाइड, जैंथोसिन और डायसोप्रोपाइल सिलिल आइसोप्रोपिल ईथर कोम्बुचा में पाए जाने वाले प्रमुख स्वाद यौगिक थे।

अल्ट्रासाउंड निष्कर्षण (UAE) की स्थितियों, उपचार समय, तापमान और विलायक-से-सैम्पल अनुपात, का प्रभाव, सी बकथॉर्न के बीज से तेल निष्कर्षण दक्षता, एंटीऑक्सिडेंट गतिविधि, और तेल की स्थिरता का अध्ययन किया गया। सी बकथॉर्न के बीज के तेल (SBSO) की निकासी के समय, तापमान और अल्ट्रासाउंड पावर ने एक महत्वपूर्ण प्रभाव ($p < 0.05$) दिखाया। यूआई प्रक्रिया ने 6.87% की अधिकतम तेल वसूली हासिल की और सॉल्वेंट एक्सट्रैक्शन तकनीक की तुलना में काफी अधिक थी। सॉल्वेंट-टू-सैम्पल रेश्यो 10:1 (mL: g) के साथ 552 W अल्ट्रासाउंड पावर पर 8.28 मिनट के लिए 50°C पर प्राप्त UAE की स्थितियों के अनुकूलतम मूल्य थे। SBSO के सैम्पल में उच्च कैरोटीनॉयड (237.04 ± 3.86 मिलीग्राम प्रति 100 ग्राम β -कैरोटीन समतुल्य), फ्री रेडिकल स्केवेंजिंग क्षमता (1.42 ± 0.01 IC50 μg

प्रति एमएल) और ऑक्सीडेटिव स्थिरता (टोटॉक्स मूल्य = 8.04 ± 0.02) का पता चला। अनुकूलतम यूई स्थितियों में निकाले गए तेल के नमूने की जीसी-एमएस प्रोफाइलिंग ने फैटी एसिड के बेहतर प्रतिधारण को प्रदर्शित किया।

एक अनूठा कार्यात्मक पेय बनाने के लिए, सी बकथॉर्न बेरी जूस को 25 डिग्री सेल्सियस पर 14 दिनों के लिए कोम्बुचा पेलिकल का उपयोग करके किण्वित किया गया। परिणामी कोम्बुचा पेय की बड़े पैमाने पर जांच की गई और मूल रस की तुलना में, बढ़ी हुई एंटीऑक्सीडेंट गतिविधि (23.49%), फेनोलिक 28.45 मिलीग्राम जीई/जी से 35.89 मिलीग्राम जीई/जी, और फ्लेवोनॉयड सामग्री 49.66 से 58.08 मिलीग्राम सीई/जी और मानव स्वास्थ्य के लिए आवश्यक तत्व (Mg, K, Fe, Cr, Cu, और Zn) बढ़े हुए मात्रा में पाए गए। शूगर के विश्लेषण से पता चला कि बेरी कोम्बुचा में मैनोज प्रमुख घटक था, जो कुल शूगर मात्रा सामग्री का 57.37% था, जबकि ग्लूकोज कुल शूगर का 42.63% था। किण्वन प्रक्रिया ने बेरी के रस में प्रारंभिक सुक्रोज (14.1%) को पूरी तरह से ग्लूकोज में बदल दिया, जिससे अंतिम बेरी कोम्बुचा में सुक्रोज नगण्य हो गया। किण्वन प्रक्रिया ने स्वाद प्रोफाइल को भी बदल दिया, जिसके परिणामस्वरूप अत्यधिक वांछनीय पेय प्राप्त हुआ।

सी बकथॉर्न प्रसंस्करण में विभिन्न संबद्ध हितधारकों को सशक्त बनाने के लिए, भारत के हिमाचल प्रदेश के लाहौल-स्पीति जिले में कई इंटरैक्टिव कार्यशालाएं, सेमिनार और हितधारकों की बैठक आयोजित की गई। यह प्रक्रियाएं उन्नत उत्पादन, विपणन कनेक्शन स्थापित करने, प्रसंस्करण विधियों में सुधार करने, सूचना का प्रसार करने और स्थानीय समुदाय में प्रचार करने के लिए था।

अंत में, यह शोध कार्य एक व्यापक ग्रामीण कार्य योजना के रूप में काम करेगा जो परिवर्तनकारी ग्रामीण प्रौद्योगिकियों का लाभ उठाता है, सी बकथॉर्न प्रसंस्करण को बढ़ाता है, स्थानीय आजीविका के अवसर पैदा करता है, संसाधनों का संरक्षण करता है, और हिमाचल प्रदेश के हिमालयी क्षेत्रों में सतत विकास को बढ़ावा देता है।

कीवर्ड: सी बकथॉर्न; नई प्रसंस्करण प्रौद्योगिकियां; प्रक्रिया अनुकूलन; प्रक्रिया गतिकी; बायोएक्टिव क्षमता; कार्यात्मक खाद्य पदार्थ; मेटाबोलोमिक्स; प्रमुख कंपोनेंट विश्लेषण; मूल्य श्रृंखला प्रबंधन

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LIST OF SYMBOLS & ABBREVIATIONS

%	Percent
<	Less than
>	Greater than
≥	Greater than or equal to
@	At the rate
ANOVA	Analysis of variance
FSSAI	Food Safety and Standards Authority of India
FSSR	Food Safety and Standards Regulations
AOAC	Association of Official Analytical Chemists
IS	Indian Standard
°C	Degree Celsius
CLA	Conjugated linoleic acid
Eq.	Equation
FA	Fatty acid
FAME	Fatty acid methyl esters
Fig.	Figure
FFA	Free fatty acid
g	gram
g.L ⁻¹	gram per litre
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectroscopy
h	Hour
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
kg	Kilogram
kg.s ⁻¹	Kilogram per second
µm	micrometer
µL	micro-Litre
MCFA	Medium chain fatty acid
mm	millimeter
m/z	mass to charge ratio

min	minute
meq	mili equivalent
mL	mili Litre
mL.min ⁻¹	mili Litre per minute
mg	mili gram
MUFA	Monounsaturated fatty acid
MS	Mass spectroscopy
°C. min ⁻¹	Degree Celsius
O ₂	Oxygen
ppb	Parts per billion
ppm	Parts per million
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
R ²	Co-efficient of determination
rpm	Revolution per minute
s	Second
SD	Standard deviation
SPME	Solid phase micro extraction
SPE	Solid phase extraction
w	weight
W	Watt
