Final Technical Report

Development of Sustainable Rural Livelihood Options Utilizing Locally Available Bio-Resources Through Transformative Rural Technologies in the Indian Himalayan Regions of Himachal Pradesh and Sikkim

Project ID- GBPNI/NMHS-2017-18/SG-17

Funded by:

National Mission on Himalayan Studies (NHMS)
Ministry of Environment, Forest & Climate Change
Government of India

Executed by:

Centre for Rural Development and Technology
INDIAN INSTITUTE OF TECHNOLOGY DELHI
Hauz Khas, New Delhi – 110016

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on

Development of Sustainable Rural Livelihood Options Utilizing Locally Available Bio-Resources Through Transformative Rural Technologies in the Indian Himalayan Regions of Himachal Pradesh and Sikkim

(Project ID- GBPNI/NMHS-2017-18/SG-17)

Submitted to

GBPIHED

National Mission on Himalayan Studies
Ministry of Environment, Forest & Climate Change
Government of India

Submitted by

Prof JATINDRA K SAHU
Centre for Rural Development and Technology
INDIAN INSTITUTE OF TECHNOLOGY DELHI
Hauz Khas, New Delhi – 110016

Acknowledgement

The aim of the current project is to develop sustainable livelihoods options through utilizing locally available bio-resources such as aromatic and herbal plants in Himachal Pradesh and Sikkim in a focused manner. The vision is to support the sustenance and enhancement of the ecological, natural, cultural, and socio- economic capital assets and values of the Indian Himalayan Region (IHR) with a mission to launch and support innovative studies and related technology-know-hows and knowledge interventions towards the sustenance and enhancement of the ecological, natural, cultural, and socio-economic capital assets and values of the Indian Himalayan Regions. In this regard, I am extremely indebted to National Mission on Himalayan Studies (NMHS), Govt of India for considering and sanctioning this project in time and releasing funds for the timely execution of the proposed objectives and activities. I take this opportunity to thank Director and Dean (R&D), IIT Delhi for forwarding this project and providing all kinds of facilities and financial approval as and when required.

I am thankful to my collaborating institutes- GBPNIHESD, Kullu Unit, Himachal Pradesh and CAEPHT, Gangtok, Sikkim. I am truly thankful to PIs Dr. Sarla Shashni and Prof N. S. Chauhan of these collaborating institutes for the cooperation and extended activities at the grassroots rendered to the project. I appreciate them for conducting various programs, seminars and workshops in the project sites successfully and providing progress reports, SoE and UC from time to time as and when desired. I also thank the local people from the project sites of Lahaul-Spiti, Himachal Pradesh and Gangtok, Sikkim for their interest, participation, and support in conducting hands-on trainings and workshops. The co-operation of the local administrations during execution of these activities is truly appreciable.

I would like to convey my sincere thanks and gratitude to Prof Virendra Singh, CSKHPKV, Palampur, Dr. Ashok Singh, Scientist, IHBT-Center for High Altitude Biology, Ribling, Mrs. Mamta Chander, Managing Director, JAGRITI, Ms. Amshu, Thapasu Foods, Bishan Dass Parsheera, President, Sea buckthorn Society Lahaul and Spiti, Dr. R.S. Rawal, Director, GBPNIHE, Er. R. K. Singh, Scientist-E, Coordinator and Regional Centre Head, GBPNIHE-HRC, Mr. Gulab Chand Negi Retiered AGM, NABARD, Dr. Tsering Stobdan, Principal Scientist, DIHAR-DRDO, Leh-Ladakh, Dr Mahesh Gupta, Pr. Scientist, CSIR-IHBT, Palampur, HP, Dr. Subrat Sharma, Head, Ladakh Regional Centre, GBPNIHE, Mr. Rishabh Singh Thakur, DDM, NABARD, Kullu, Dr. Renu Lata, Scientist-C, GBPNIHE-HRC, Ms. Sonam Chorel, Field Coordinator, Ladakh Regional Centre, GBPNIHE, and Mr. B D Parsheera, President, Seabuckthorn Society, Lahaul, HP. I also thank the Review Committee for giving encouraging feedback from time to time and helping us improving the quality of research and tangible deliverables.

I would also like to acknowledge handwork, perseverance, and sincerity of Ms Nikita Sanwal - Senior Research Fellow of the project for her untiring efforts the execution of the project activities successfully on-field and off-field.

Jatindra K Sahu, PhD
Principle Investigator

Part A: Project Summary Report

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NMHS-FINAL TECHNICAL REPORT (FTR)

Demand-Driven Action Research Project Grant

NMHS Reference No.:	GBPNI/NMHS-2017-18/SG 17	Date of Submission:	1	5	1	2	2	0	2	2
			d	d	m	m	y	y	y	y

Development of Sustainable Rural Livelihood Options Utilizing Locally Available Bio-resources through Transformative Rural Technologies in the Indian Himalayan Regions of Himachal Pradesh and Sikkim

Project Duration: *from* (**30.07.2018**) *to* (**30.09.2021**).

***Submitted to*:**

National Mission on Himalayan Studies
Ministry of Environment, Forest & Climate Change
Government of India

***Submitted by*:**

Prof Jatindra K Sahu

Centre for Rural Development & Technology
Indian Institute of Technology Delhi
Hauz Khas - 110 016, New Delhi
Tel: +91-11-2659-6349
Email: *jksahu@iitd.ac.in*

NMHS-Final Technical Report (FTR)

Demand-Driven Action Research Project

DSL: Date of Sanction Letter

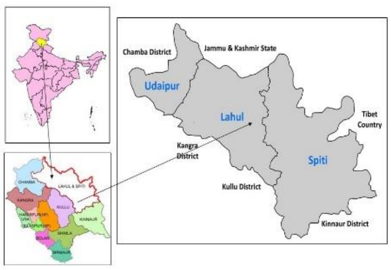
2	8	0	3	2	0	1	8
d	d	m	m	y	y	y	y

DPC: Date of Project Completion

3	0	0	9		2	0	2	1
d	d	m	m		y	y	y	y

Part A: Project Summary Report

1. Project Description

i.	Project Reference No.	GBPNI/NMHS-2017-18/SG 17					
ii.	Type of Project	**Small Grant**	√	~~Medium Grant~~		~~Large Grant~~	
iii.	Project Title	Development of Sustainable Rural Livelihood Options Utilizing Locally Available Bio-resources through Transformative Rural Technologies in the Indian Himalayan Regions of Himachal Pradesh and Sikkim					
iv.	State under which Project is Sanctioned	Himachal Pradesh and Sikkim					
v.	Project Sites (IHR States covered) (Maps to be attached)	Himachal Pradesh - Lahaul and Spiti District Sikkim - Pangi, Churah, Saluni, Geying, Soreng, Ranipool, Pakyong and Sajong   					
vi.	Scale of Project Operation	~~Local~~		**Regional**	√	~~Pan-Himalayan~~	
vii.	Total Budget/ Outlay of the Project	**Rs. 51,33,600.00**					

viii.	Lead Agency	Indian Institute of Technology Delhi, New Delhi
	Principal Investigator (PI)	Prof Jatindra K Sahu
	Co-Principal Investigator (Co-PI)	Prof. S N Naik
ix.	Project Implementing Partners	Dr Sarla Shashni, Scientist D G.B. Pant Institute of Himalayan Environment & Development, Himachal Regional Centre, Mohal- Kullu, Pin: 175101, Himachal Pradesh Prof N S Chauhan College of Agricultural Engineering & Post-Harvest Technology, (Central Agriculture University), Gangtok, Pin: 737135, Sikkim
	Key Persons / Point of Contacts with Contact Details, Ph. No, E-mail	Prof Jatindra K Sahu Centre for Rural Development & Technology Indian Institute of Technology Delhi Hauz Khas - 110 016, New Delhi Room no. 382, Block No. III Tel: +91-11-2659-6349 Email: jksahu@iitd.ac.in

2. Project Outcome

The major outcomes of the project are bulleted as below.

- Population assessment and bio-functional profiling of Sea buckthorn species was organised in Lahaul Valley of Lahaul & Spiti district in Himachal Pradesh and explored their conventional harvesting, processing, utilization pattern, and their SWOT analysis.

- The rural appraisal survey conducted to develop an information database of the plants such as their species, uses, ethnobotany, phenology of fruits and flowers, distribution, etc. The information collected through formal and informal meetings and discussion with local people, study tours and field visits and published literature, a data-book titled "Aromatic, Medicinal and Agricultural Plants in Indo Trans Himalayan Region- A Compilation" by Sanwal N, Satheeshkanth SSM, and Sahu JK with ISBN: 978-93-92513-39-8 has been published.

- Awareness creation programs on good/sustainable harvesting, post harvesting, and value addition practices of sea buckthorn and large cardamom was organised successfully.

- Organized around 11 workshops/seminars on value chain management of Sea buckthorn which were participated by local communities and government and non-government officials.
- Four awareness programmes were undertaken in three different locations of Sikkim. During the demonstration, working of improved large cardamom harvesting knife was demonstrated to the farmers. The farmers expressed their willingness to use the improved hand tool. On an average 120 farmers participated in the organised programmes.
- Involvement of local women and youth for livelihood generation and entrepreneurial activities during the awareness programmes.
- Technology standardization and optimization studies was carried out and demonstrated for the production of sea buckthorn products like juice, jam, and jelly.
- Established and demonstrated a low-cost-quick technology for sea buckthorn seed oil extraction utilizing ultrasound-assisted extraction method.
- Novel tea processing standardization was studied and demonstrated for the value addition of sea buckthorn leaves and established functional sea buckthorn tea with enhanced flavor and nutritional properties.
- Sea buckthorn berry was utilized to develop functional beverage through kombucha fermentation. Process technology was established for the standardization of the beverage.
- A hybrid dryer was developed, and standardization for drying of cardamom capsules, sea buckthorn leaves and berries for their primary processing for the development of value-added products.
- A Technology Incubation Centre (TIC) was established and is in operation at Kirting village, Lahaul & Spiti for processing and value addition of sea buckthorn at grassroots.
- Established marketing linkages through regional level marketing agencies and FPO's and FBO's meetings.
- The improved basket holder was demonstrated in villages East Sikkim district to reduce the efforts and drudgery associated in material handling. The improved basket holder with head strap supports the basket through head strap and waist. This additional support at waist is very helpful in reducing drudgery associated with the transportation of load in hills. The farmers observed that improved basket holder was very useful in reducing discomfort/pain in neck and forehead.
- The improved basket was also demonstrated in Pantham village of East Sikkim.
- Technology and Machinery Demonstration Mela was organised by CAEPHT, Gangtok to showcase the technologies and farm machineries developed under this project.

- In order to promote improved technologies and large cardamom harvesting, 98 number of improved large cardamom harvesting knives were procured from CAEPHT centre of All India Coordinated Research Project on Ergonomics and Safety in Agriculture and distributed among farmers. In addition, project team also participated in number of Technology Demonstration Mela organized by CAEPHT, Gangtok.
- Two awareness programmes were undertaken in three different locations of the Sikkim. During the demonstration, working of improved large cardamom harvesting knife was demonstrated to the farmers. The farmers expressed their willingness to use the improved hand tool. Total 60 farmers participated in both programmes.

2.1. Overall Project Abstract

Despite current attractive economic growth in India, poverty remains a major development challenge, particularly in the Indian Himalayan regions, for several biophysical and socioeconomic reasons. The objective of this proposal is to harness the rural livelihood options through utilization of locally available wild bio-resources very specifically sea buckthorn and cardamom for the people residing in the Indian Himalayas regions of Himachal Pradesh and Sikkim. This project is perceived as a rural action plan using transformative rural technologies that could provide viable livelihood options for improving the yield potential of sea buckthorn and large cardamom, income generation from on-farm and off-farm activities as well as conservation and efficient management of existing natural resources while developing appropriate low cost technologies and disseminating them for sustainable rural development in the Himalayan regions of Himachal Pradesh and Sikkim. The objective of the project is to demonstrate, train and build capacities of local tribes and other user groups, and to make them adopt some of the simple, low cost, hill specific rural technologies in participatory mode. Further, developing appropriate scientific and technological interventions for value addition of sea buckthorn and large cardamom. A rural appraisal survey was applied for the development an information database of the plants such as their species, uses, ethnobotany, phenology of fruits and flowers, distribution. Further, two biological commodities i.e., sea-buckthorn (for Himachal Pradesh) and large cardamom (for Sikkim) were adopted for inclusive study. As a result, one Technology Incubation Center (TIC) was established in the region for the primary processing of sea buckthorn. In order to build the capacities of stakeholders for processing of sea buckthorn, many interactive workshops were organized in Lahaul & Spiti district. Further to identify and develop a value chain management of sea buckthorn, many seminars were organised at Keylong, Lahaul & Spiti district. Many participants and associated stakeholders including farmers, women members of self-help groups (SHGs), Mahila Mandals, NGOs, marketing agencies, and research organisation participated in the programmes. Overall recommendation and suggestion of the meeting were to explore the possibility of forest areas for high production of sea buckthorn, linkages with marketing agencies at local level, development of processing tools and

techniques, dissemination of information at ground level for monetary benefit and promotion of local societies providing benefit to the locals especially women. The TIC with equipment such dryer, packaging unit, corking machine, bottling machine, and pulping machine for processing of sea buckthorn leaves and berry has been established at Kirting, Lahaul & Spiti district. Various marketing agencies were approached for value chain management of sea buckthorn in Himachal Pradesh. Creation of awareness for cultivation, harvesting and processing of large cardamom in Sikkim was accomplished awareness programmes in different locations in Sikkim. Many local farmers participated in the programmes. Technology demonstration of cultivation, harvesting and processing of large cardamom in Sikkim was demonstrated. Regular interactions among scientists and primary stakeholders ensured that rural people acquire all necessary knowledge related to a technology and entrepreneurship. In overall, the outcomes of the project can be utilized for sustainable management of resources through exploration of possible options for increasing production in a sustainable way, such as improving production from the wild, improving harvesting technologies, and promoting local value addition by introducing or developing appropriate processing technologies. Ensuring equitable distribution of benefits within forest user groups by empowering disadvantaged sub-groups is another way forward of this project.

Background of the Overall Project:

Two Indian Himalayan states, Himachal Pradesh and Sikkim have been considered as the study sites in the proposal. Detailed situation analysis and description of the project sites are given below. The project sites remain in the rain shadow area of the development process of the country. The communities that inhabit this infinitely tough terrain, reside in a scatter of small villages across the valleys and plateaux, and cut-off from the main land due to climatic and infrastructural adversities. They suffer multiple forms of poverty, and exacerbated by environmental vulnerability. Economic stagnation and poverty are severe among population, with 30-40% lying below poverty line and average 50% of underemployment as well. Frustrated youth are migrating in large numbers to the urban and industrial regions in the plains in search of employment. Two biological commodities i.e., sea-buckthorn (for Himachal Pradesh) and large cardamom (for Sikkim) adopted for inclusive study.

Sea buckthorn (*Hippophae spp*.) belonging to family Elaeagnaceae, found in the icy heights of the Himalaya, is a deciduous, thorny plant species native to Europe and Asia. In India, it has been recognized to be plant of high altitudes regions (2000-5500 m above mean sea level (amsl) such as Lahaul & Spiti, Kinnaur and Chamba districts of Himachal Pradesh and Ladakh of J&K. It is a multipurpose fast-growing species which is serving as a measure of biodiversity conservation, soil protection, medicines, food, fodder and fuel wood (Geetha *et al.*, 2009). It has an extraordinary capacity to grow and survive under adverse climatic conditions (-40 to 40o C) and has extensive root system with strong soil binding ability, which is useful for reducing soil erosion, land reclamation, wildlife habitat enhancement and water retention (Li *et al.,* 2009). Sea buckthorn berry is a very rich source of vitamins especially vitamin

C and is called treasure of bio-activity substance because of its over 190 bio-activity substances possessing unique medicinal properties. For these reasons, it is also called a wonderful plant. The berry and seed of sea buckthorn are the main sources of its nutritional and therapeutic values. These beneficial plant parts have provided raw material for various products, such as oil, juice, tea and food additives to candies, jellies, cosmetics and shampoos and medicinal and cosmetic purposes (Anderson *et al.*, 2008). A vast research work has been conducted by the scientific community in Himachal Pradesh especially a value chain starting from nursery to plantation and management, which supports ecological conservation of cold desert, control in soil erosion, improvement of soil fertility, etc. (Sharma and Singh, 2017; Sharma *et al.* 2019). But very less work has been conducted on the post-harvest techniques of sea buckthorn plant produce. Since seabuckthorn plant provides vast raw material such as fruit berry, pulp, seeds, leaves and stem, which could be developed into value-added products, which can further strengthen the economic status of most of the farmers in Lahaul and Spiti district. But there is very less knowledge of post harvesting techniques such as how and when to harvest leaves or fruits, its drying, processing, value addition, and packaging. Also, how and where they can market their produce so that they can get good or timely economic return which is not there at current scenario.

Sea buckthorn has great potential for sustainable environmental protection and commercial exploitation in cold desert areas of the Himalayas. The fruits are the rich source of vitamin C and used as raw material for producing food, cosmetic, medicine and nutraceutical products. The fruit pulp and seeds contain high quality medicinal oil. The fruit skin (after depulping) is utilized for making tea. Sea buckthorn is an excellent source of antioxidants and other healing nutrients. It has been used to heal psoriasis, make skin glow, boost immunity, slow aging, and lower cholesterol level in human body. Therefore, there is an urgent need to introduce the scientific method for the harvesting and drying techniques of the produce for preparing value added products and provide economic return to farmers. Sea buckthorn berries are highly perishable which need processing within a same day of its harvest. Therefore, in order to make a quality product of the produce regional level primary processing of the species is very important.

North Sikkim is specialized for production of large cardamom in India. Total 117.3 ha land is used for cardamom cultivation in North Sikkim. Large cardamom has high positive impact on gastro-intestinal tract. It stimulates the gastric and intestinal glands to secret essential juices with help of its simulative properties. However, processing and value addition technologies are lacking at the farmers level for these products. In order to utilize the natural bio-resources in a sustained and focused manner, it is important that these resources be harnessed efficiently and eco-friendly to meet the people's development aspirations without degrading them and therefore, urgent need for large scale establishment of technology resource centre is the need of the hour. Poor access to appropriate technologies due to difficult topographies and tough mountain conditions is one of the major causes of poverty, drudgery and natural resources degradation in the regions. Scientific and technological interventions would be perceived as a means of developing and disseminating improving technologies through action and participatory research. This approach, would be able to diversify livelihood earning options for local communities and may also help conserve natural resources on which these options depend on the other. Transformative rural technology is widely recognized as one of the major determinants of socio-economic development, and the idea that the simple and hill specific transfer of technology from lab or field lab to field/land will result in growth and thereby poverty is alleviating.

Objectives/ Aim:

[1] Development of scientific and sustainable strategies for cultivation and harvesting of natural bio-resources such as aromatic and herbal plants, crops and scrubs, agro produce, and timber and non-timber forest products in the Indian Himalayan regions.

[2] Development of appropriate scientific and technological interventions for processing and value addition of these local bio-resources into high value products.

[3] Establishment of replicable community models through rural transformative technologies and participatory rural action research for sustainable utilization of the bioresources in collaboration with local grassroots organizations.

Methodology:

The objectives were executed with following specific activities and methodologies:

Objectives	**Methodology and Implementation**
1. Development of scientific and sustainable strategies for cultivation and harvesting of natural bio-resources such as aromatic and herbal plants, crops and scrubs, agro produce, and timber and non-timber forest products in the Indian Himalayan regions.	• A rural appraisal survey conducted to develop an information database of the plants such as their species, uses, ethnobotany, phenology of fruits and flowers, distribution, etc. • The information collected through formal and informal discussion with knowledgeable people of the regions. • The published literature consulted to cross check the information gathered from local people and rectified accordingly. The known conservation status of these species was also worked out following the published literature. • Characterization, germplasm exploration, vegetative propagation, genetic selection, and incorporation into a sustainable land-use system was done utilizing the promising, under-exploited bioresources such as sea buckthorn and large cardamom

2. Development of appropriate scientific and technological interventions for processing and value addition of these local bio-resources into high value products.	• Technology for cultivation, processing and value-addition of sea-buckthorn in Himachal Pradesh and large cardamom in Sikkim. • Drying of sea buckthorn berries and leaves and large cardamom – 01 hybrid dryer • An improved sea buckthorn oil extraction method – 01 • Sea buckthorn tea – 01 • Packaging of dried sea buckthorn leaves - 01 • Processing of sea buckthorn berries into various products (juice, jam, jerry) – 01 each • Harvesting of large cardamon capsules – 01 • Field visits and awareness programs: 12 • Hands-on training programs – 08 • Seminars – 6 • FBO meet – 01
3. Establishment of replicable community models through rural transformative technologies and participatory rural action research for sustainable utilization of the bioresources in collaboration with local grassroots organizations.	• Development of community-based processing enterprise in the project sites • Organization of local Trade Fair and Mela for creating awareness and promotion of local high value products in the regions • Organization of conservation education and training programs • Organization of workshops and hands-on training programs. • Establishment of direct linkages between private agencies and the local tribes for creating sustainable marketing network in the project sites. • Linkage with private farms and industries

Results:

- Population of sea buckthorn in Lahaul Valley of Lahaul & Spiti district was assessed and areas with rich sea buckthorn diversity were identified such as *Tod* Valley with maximum followed by *Myar* Valley and *Tinnan* Valley.
- Stakeholders in the value chain of sea buckthorn were identified such as Lahaul Sea buckthorn Society, Yuvak Mandals, research organization, private companies, local communities, women self-help groups, etc.

- Identification of the problem in sea buckthorn value chain as lack of technological intervention at regional level.
- Established a Technology Incubation Centre (TIC) at Kirting Village, Lahaul & Spiti with technologies for primary processing of the sea buckthorn at local level.
- Training on value addition processes of sea buckthorn in terms of juice, tea and seed oil was also done with the stakeholders at TIC at Kirting, Lahaul&Spiti.
- Market linkages for the produce was developed among farmers and marketing agencies for the products developed under the project.
- National level seminars were organized at G B pant institute of Himalayan Environment Mohal Kullu on ‘Creation of the Sea buckthorn value chain in the Trans-Himalayan region’.
- Awareness on the good/sustainable harvesting and post harvesting method of Sea buckthorn. Workshops/ seminars were organized on value chain management of Sea buckthorn which were participated by many local people.
- Involvement of 2 Women Self Help Groups for livelihood generation and entrepreneurial activities of sea buckthorn.
- In order to study the difficulties faced by the farmers, as a part of pilot study, physiological measurement of the workers during harvesting using traditional knife were undertaken. The physiological data of 5 subjects obtained from Human Energy Measurement System was analyzed to find out the heart rate, oxygen consumption rate, and energy expenditure rate.
- Furthermore, to promote improved technologies and large cardamom harvesting knife, 98 number of improved large cardamom harvesting knives were procured from CAEPHT centre of All India Coordinated Research Project on Ergonomics and Safety in Agriculture and distributed among farmers. In addition, project team also participated in number of Technology Demonstration Mela organized by CAEPHT Ranipool. Correspondingly, a drying system was designed which can be used to dry large cardamom and Sea buckthorn berry.
- The drying system mainly consists of two chambers; one chamber is for pre-treatment of microwave and the second one is hot air convection drying where the material will flow in a belt conveyor system. The product will be pre-treated by microwave till its latent of vaporisation. The idea is to bring down the temperature of product to end point of sensible heat.

Conclusion:

The present work attempted to develop sustainable rural livelihoods utilising locally available biological resource in the Himalayan regions of Himachal Pradesh (Sea buckthorn) and Sikkim (Large cardamom). The three broad thematic groups, viz., (i) conservation and sustainable use of biodiversity, (ii) supplementary livelihood options and (iii) awareness and capacity building are well addressed. Local people remain the main beneficiaries of the project where employment for youth and women are increased along with improved market network. The current study contributed in the development and management of sea buckthorn and large cardamom in the forest areas for income and employment opportunities. Consequently, improvement in capacities of local people through adoption of technologies in the Indian Himalayan regions of Himachal Pradesh and Sikkim is expected. Participation of private sectors in production of medicinal and aromatic herbs and plants. This will not only help in exploiting the vast potential in this field, but will also help the rural poor living around the forest areas to improve their livelihoods and sustain them in the long run. Development of sustainable product and marketing through promoting local value addition by introducing or developing appropriate processing technology, creating marketing support services to local groups through analysing market system to generate information needed for sustainable management, fair prices, and efficient market, providing good marketing information (current and future), helping identify potential markets, providing credit facilities for processing and trade, providing rural entrepreneurship development training and promoting market competition to provide fair prices for collectors and FUGs. Local women and youths, from scheduled castes and scheduled tribes, from different villages are benefitted by this project. Apart from these, local PRI agencies and stake holders, local state govt. agencies like Department of Forestry, Water Resources, Panchayat Raj, Health and Family Welfare, Department of Tribal Welfare, Department of Agriculture, Department of Minority, and Ministry of AYUSH, small scale federations and industries – KVIC and Medicine and Herbal Farms and Industries may benefit from the outcomes of this project.

Recommendations:

- Establishment of FBO models may take forward the successful outcomes of the project. Food processing entrepreneurs may intervene under ‘Make in India’ campaign and establish small and large-scale industries.
- Marketing network for sea buckthorn and cardamom products must established through private sector entities (business-to- business partnerships), donor agencies, and the public sectors.
- Mobile app connecting sea buckthorn/large cardamom growers and retailers or consumers is recommended for smooth establishment.
- Sustainable harvesting practices for sea buckthorn and/or large cardamom established through technological intervention must applied by the local farmers.
- Plantation of the sea buckthorn in barren land, community and marginal lands and river side of the region for the maximum financial and environmental benefit is recommended.
- Geographical Indication (GI) of sea buckthorn/large cardamom is also recommended for its uniqueness in the region. Further, quality processing and packaging of raw material at local level for better market demand is necessary.
- For overall sustainability of the enterprise’s collaborative efforts among the farmer groups, society, government and scientific organisation is also recommended.

- Short term training programmes and vocational training programmes are suggested for capacity building of various stake holders such as farmers, farm women, rural youth, school dropouts, and rural artisans, extension officials from state governments and KVKs, and personnel from NGOs/SHGs.
- It is proposed to attain a higher growth trajectory through significant increase in investment for strengthening supply chain infrastructure and expansion of processing capacity particularly in perishables like sea buckthorn.
- Establishment of long-term sustainability in growth of the sector through efficient use of water, energy, adoption eco-friendly technology in processing, storage, packaging and use of waste from FPI industry.
- The Production Linked Incentive Scheme (PLIS) of MoFPI, GOI, proposes financial incentive to modernize and enhance competitiveness of the food processing industry by manufacturing specific categories of food products having high potential for growth in output and value addition. Pradhan Mantri Formalisation of Micro Food Processing Enterprises (PMFME) scheme focuses on capacity building, entrepreneurship development, essential functions of enterprise operations, marketing, bookkeeping, registration, FSSAI standards, Udyog Aadhar, GST Registration, general hygiene. The deliverables of our project may be merged with these central funded schemes and/or programmes.
- Specific training designed for One District One Product (ODOP) product (Sea buckthorn and cardamom both are included in this scheme) or the product produced by the unit including operations of necessary machines, hygiene issues, packaging, storage, procurement, new product development etc.
- FPOs/SHGs/ producer cooperatives along their entire value chain for sorting, grading, assaying, storage, common processing, packaging, marketing, processing of agri-produce, and testing laboratories. Also, a large number of trained resource persons are available with State Rural Livelihood Missions (SRLMs). These local resource persons of SRLM having expertise in agro-produce would be utilized for training, upgradation of units, DPR preparation, handholding support, etc.

2.2. Objective-wise Major Achievements

S. No.	Objectives	Major achievements (in bullets points)
1	Development of scientific and sustainable strategies for cultivation and harvesting of natural bio-resources such as aromatic and herbal plants, crops and scrubs, agro produce, and timber and non-timber forest products in the Indian Himalayan regions.	• Identification of the sea buckthorn (*Hippophae* spp.) as one of the potential natural bioresource of Lahaul & Spiti district, Himachal Pradesh for developing a sustainable strategy for its harvesting and post harvesting management. • Areas rich in sea buckthorn diversity in Lahaul valley of Lahaul & Spiti district were identified. These areas include Tod Valley, Miyar Valley, Patan Valley and parts of Tinnan Valley in Lahaul Subdivision of Lahaul & Spiti district. • Stakeholders in the value chain of sea buckthorn were also identified such as Lahaul Sea buckthorn Society, Yuvak Mandals, research organization, private companies, Women Self Help Groups (SHGs) and local communities, etc. • Awareness on the good/sustainable harvesting and post harvesting method of Sea buckthorn and Cardamom. • Awareness creation programs on good/sustainable harvesting, post harvesting, and value addition practices of sea buckthorn and large cardamom was organised successfully. • Two awareness programmes were undertaken in three different locations in Sikkim. During the demonstration, working of improved large cardamom harvesting knife was demonstrated to the farmers. The farmers expressed their willingness to use the improved hand tool. Many local farmers participated in the programmes.

2	Development of appropriate scientific and technological interventions for processing and value addition of these local bio-resources into high value products.	• By shedding insight on dioecism in *Hippophae sp*., a study was performed to help to understanding the potential use of male and female sea buckthorn plants in tea / nutraceutical processing based on their bioactive components. • Established a low cost, quick technology for the extraction of sea buckthorn seed oil. In recent times sea buckthorn oil is very much expensive in the market. Applying the established ultrasound assisted extraction technology, it will be a great help in reducing the prices and improving the quality of the oil. Also, the oil extraction time can be reduced. • A novel technology developed for the processing of sea buckthorn leaves will provide a good market for a decent quality with a pleasant flavor and aroma tea. Along with generating livelihood, it will be beneficial in combating several medical problems. • Development of microwave-assisted-vibro-fluidized hybrid dryer system is under experimentation for the preparation of good quality, bright colour and pleasant aroma containing dried sea buckthorn berry/cardamom. • To reduce the efforts and drudgery associated in material handling, the improved basket holder developed by NERIST, Nirjuli, Itanagar, Arunachal Pradesh was demonstrated in villages East Sikkim district. The improved basket holder with head strap supports the basket through head strap and waist. This additional support at waist is very helpful in reducing drudgery associated with the transportation of load in hills.

3	Establishment of replicable community models through rural transformative technologies and participatory rural action research for sustainable utilization of the bioresouces in collaboration with local grassroots organizations.	• The rural appraisal survey was conducted to develop an information database of plants such as their species, uses, ethnobotany, phenology of fruits and flowers, and distribution. The information collected through formal and informal discussion with local people of the Indian trans Himalayan region of Himachal Pradesh and through review literature, a data-book titled "Aromatic, Medicinal and Agricultural Plants in Indo Trans Himalayan Region- A compilation (ISBN: 978-93-92513-39-8) is published. • Established a Technology Incubation Centre (TIC) at Kirting, Lahaul & Spiti in Himachal Pradesh. • Developed various value-added products like sea buckthorn leaves teas, jam, jelly, juice and pulp juice at the TIC. • Organised hands-on training programmes on value addition processes of Sea buckthorn in terms of juice, tea and seed was also done with the stakeholders at TIC at Kirting village in Lahaul & Spiti. • Marketing linkages for the produce has been developed between farmers and marketing agencies for the products developed under the project. • Marketing of the produces through Entrepreneurship Cell of the Institute and Thapasu Foods LLP (Himachal Pradesh Start up) was established. • National level seminars were organized at G B Pant Institute of Himalayan Environment, Mohal-Kullu on "Creation of the Sea buckthorn value chain in the Trans-Himalayan region". • Organized 11 workshop/seminar on value chain management of Sea buckthorn which were participated by an average 200 local inhabitants.

		• Involvement of various women self-help groups for livelihood generation and entrepreneurial activities. • In order to promote improved technologies and large cardamom harvesting knife, 98 number of improved large cardamom harvesting knives were procured from CAEPHT centre of All India Coordinated Research Project on Ergonomics and Safety in Agriculture and distributed among farmers. In addition, project team also participated in number of Technology Demonstration Mela organized by CAEPHT, Gangtok.

2.3. Outputs in terms of Quantifiable Deliverables

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, and Reason thereof:
1.	A Technology Incubation Center (TIC) has been established at Kirting village, Lahaul & Spiti.	Local level primary and secondary processing of Sea buckthorn leaves and berries	• Regional level processing unit • Easy processing of perishable raw materials • Maintains the quality of the final products • Development of entrepreneurial skills among local community	
2.	Supplementary livelihood options for local communities and other rural population	Local level procurement and processing of the Sea buckthorn berries, and leaves has motivated the local people to collect and sell it	• Alternative livelihood option for local community especially women self-help groups • Environmental sustainability and conservation of species • High economic return and easy processing at local levels	

			• Employment generation and increase in livelihood of locals specially women groups through involvement in the value chain at various stages	
3.	Capacity building programmes among local inhabitants	Organized workshops / seminars on sea buckthorn status and value chain management Total participants (in each event) more than 150 (~ 60 women, ~ 90 male) 1 National level Seminar was organized for the value chain creation of Sea buckthorn in Trans Himalaya which was supported by NABARD Total participants: 60	• Awareness on present status and prospects of Sea buckthorn • Increase in knowledge on value addition and potential markets for Sea buckthorn and its ancillary products • Seminar gave the platform to the farmers to discuss the issues and mitigation measures in sea buckthorn-based enterprise from producer to consumer. • The platform also gave the chance to farmers to interact with the experts from scientific and private sectors.	
4.	Environmental Conservation	Awareness on the species and its livelihood aspects results in conservation of the species at its natural habitat	• Conservation of species in their natural habitat • Due to nitrogen fixation capacity, it is being used to address soil erosion in fragile terrains of the valley • Niche value added products with high nutrient value.	

(*) As stated in the Sanction Letter issued by the NMHS-PMU

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number and Brief Details	Details of Attachment / Supporting Document
1.	New Models/ Process/ Strategy developed:	5 nos. (Drying of leaves and berries, drying of large cardamon, harvesting of large cardamon, sea buckthorn oil extraction technique, kambucha, jam, jelly, juice and pulp making from sea buckthorn) Established processing and value addition technologies for Sea buckthorn and large cardamom	Kindly refer to *Part B*
2.	New Species identified:	3 nos. New species of sea buckthorn plant identified and studied	Kindly refer to *Part B*
3.	New Database established:	1 no. Databook on Indian Himalayan plants was published	Kindly refer to *Part B*
4.	New Patent, if any:		
	I. Filed (Indian/ International)	-	
	II. Granted (Indian/ International)	-	
	III. Technology Transfer (if any)	A hybrid dryer system for sea buckthorn leaves and berries drying was transferred to TIC, HP. Sea buckthorn processing techniques were transferred to Lahaul Sea buckthorn Society, Yuvak Mandals, research organizations, Private companies, Women Self Help Groups (SHGs) and local communities.	Kindly refer to *Part B*
6.	Others (if any):		Kindly refer to *Part B*

3. Technological Intervention

Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
Development and deployment of indigenous technology	Drying of leaves, preparation of kambucha, processing and value addition of sea buckthorn berry juice, jam, pulp, seed oil.	
Diffusion of High-end Technology in the region	A Technology Incubation Centre established in the Kirting Village, L&S district, HP for the easy primary processing of the sea buckthorn berries and leaves.	Seven Panchayats of Lahaul and Spiti district who can easy access the TIC and process their raw material.
Induction of New Technology in the region	Novel sea buckthorn processing and value addition techniques were developed through lab experimentations. Large cardamom harvesting and drying tools were improved and demonstrated.	Published articles may benefit the scientific community and the stakeholders.
Publication of Technological / Process Manuals	A databook on medicinal and aromatic plants from Himalayan region is published, research papers and conferences proceedings for creating knowledge base on sea buckthorn and large cardamom.	The database will be immensely useful for the collectors, growers, and traders of medicinal and aromatic plants along with the researchers of botany, phyto-chemistry, pharmacy, agriculture, and biomedicine.
Others (if any)		

4. Demonstrative Skill Development and Capacity Building/ Manpower Trained

S. No.	Type of Activities	Details with number	Activity Intended for	Average Participants/Trained			
				SC	ST	Woman	Total
1.	Workshops	05	Sea buckthorn status and value chain management	-	200	60	200
2.	On Field Trainings	08	• Sea buckthorn: Status and Prospects organized by GPB and IITD in Lahul and Spiti, Himachal Pradesh	40	65	105	300
			• Sea buckthorn: Processing and value addition organised by GPB and IITD in Lahul and Spiti, Himachal Pradesh	40	55	30	125
			• FBO meeting for sea buckthorn processing and marketing by GPB and IITD in Lahul & Spiti, Himachal Pradesh	12	15	8	35
			• Stakeholders meeting for sea buckthorn upliftment by GPB and IITD in Lahul and Spiti, Himachal Pradesh	35	40	25	100
			• Technology and Machinery Demonstration Mela-2019 organized by CAEPHT Ranipool	30	60	20	110
			• Technology and Machinery Demonstration Mela-2020 organized by CAEPHT Ranipool	50	80	30	160
			• Technology and Machinery Demonstration Mela-2021 organized by CAEPHT Ranipool	30	60	40	130

3.	Skill Development		Demonstration on use of large cardamom harvesting knives		15	10	50
4.	Academic Supports	01	PhD	-	-	-	01
	National Seminar	04	Seminars on value creation of sea buckthorn and large cardamom	40	160	50	350

5. Linkages with Regional & National Priorities (SDGs, INDCs)

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Average Beneficiaries
1.	Sustainable Development Goal (SDG)	The project linkages the following SDGs - Goal 1- No poverty, Goal 2- Zero hunger, Goal 3- Good health and well-being, and Goal 8- Decent work and economic growth Kindly refer to ***Part B***	07	800
2.	Climate Change/INDC targets	The project outcomes put forward and further propagate a healthy and sustainable way of living based on traditions and values of conservation and moderation, including through a mass movement for ‘LIFE’– ‘Lifestyle for Environment’ as a key to combating climate change".		
3.	International Commitments	-		
4.	Bilateral engagements	-		
5.	National Policies	-		

6.	Other's collaborations	1. G.B. Pant Institute of Himalayan Environment and Sustainable Development, Himachal Unit, Himachal Pradesh 2. College of Agricultural Engineering and Post-Harvest Technology, Gangtok, Sikkim.	02	Exchange of faculty members and research students.

6. Project Stakeholders/ Beneficiaries and Impacts

S. No.	Stakeholders	Support Activities	Impacts
1.	Gram panchayat: 7-gram panchayats Jahalma, Shansha, Nalda, Jobrang, Warpa, Ranika, Gohrma in HP, and four villages each in Namchi and Ravong sub-divisions of South Sikkim, six villages each in Gangtok, Pakyong and Rongli sub-divisions of East Sikkim	-Awareness programs -Hands-on trainings -Field Meetings -Field Survey -Field Demonstration	Population of these 7-gram panchayats are 3254 which can get a direct assess of the Technology Incubation Centre (TIC) established in the region for primary processing of their produce like drying of sea buckthorn leaves and berry pulp extraction. Improved harvesting tools and awareness programs in Sikkim may impact the social status and income pattern of the local people. -Kindly refer to ***Part B***
2.	Govt Departments: (Agriculture/ Forest) MoEF&CC, MoRD, MoHFW, Min. of Tribal Affairs, Min of Agriculture & Farmers Welfare, Min of Minority Affairs, Min of AYUSH	-Awareness programs -Hands-on trainings -Field Meetings -Field Survey -Field Demonstration	Various schemes under Govt of India may help the rural people in generating sustainable livelihood income for the local people of Himachal Pradesh and Sikkim.

3.	Villagers: 6 villages in Lahaul and Udaipur tehsil with approximately 40 households. 5 villages each in Gyalshing and Soreng sub-divisions of West Sikkim	-Awareness programs -Hands-on trainings -Field Meetings -Field Survey -Field Demonstration	They were sensitized on the importance of the sea buckthorn species and its economic and environmental benefits
4.	SC Community: In Sikkim, SC and ST population, from four villages each, in Namchi and Ravong sub-divisions of South Sikkim.	-Awareness programs -Hands-on trainings -Field meetings -Field survey -Field demonstration	During the Awareness programs and workshops considerable number of SC community people benefitted. Kindly refer to ***Part B***
5.	ST Community: Lahaul & Spiti is a tribal district therefore, all the stakeholders are schedule tribe. Six villages each in Gangtok, Pakyong and Rongli sub-divisions of East Sikkim	-Awareness programs -Hands-on trainings -Field Meetings -Field Survey -Field Demonstration	
6.	Women Group: 2 Women Self Help Group	-Awareness programs -Hands-on trainings -Field Meetings -Field Survey -Field Demonstration	Women Self Help Groups (SHGs) like - Jhansi and Saraswati from the Shansha and Nalda panchayat were trained on harvesting techniques and involved in collection of berries.
	Others (if any)	-	-

7. Financial Summary (Cumulative) (will be submitted with UC and SoE)

S. No.	Financial Position/Budget Head	Funds Received (INR in Lacs)	Expenditure/ Utilized (INR in Lacs)	% of Total cost
I.	Salaries/Manpower cost	16.21	14.46	89.20%
II.	Travel	5.80	5.24	90.34%
III.	Expendables &Consumables	4.35	4.31	99.08%
IV.	Contingencies	2.45	4.19	171.02%
V.	Activities & Other Project cost	9.20	9.48	103.04%
VI.	Institutional Charges	4.70	5.00	106.38%
VII.	Equipment	7.00	6.73	96.14%
	Total	**49.7168**	**49.4382**	**99.44 %**
	Interest earned			
	Grand Total			

** Tentative SoE of the overall project*

8. Major Equipment/Peripherals Procured under the Project** (if any)

S. No.	Name of Equipment	Cost (INR)	Utilisation of the Equipment after project
1.	Microwave conveyor dryer (1 no)	1,97,000/-	The equipment have been installed in the TIC established in Kirting village of Lahaul-Spiti for the benefit of the local people and the equipment are in operational. IITD with the approval of the NMHS has transferred the equipment inventory to GBPIHED Kullu region where they will provide the technical assistance from time to time.
2.	Packaging machine (1 no)	99,750/-	
3.	Bottle filling machine (1 no)	83,475/-	
4.	Cap sealing Machine (1 no)	47,250/-	
5.	Steam jacketed kettle (1 no)	91,250/-	
6.	Sieve shaker (1 no)	39,942/-	

Details should be provided in detail (ref* ***Annexure III &IV).

9. Quantification of Overall Project Progress

S. No.	Parameters	Total (Numeric)	Details of Attachments/ Supporting Documents
1.	IHR States Covered	2	Himachal Pradesh and Sikkim
2.	Project Site/ Field Stations Developed	1	Kindly refer to *Part B*
3.	New Methods/ Modelling Developed	11	Kindly refer to *Part B*
4.	No. of Trainings arranged	8	Kindly refer to *Part B*
5.	No of beneficiaries attended trainings	1050	
6.	Scientific Manpower Developed (Phd/M.Sc./JRF/SRF/ RA):	1 PhD/SRF	
7.	SC stakeholders benefited	250	
8.	ST stakeholders benefited	380	
9.	Women Empowered	110	
10.	No of Workshops Arranged along with level of participation	5 250 participants	Kindly refer to *Part B*
11.	On-field Demonstration Models initiated	1	
12.	Livelihood Options promoted	5	
13.	Technical/ Training Manuals prepared	1	
14.	Processing Units established	1	
15.	No of Species Collected	3	
16.	New Species identified	3	
17.	New Database generated (Types):	1	
	Others (if any)	-	

10. Knowledge Products (KPs) and Publications

S. No.	Knowledge Products (KPs)/ Publication	Number		Total Impact Factor	Remarks/ Enclosures
		National	International		
1.	Journal Research Articles/ Special Issue:	1	4		*Kindly refer to Part B*
2.	Book Chapter(s)/ Books:	4	-		
3.	Technical Reports	1	-		
4.	Training Manual (Skill Development/ Capacity Building)	3	-		
5.	Papers presented in Conferences/Seminars	2	4		
6.	Policy Drafts/Papers	-	-		
7.	Others:	-	-		

**Please append the list of KPs/ publications (with impact factor and further details) with due Acknowledgement to NMHS.*

11. Success Model(s)/ Best Practice(s) under the Project:

Parameters	***Description***	***Details of Attached supporting documents***
(1) Adaptability of the Technology:	Technology deployment in the region is very easy to use thus adaptability of these technology by the locals are quite high.	Kindly refer to *Part B*
(2) Acceptability (interest of the local people):	Local people of the region are very much interested in the project activity mainly due to the technological interventions done in the region. The technology is beneficial for them in terms of value addition of the sea buckthorn/large cardamom and improved harvesting tools of cardamom which is reducing their drudgery in terms of its processing time in traditional manner and at the same time improving the quality of their produce. The improved quality of produce therefore getting good market price for them.	

(3) Improvement in Ecological Variables:	Due to the increased awareness on the species has also supported the conservation of the species in its natural habitat which was earlier not being practiced by the locals. Earlier they used to uproot the species for their agricultural purpose or to use it as fuelwood.	
(4) Outcomes of the Scientific Publications, Knowledge Products:	▪ One Technical Booklet titled 'Value Chain Creation of Sea buckthorn in Trans Himalaya'. ▪ One Databook titled 'Aromatic, Medicinal and Agricultural Plants in Indo Trans Himalayan Region- A Compilation'. ▪ 3 nos. original research articles, 2 nos. review articles, 2 nos. book chapters, 10 nos. conferences.	

12. Recommendation on Utility of Project Findings, Replicability and Exit Strategy

Particulars	***Recommendations***
Utility of the Project Findings:	The project outcome will help locals, particularly women for their livelihood generation and overall conservation of the resources. This will further ensure the quality aspect of niche value added products from Sea buckthorn and cardamom along with its sustainable marketing. Establishment of FBO models may take forward the successful outcomes of the project. Food processing entrepreneurs may intervene under 'Make in India' campaign and establish small and large-scale industries. Marketing network for sea buckthorn and cardamom products must established through private sector entities (business-to- business partnerships), donor agencies, and the public sector (local governments).

Replicability of Project:	The project is a baseline for livelihood generation through appropriate scientific and technological interventions for processing and value addition of local bio-resources into high value products in the Indian Himalayan Region. Mobile app connecting sea buckthorn/large cardamom growers and retailers, or consumers is recommended for smooth establishment. Sustainable harvesting practices for sea buckthorn and/or large cardamom established through technological intervention must applied by the local farmers.
Exit Strategy:	Value chain identification from producer to consumer for sea buckthorn/cardamom -based products has been established involving locals, sea buckthorn/cardamom society of the region, research institutions and marketing agencies. Further, Start-up company of the Himachal Pradesh and Sikkim state has been also linked in SBT based enterprise for further step up in the value chain especially through creation of Farmer Producer Organisation (FPOs). Short term training programmes and vocational training programmes are also suggested for capacity building of various stake holders such as farmers, farm women, rural youth, school drop outs, and rural artisans, extension officials from state governments and KVKs, and personnel from NGOs/SHGs.

19-12-2022

Prof Jatindra Kumar Sahu
(**Project Coordinator**)
(Signed and stamped)

Prof Sunil K Khare
Dean (R&D), IIT Delhi
(Signed and stamped)

Place: New Delhi

Date: 19/12/2022

Part B: Detailed Project Outcomes

INDEX

1. Project Description

1.1 Rationale of the project

Despite current attractive economic growth in the country, poverty remains a major development challenge, in the Indian Himalayan Regions, for several biophysical and socioeconomic constraints. Development of a sustainable livelihood option in Himachal Pradesh and Sikkim is considerably more complex and challengeable because of vulnerabilities arising from topographical complexity, altitudinal gradient and poor physical and socioeconomic infrastructure. A majority of the poor in the regions live in remote areas and depend heavily on biological occurring natural resources. Realizing the importance of medicinal and aromatic plants, herbs, scrubs, timber and non-timber forest and agro products for generating sustainable rural livelihood options through stringent R&D and extensional activities are needed for sustainable utilization of locally available and commercially valuable natural bio-resources in a focused manner. Two prominent states in IHR, Himachal Pradesh and Sikkim harbour a wide range of rare endemic, threatened and medicinally important species of plants, herbs and scrubs

1.2 Project Site

Lahaul and Spiti district of Himachal Pradesh are located at 31°44' 57" and 32°59'57"N Latitudes and 76°46'29" and 78°41'34" E Longitudes which extends from 2,400 to 7,000 m amsl (Fig.1). The geographical area of the district is 13,841 sq. km, with total population of 31,564 averaging 2 persons per sq. km (Census, 2011). The district faces severe climatic conditions with average rainfall of 25 mm to a maximum of 400 mm (low rainfall); snowfall 3–12 feet (high snowfall) and temperature ranges from -20 to 33°C. Seabuckthorn *(Hippophae* spp.) are major dominant tree/shrubby woody species in the riverine, along the roadside, irrigation channel side and grasslands habitats. Lahaul & Spiti district is considered as the largest producer of Seabuckthorns in the country. The district has annual potential of 500 tonnes of seabuckthorns. Around 400 farmers have readily planted it in 100 ha marginal lands in 25 villages, mostly in Chandra valley of Lahaul & Spiti district. Sea buckthorn has great potential for sustainable environmental protection and commercial exploitation in cold desert areas of the Himalayas. The fruit is the richest source of vitamin C and used as raw material for producing food, cosmetic, medicine and nutraceuticals. The fruit pulp and seeds contain high quality medicinal oil. The fruit skin (after extracting pulp) is utilized for making tea. Sea buckthorn is an excellent source of antioxidants and other healing nutrients. It has been used to heal psoriasis, make skin glow, boost immunity, slow aging, and lower cholesterol level in human body.

Sikkim is a thumb-shaped state having geographical area of 7096 sq. km. East Sikkim, North Sikkim, South Sikkim and West Sikkim are the four administrative districts in Sikkim. According to 2011 census, the population of South Sikkim is 146850 among which 76663 are male and 68241 females. There are two sub-divisions (Namchi and Ravong) in South Sikkim. There are 136 villages in South Sikkim among which 91 in Namchi and 45 in Ravong sub-division. South district recorded the second highest proportion of Scheduled Caste (6053) and third highest in Scheduled Tribes (41392).

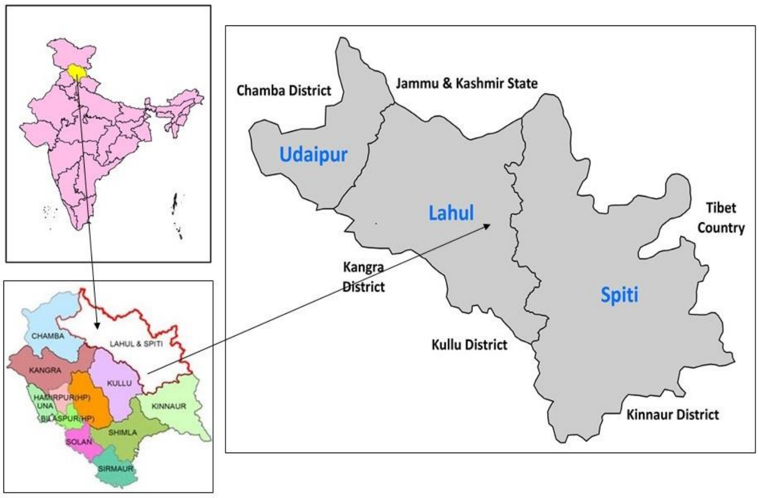


Fig.1 Lahaul & Spiti district in Himachal Pradesh

Fig.2 Locational of East, West and South districts in Sikkim

East Sikkim occupies the south-east corner of the state, touching China on one side and Bhutan on the other side. The district is a very sensitive area with the Indian army having control over most of the areas. The population of the district is 283583 according to 2011 census. The district has a literacy rate of 83.85%. East district is the third highest (954 sq. km.) in area, but in term of population and literacy rate has reflected the highest positioned with 283583 and 83.85% during Census 2011. The district accounts for almost 50% of the total unemployed. The district has three sub-divisions (Gangtok, Pakyong and Rongli). There are 107 villages in the district out of which 65 in Gangtok, 26 in Pakyong and 16 in Rongli sub-division. East district records the highest proportion both in Scheduled Caste (15305) and Scheduled Tribes (78436) population according to 2011 census.

West district is the second largest (1166 sq. km.) in area, but in term of population it is the third highest 136435 with 77.39% of literacy rate according to 2011 census. The district comprised of two subdivisions i.e., Gyalshing and Soreng. There are 112 villages in west Sikkim, out of which 65 in Gyalshing and 47 in Soreng sub-division. The district records the third highest proportion of Scheduled Caste (5935) and second highest in Scheduled Tribes (57817) population. The people are primarily of Nepali descent; other ethnic groups include the Lepcha and Bhutia communities. The location of these two districts is shown in the state of Himachal Pradesh is shown in Fig.2.

1.3 Major problems in the project sites

- Lack of sustainable rural livelihood options
- Lack of awareness about high value bio-resources
- Underutilization/ overexploitation of aromatic and medicinal plants, herbs, scrubs, timber and non-timber products
- Lack of primary processing technologies and scientific knowledge for value addition of tradition plants and produce
- Lack of primary processing facility at the door steps of the tribal community
- Less field intensive technology demonstration.
- Lack of training on value addition of indigenous bio-resources.
- No scientific study, technology for collection, processing and packaging of high value products.
- No skill development programme and capacity building facility through extension activities.

1.4 Justification for the Project

- The project is perceived as an action plan using transformative rural technologies that could provide viable livelihood options for improving income generation from on-farm, off-farm and non-farm activities as well as conservation and efficient management of natural resources while developing appropriate low-cost technologies and disseminating them for sustainable development in Himachal Pradesh and Sikkim.
- Of more relevance to tribal development is the prospect of village level processing. A range of small-scale enterprises may be possible for aromatic, medicinal, timber and non-timber forest products collectors, user groups or cooperatives in the region associated with improved cleaning,

grading, product drying, packaging and distribution and harvesting nearer to maturity with excel at quality and safety.

- Public interest in natural therapies, namely herbal medicines has increased dramatically. In India, nearly 9,500 licensed herbal industries and a multitude of unregistered herbal units depend largely upon supply of medicinal plants for manufacturing of herbal formulations. It is estimated that more than 2,400 traditional higher plant species available in Himachal Pradesh and Sikkim are used in the codified Indian system of medicine and Western medicine system. The World Health Organization report indicates that more than 80% of South Asian population uses plant-based medicines for maintaining & improving its health.
- The current practice of medicinal, aromatic and forest products utilization in the regions involves only harvesting during certain period that is convenient to collectors. The management options for increasing cultivation and production while making it sustainable, are not found in practice. There is a need to consider several possible options for production management depending upon commercial opportunities. The key for the sustainable benefit is the reconciliation of biological sustainability with commercial viability. There are several possibilities to increase the production of medicinal, aromatic and forest products in a sustainable way like improving production from the wild, through domestication and improving harvesting technologies.
- Promotion of cultivation on private land seems the best way forward for threatened and unmanaged populations of high-altitude aromatic, medicinal, timber and non-timber forest products. Cultivation could take the pressure off collection from the wild. It could also support small-scale processing plants through greater quality control & stability of supply.
- In order to utilize the natural bio-resources in a sustained and focused manner, it is important that these resources be harnessed efficiently and eco-friendly to meet the people's development aspirations without degrading them and therefore, urgent need for large scale establishment of technology resource centre is the need of the hour. Poor access to appropriate technologies due to difficult topographies and tough mountain conditions is one of the major causes of poverty, drudgery and natural resources degradation in the regions. Scientific and technological interventions would be perceived as a means of developing and disseminating improving technologies through action and participatory research. Transformative rural technology is widely recognized as one of the major determinants of socio-economic development, and the idea that the simple and hill specific transfer of technology from lab or field lab to field/land will result in growth and thereby poverty is alleviating.
- Conservation of bio-diversities and protection of endangered of aromatic and medicinal plants species in IHR is another justification for the current proposal.

1.5 Goal towards which the project will contribute in terms of SDGs

The goal of the proposal is to develop sustainable livelihoods options through utilizing locally available bio-resources such as aromatic and herbal plants, crops and scrubs, agro produce, timber and non-timber forest products in Himachal Pradesh and Sikkim in a focused manner.

1.6 Project Objectives

1. Development of scientific and sustainable strategies for cultivation and harvesting of natural bio-resources such as aromatic and herbal plants, agro produce, and timber and non-timber forest products in the Indian Himalayan regions (IHR) of Himachal Pradesh and Sikkim.
2. Development of appropriate scientific and technological interventions for processing and value addition of the local bio-resources into high value products.
3. Establishment of replicable community models in the selected project sites through rural transformative technologies and participatory rural action research for sustainable utilization of the natural product resources into various high value commercial products in collaboration with local grassroot organizations.

2. Project Outcomes

Objective-wise outcomes of the project have been discussed in the following sections.

2.1. Objective 1: Development of scientific and sustainable strategies for cultivation and harvesting of natural bio-resources such as aromatic and herbal plants, agro produce, and timber and non-timber forest products (Sea buckthorn and Large Cardamom) in the Indian Himalayan Region (IHR) of Himachal Pradesh and Sikkim

Abstract: Livelihood strategies are highly vulnerable to many uncertainties of nature in Himachal Pradesh and Sikkim. The climatic conditions do not permit growing of more than two crops a year. This makes people to diversify their livelihood strategies other than cultivation. Relatively small size of operational holdings results in adoption of supplementary strategies in the states of Himachal Pradesh and Sikkim. The observation that majority of the produce out of cultivation is used for self-consumption and a very little of it is marketed is sufficient to infer that a large proportion of population has to supplement their earning with supplementary strategies. Scope of intensification of farm operations being limited, a high dose of technological inputs is required to improve productivity and ensure sustainability in the regions. Another constraint to the livelihoods strategies is the frequent landslips and landslides. The climatic conditions and various anthropogenic activities together with highly fragile nature of the strata of regions make vulnerable to this phenomenon. Extreme loss of soil fertility and damage to land basin caused by soil erosion is another event which makes the sustainability of farm dependent rural poor vulnerable. The steep slopes in hilly terrain have the terraced farming prevalent in them and these farms are more prone to soil erosion with the slightest of rains. The vision of the NMHS is to support the sustenance and enhancement of the ecological, natural, cultural, and socio- economic capital assets and values of the Indian Himalayan Region (IHR) with a mission to launch and support innovative studies and related knowledge interventions towards the sustenance and enhancement of the ecological, natural, cultural, and socio-economic capital assets and values of the Indian Himalayan Regions. One of the important goals of the NMHS is fostering conservation and sustainable management of natural resources where it also commits to enhance supplementary and/or alternative livelihoods for IHR peoples and overall economic and ecological well-being of the region. Therefore, the present proposal is quite relevance to NMHS where it is attempted to develop sustainable rural livelihoods utilising locally available biological resource in Himachal Pradesh and Sikkim. The three broad thematic groups viz. conservation and sustainable use of biodiversity, supplementary livelihood options and awareness and capacity building are well. addressed by the proposal. Local people remain the main beneficiaries of the project where employment for youth and women are increased along with improved marketing network.

2.1.1 Participatory Rural Appraisal (PRA) survey in the project sites to identify biological resources available and their processing and utilization pattern

Two Himalayan states Himachal Pradesh and Sikkim have been considered as the project area in the proposal. Lahaul & Spiti and Chamba in Himachal Pradesh and Geying, Soreng, Ranipool, Pakyong and Sajong in Sikkim will be concentrated in the project study. To analyse actual scenario at local level investigation is designed to prepare the authentic blue print of natural biological sources in the districts and their commercial importance. Following three main broad areas planned to examine as proposed:

1. Aromatic and medicinal plants, herbs and scrubs
2. Agro produce
3. Forest products - both timber and non-timber forest products

From farm to fork- each information is planned to gain from the local people, vendors and concerned individuals by doing one to one communication. The process of cultivation, methods of irrigation, pre-harvesting cautions, harvesting equipment, storage problems, processing challenges and marketing scopes are important parameters to know it well and further work for the betterment of the techniques.

Cultivation: The patterns of the cultivation of aromatic and medicinal plant, herbs and scrubs, agro produce, timber and non-timber forest products planned to be counselled. The main points to be discussed are:

- Naturally grown produces and particular areas
- Irrigation requirements and problems
- Mapping of the produce
- Requirements for the particular cultivations

Pre-Harvesting: The basic investigation about following points will give a clear picture about the pre-harvesting problems and scope of the area

- Part of the plant focused to harvest and its uses
- Identification of the correct timing of the particular part to be harvested
- The maturity indicators local people use to harvest the produce.
- Precautions taken to avoid the spoilage of the produce

Harvesting: Harvesting is an important step in the food processing. The protocol used is an important parameter to know the produce and local domain. Following points can be considered while gaining the information through Participatory Rural Appraisal (PRA) survey.

- Produce is harvested manually or any technology is used
- What are the constraints faced during the harvesting
- Precooling methods used to keep the produce fresh for longer period
- Type of containers used during harvesting

Storage: Shelf life of any produce is considerable for any kind of processing. Storage methods have a great impact on it. By exploring the following points efficient data can be obtained:

- Temperature and other conditions during storage
- Average storage time
- Particular preservatives used
- Spoiling parameters during storage (rodants, moisture, temperature etc)

Processing and Marketing: The local methods to process the produces can give a basic foundation for accelerating the uses and livelihood options in the particular area of the state.

- Products made by the produce in local level (juice, jam, tea, pickles, medicines etc.)

- Over all wastage
- Unused produces
- Raw uses
- Properties of the particular plant known by local people
- Problems in marketing
- Livelihood options from particular produce

Therefore, Participatory Rural Appraisal (PRA) survey is planned to be conducted for designing a data base according to the flow chart (Fig.3 & 4). This activity was planned during the month of October-November, but due to the weather conditions it is postponed to May-June. Meanwhile, the local vendors were contacted for the local information about the produces and utilization.

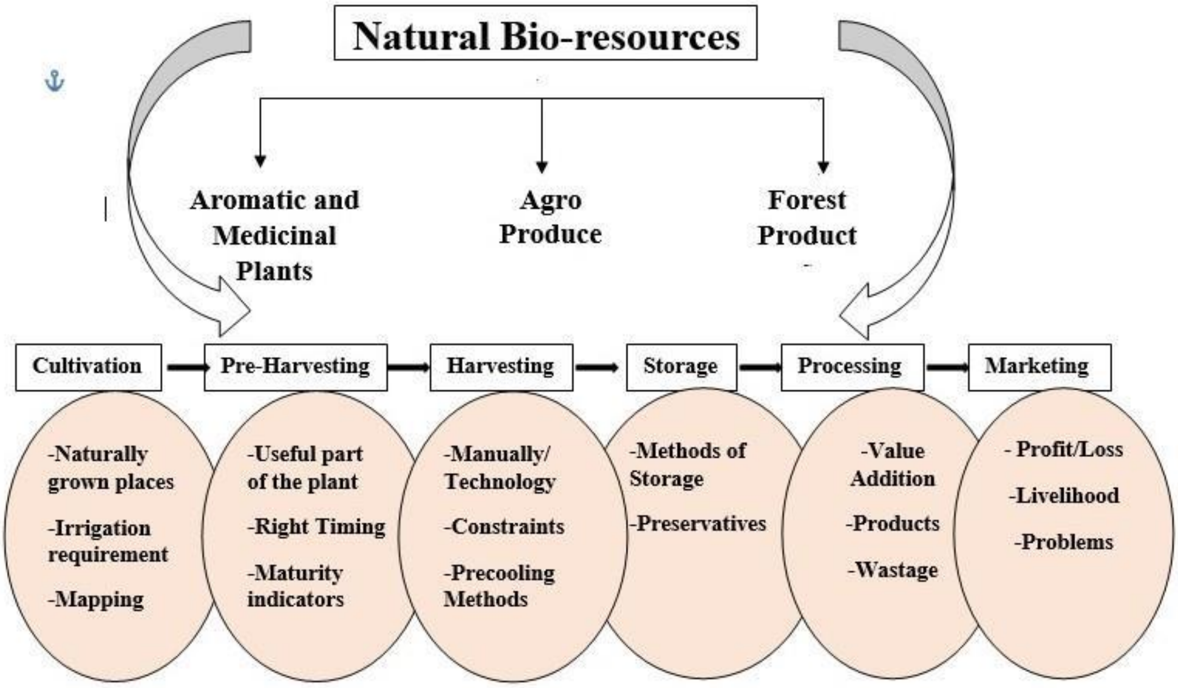


Fig.3 Flow chart for Participatory Rural Appraisal (PRA) Survey

Fig.4 Discussion over production and utilization of sea buckthorn at Lahaul Spiti at IIT Delhi

2.1.2 One-day workshop cum interactive meeting on "Sea buckthorn: current status, problems and future prospects" organised on June, 2019

A one-day workshop cum interactive meeting on "Sea buckthorn: current status, problems and future prospects" at Forest rest house of the Jahlama village in Lahaul- Spiti, district of Himachal Pradesh. The objective of the workshop was to explore the status of sea buckthorn in the region. Around 30 local people enthusiastically participated (Fig. 5 to 8) in the program. Followings are the main focusing points we discussed, and the problems related to the crop observed in the meeting:

- Sea buckthorn berry is a very rich source of vitamins and is called treasure of bio-activity substance because of its over 190 bio-activity substances possessing unique medicinal properties.
- The species of Sea buckthorn that is cultivated in Lahaul valley by around 400 farmers is *Hippophae salcifolia*, which is a valuable field crop.
- A vast research work has been conducted by the scientific community in Lahaul-Spiti especially a value chain starting from nursery to plantation and management. But very less work has been conducted on the post-harvest techniques of Sea buckthorn plant produce.
- In the district, there is very less knowledge of post harvesting techniques such as how and when to harvest leaves or berries, its drying, processing, value addition, packaging etc.
- Also, how and where they can market their produce so that they can get good or timely economic return which is not there at current scenario.

- Sea buckthorn bushes being a thorny species it is quite difficult to harvest the berries from the plant. Traditionally farmers used to harvest the berries by beating bushes using stick and shaking the branches which in turn deteriorating the quality of berries for its further processing and destroying leaves.

Therefore, there is an urgent need to introduce the scientific method for the harvesting and drying techniques of produce for making good quality product and economic return to the farmers.

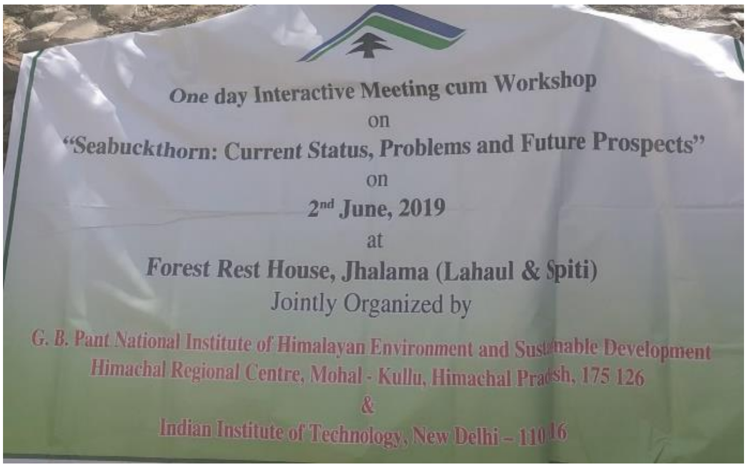


Fig.5 One day interactive meeting cum workshop at Jahlama, Lahaul-Spiti, Himachal Pradesh

Kullu | Main | 03 June 2019

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जाहलमा में सीबकथोर्न के बारे में बताया

उदयपुर (लाहौल-स्पीति)। जीबी पंत राष्ट्रीय सतत विकास संस्थान क्षेत्रीय केंद्र मौहल, भारतीय औद्योगिकी संस्थान दिल्ली की ओर से लाहौल के जाहलमा में सीबकथोर्न पर कार्यशाला का आयोजन किया गया। वैज्ञानिक सरला शाशनी ने सीबकथोर्न से संबंधित परेशानियों को जाना। आईआईटी दिल्ली से आए प्रोफेसर जतिंद्र कुमार साहू ने छरमा प्रोसेसिंग से संबंधित नई तकनीक बताई। ब्यूरो

Fig.6 Publication in the local newspaper (Amar Ujala; Kullu district; 03.06.2019; page 06)

Fig.7 Discussion over production and utilization of sea buckthorn during the workshop

Fig.8 Field visit and discussion with the local people in Jahlama, Lahaul-Spiti, Himachal Pradesh

2.1.3 Field visit to know the current status, problems and future-prospects of cardamom at Sikkim

A survey has been conducted in Sikkim state to understand the traditional practices of cardamom processing by the local farmers. A sustainable livelihood can be generated by giving them low-cost techniques.

Fig.9 Visit to cardamom fields and local drying unit

2.1.4 Survey on traditional cultivation, harvesting, processing, value addition and marketing network for large cardamom in Sikkim

Major field operations of cultivation of large cardamom includes plantation establishment & management, site selection, planting, application of manure, irrigation, plant protection practices, harvesting & curing, packaging & management. Based on the feedback of the farmers, harvesting is one of most tedious operation and is performed manually with traditional knife (Fig.10)

Fig.10 Physiological measurement for modified Cardamom harvesting knife

Table 1 Statistical analysis of physiological parameters of subjects while harvesting large cardamom using traditional knife

Physiological parameter	Traditional					
	Mean	Max	Min	SD	5th	95th
Heart rate (HR)	106.35	129.20	87.24	13.20	84.64	128.06
Oxygen consumption rate (OCR) (L/min)	0.91	1.41	0.60	0.02	0.52	1.40
Energy expenditure rate (EEm) (Kcal/min)	4.41	5.99	3.54	0.73	3.22	5.60

In order to study the difficulties faced by the farmers, as a part of pilot study, physiological measurement of the workers during harvesting using traditional knife were undertaken (Fig. 10). The physiological data of 5 subjects obtained from Human Energy Measurement System was analyzed to find out the heart rate (or HR), Oxygen consumption rate (OCR), and energy expenditure rate (EEm). The average value of each parameter for individual subject for 10 min data (recorded every after 1-2s) was calculated and summarized in Table 1. It is evident from Table 1 that average values of heart rate, oxygen consumption rate 0.91 L/min, and 4.41 Kcal/min respectively.

2.1.5 A one-day seminar was organized on the topic 'Value Chain Management of Sea buckthorn' on July 6, 2019

To create awareness among the local communities and develop a value chain system starting from ground to consumer, a seminar was organized on July 6, 2019, with the topic 'Value Chain Management of Sea buckthorn' at Keylong, Lahaul and Spiti. The seminar (Fig. 11 & 12) was jointly organized by GBPNIHESD, Himachal Regional Centre, Mohal – Kullu, Himachal Pradesh and Indian Institute of Technology, New Delhi under a collaborative project funded by NMHS, MoEF&CC, New Delhi. The seminar was attended by 136 participants, including local farmers, scientists from various organizations, researchers, NGOs, societies. Shri Amar Negi, SDM, Lahaul & Spiti was the chief guest of the seminar.

Lectures by experts

1. Sarla Shashni, Scientist & Convener, briefed about the overall programme and sustainable environmental and economic development of the region with SBT.
2. Virendra Singh, Professor, CSKHPKV, Palampur elaborated about the cultivation of SBT as soil holding plant and overall benefits through developing various nutraceutical products.
3. Ashok Singh, Scientist, IHBT-Centre for High Altitude Biology, Ribling discussed about the conservation of the SBT through various in-situ and ex-situ conservation.
4. Nikita Sanwal, Research Scholar, IIT, New Delhi introduced the technological aspects involved in the value addition process of SBT for developing high quality products.
5. Mamta Chander, Managing Director, JAGRITI a community-based organization told participants about women empowerment through region specific small intervention for overall environmental

sustainability and livelihood generation. She had also stressed on the steps involved for successful enterprise development.

6. Amshu, Thapasu Foods, a Himachal based startup company briefed participants about their mission to promote traditional grains and resources available in the region by providing them a marketing platform. She had explained how their company can help farmers in the region during various steps involved in the value addition process of SBT.
7. Bishan Dass Parsheera, President, Seabuckthorn Society Lahaul and Spiti told the participants about the issues coming across the society while working with the farmers at grassroot levels. He had also emphasized on the need of capacity building and need of proper chain management for its successful implementation.

Discussion with Participants:

- Due to the limited agricultural land and enough forest land in the region, the farmers requested for forest land to be allotted for the SBT cultivation under Anderson Settlement, 1886.
- Identification of target markets, potential customers, agro economics and the pricing policies for the developed products need to be fixed. The process of the marketing of the SBT and continuous skill development programmes of the farmers at various levels of value addition should be done.
- Development of SBT sustainable harvesting technologies and availability of tools for the plucking leaves and plants is needed to be standardized. There is also a need for technology transfer from developing agency to implementing agency at the local level.
- Identification of the high yield species for the demonstration and development of thorn less plants, its field testing and mass multiplication at the suitable sites should be done while emphasizing on organic farming of the same.
- Information dissemination and capacity building on the identification of male & female plants, cultivation, harvesting, post-harvesting, and incentives through social media, leaflets, flyers, etc. for the beneficiary’s knowledge is required.
- Identification and promotion of local NGOs, SHGs, and local organizations for providing maximum benefits of SBT to local communities.
- Development of sole SBT industry and registration of the local company for dealing exclusively with SBT and provision of government subsidies and grants for the same is required.

Overall outcome and recommendations

The seminar successfully gathered the information about the needs, suggestions and future scenarios about the development of the SBT and its products in the Lahaul and Spiti. Various expertise, stakeholders and local people mutually exchanged their views through a seminar organized at Keylong, Lahaul & Spiti on July 06, 2019. The broad outcomes of the seminar were:

- Possibility of forest area provision for cultivation of SBT for high level production.
- Linkages with marketing agencies at local level is needed.

- Development of technologies for value addition of the produce such as harvesting tools, drying techniques and quality assurance should be done.
- Dissemination of information about the monetary benefits of SBT and its allied products at various levels.
- Promotion of local societies for providing benefits to local people especially women.

The seminar helped the organizers in identifying the gaps in the development of SBT as a major industry in Lahaul & Spiti, a cold desert district of Himachal Pradesh, where the resources and biodiversity is limited and fragile, which needs to be conserved.

Fig. 11 Demonstration of sea buckthorn products at seminar

Fig.12 One day seminar cum sea buckthorn products display at Keylong, Himachal Pradesh

2.1.6 Development of a database of high value and commercial plants in Indian Himalayan Region

India has a very rich plant biodiversity due to prevailing very highly divergent ecosystem. The Indian Himalayan Region (IHR) constitute one of the richest ecosystems, endowed with an enormously rich flora of medicinal and aromatic plants. The Indian Himalayan region includes Jammu & Kashmir, Ladakh, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya, Assam and West Bengal. Trans-Himalaya harbors a fragile biome, characterized by low productivity, high intensity of solar radiation, and high degree of resource seasonality.

Plant Biodiversity has always been the source of food, medicine and other necessities of life since the beginning of human civilization. The Himalayas ranges are one of the best examples, which abound approximately 18,440 species of Angiosperms, Gymnosperms, Pteridophytes, Bryophytes, Lichens and Fungi, of which about 25% species are endemic to the Himalayas. Avery harsh and characteristic climate prevails in trans Himalayan cold deserts where the summer temperature touches up to 40°C while the winter temperature drops below minus 35°C and are characterized by barren mountains, nail biting winters, low humidity and sparse vegetation. The cold arid regions of Jammu & Kashmir and Himachal Pradesh may be divided into seven major geographical valleys for exploration and enlisting the plant biodiversity viz. Indus, Nubra, Changthang, Suru, Zanskar, Lahaul and Spiti.

The trans Himalayan cold desert has a characteristic vegetation differing from other parts of Himalayas due to prevailing unique climatic conditions and physiography. The vegetative growth starts at the commencement of summer when the melting snow provides abundant moisture. The alpine mountain slopes, meadows and alpine pasture lands abound in varieties of alpine and high alpine flowers making trans Himalayan barren mountains lush green for brief summer period. Most parts of trans Himalayan cold desert of Ladakh and Lahaul-Spiti are inaccessible and remain cut off from the rest of the world due to rugged and difficult terrain and prolonged subzero temperatures which have forced the local people to be self-sustained for most parts of the history. Like other parts of the Himalayas, these regions are considered treasure of medicinal, aromatic and other important plants which have been in use by the local tribes for day to day needs and this long dependence on plant wealth had enriched their knowledge about multifarious uses of plant biodiversity. Much of such valuable information are confined to tribes and ethnic groups.

Lack of knowledge on agro-technologies of medicinal plants is the major bottleneck in wholehearted acceptance by farmers towards their organic cultivation. This set of data elaborately describes the botanical, phytochemical and therapeutic aspects of medicinal plants and agro produce in the Trans Himalayan region. To encourage people’s participation for need based scientific management, PRA survey was conducted in the regions of Lahaul-Spiti about the local aromatic, medicinal plants and agro-produce and the information was collected. The ethnobotanical information was verified with local physicians, senior citizens and available literatures. There has been always a search of ways to find cure and relief from mental and physical illnesses. India has the most diverse cultural traditions associated with the uses of medicinal plants. The people of Ladakh and Lahul-Spiti of the trans Himalayan region has its own medical system called "Amchi system of medicine" (Tibetan Medicine) and the practitioners are called Amchis (Superior of all). Amchi medicine is based on principally on a skilful use of plants, minerals and animal products.

It has become urgently imperative to recognize new opportunities for income and employment generation in the rural area of Trans Himalaya out of the resources that are presently available naturally. The traditional remedies of the ancient world were all based on natural products. Conservation of biological diversity, particularly medicinal and aromatic plants is one of the 5 top priority environmental problems facing the globe. The increasing demand of phyto-medicines calls for concerted efforts for domestication and cultivation of genetic resources of medicinal plants on large scale through herbal gardens.

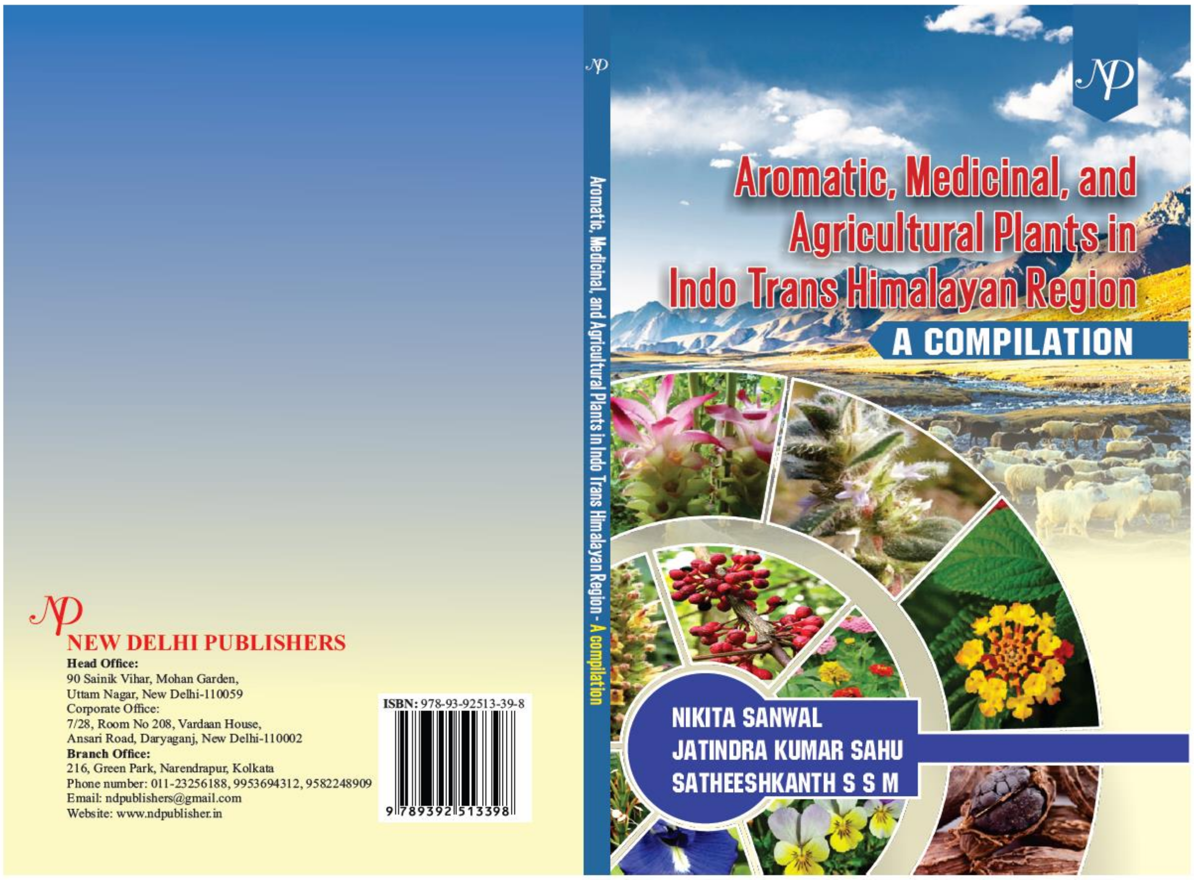


Fig. 13 A Databook entitled "Aromatic, Medicinal and Agricultural Plants in Indo Trans Himalayan Region - A compilation" Sanwal N, Satheeshkanth SSM, Sahu JK. ISBN: 978-93-92513-39-8

The important medicinal plant species of Indian trans Himalayan region have been collected along with therapeutic uses during the botanical survey by the authors. This book (Fig. 13) has been brought out to provide a comprehensive database on medicinal and aromatic plants along with agro produce present in the Indian Trans Himalayan region. Attempt has been made to present a concise account of the valuable plants from point of view of practical utility to the farmers. The repository of accumulated experience as well as knowledge and practices of traditional medicine from the senior citizens are also reflected in this book. The database will be immensely useful for the collectors, growers and traders of medicinal and aromatic plants along with the researchers of botany, Phyto-chemistry, pharmacy, agriculture and biomedicine.

2.2 Objective 2: Development of appropriate scientific and technological interventions for processing and value addition of the local bio-resources (sea buckthorn & large cardamom) into high value products

This objective was accomplished in four sub objectives and these three objectives were described in the foregoing sections.

a. Evaluation of the volatile components, phytochemicals, and antioxidants of sea buckthorn (*Hippophae* sp.) leaves using GC-MS and HPLC-PDA analysis – a gender-based comprehensive metabolic profiling
b. Development of a functional tea from sea buckthorn (*Hippophae* sp.) leaves through fermentation with kombucha consortium
c. Development of a low-cost- quick technology for extraction of sea buckthorn oil
d. Design and Development of a Hybrid-Multi Mode Microwave Vibro-Fluidized Hybrid Dryer for Sea buckthorn (*Hippophae Sp*.) Berries and Large Cardamom

2.2.1 Evaluation of the Volatile Components, Phytochemicals and Antioxidants of Sea buckthorn (*Hippophae* sp.) Leaves using GC-MS and HPLC-PDA Analysis – A Gender-based Comprehensive Metabolic Profiling

Abstract: Sustainable utilization of sea-buckthorn and large cardamom was studied with reduced postharvest losses due to proper S&T interventions at the grass root levels for livelihood generation and employment opportunities. The study assessed comparative polyphenol profiles, antioxidant properties, minerals, and flavour components of male and female leaves of two sea buckthorn species (*Hippophae rhamnoides* and *salicipholia*) to comprehend the selection of dioecious sea buckthorn leaves for tea and/or nutraceutical formulations based on gender and species. Advanced analytical processes and apparatus such as GC-MS, HPLC, and ICP-MS were employed for authentic analysis. Key components such as gallic acid, epigallocatechin, sesamin, epigallocatechin-gallate, rutin and quercetin were observed to be significantly present in males unlike female leaves. Consequently, male leaves exhibited better antioxidant activity as the ranges of IC_{50} and FRAP values of the males and females were calculated as 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, significant aroma-producing components like linalool and geraniol were only discovered in female leaves, not in male. For better understanding of the relationships between constituents identified using GC-MS (classified based on their bioactivities) and the factors (gender, species, and extraction methods) considered in this study, principal component analysis (PCA) based on correlation coefficients was applied to the experimental results. Furthermore, the sea buckthorn leaves contained minerals such as Mg, K, Ca, Cr, Fe, Cu, and Zn, and the investigation revealed a significant difference ($p<0.05$) between both the gender and between two species. Hereafter, the comprehensive profiling of phytocomponents in the present study can establish the preferred gender and species for nutraceuticals and nutritionally-rich-flavourful tea based on investigated bioactive functionalities.

Introduction

Sea buckthorn *(Hippophae sp.)* is a dioecious plant which occupies a widespread natural habitat in the trans-Himalayan region of India. It is extensively disseminated throughout the temperate zone of Asia and the north-western Europe including subtropical zones, especially at high altitudes. The whole sea buckthorn plant viz. berry, leaves, seeds, and roots have significant medicinal properties (Bal et al., 2011) and is a rich source of bioactive compounds and micronutrients. Sea buckthorn berries contain a decent amount of omega 3, 6, 7, and 9 fatty acids and reported to stand alone in the plant source that offers all fatty acids including omega-7 (Ciesarová et al., 2020). Sea buckthorn leaves are reported to contain significant quantity of phenolic components and exhibit better antioxidant activity than berries. Conventionally sea buckthorn leaves are utilized in the preparation of tea and nutraceuticals. Presence of phenolics, flavonoids and tannins constitutes a significant set of phytochemicals that exhibit potent antioxidant, antibacterial, anti-inflammatory and other bioactivities in sea buckthorn leaves(Kim et al., 2011; Yogendra Kumar et al., 2013).

Most of the work published yet aimed for determining the biological activities and medicinal properties of *Hippophae sp*. without specifying the gender and flavour profile of leaves. (Criste et al., 2020; Dong et al., 2017; Skalski et al., 2018) However, significant differences between male and female plant were recognized by many researchers working on various dioecious plants which is associated with the secondary metabolites and corresponding antioxidant activity (Brandt et al., 2020; Carsten F. Dormann & Christina Skarpe, 2002; Hjältén, 1992; Paul et al., 2020). Some studies revealed that the females from dioecious plants showed lower productivity compare to males and invest higher amount of nitrogen and carbon during reproduction process (Mesgaran et al., 2021; Olukayode Olugbenga et al., 2020). Ecological theory suggest that the females invest dominantly in the defence and reproduction while males are said to be more growth-oriented (Bañuelos et al., 2004). Ågren et al. revealed that these gender-related differences were related to chemical composition, leaf morphology, vegetative phenology, and the nutrient content of the plants. In this prospective, a gender-based comparative analysis of polyphenolic and volatile components including flavour profile of sea buckthorn leaves is needed to generate useful scientific information which is not available so far to the best of our knowledge. To increase the understanding of the chemical diversity of sea buckthorn leaves, a comparative research based on different species, gender and extraction method was undertaken in this study. Hence, the present study was designed to investigate significant constituents of volatiles along with phytochemical and antioxidant properties of male and female leaves extracts, prepared by cold percolation (CP) and ultrasound assisted extraction (UAE) methods. It is expected that the study would help in understanding the dioecism in *Hippophae* sp. of sea buckthorn leaves and enhance the understanding towards the adaptation of sea buckthorn plants based on gender and their species for tea and/or nutraceutical formulation in food and nutraceutical industries.

Materials and Methods

Plant Materials

Sea buckthorn leaves of two species *Hippophae salicifolia* and *Hippophae rhamnoides* were supplied by G.B. Pant National Institute of Himalayan Environment & Sustainable Development, Kullu, Himanchal Pradesh (HP), India. The leaves were harvested from Lahaul- Spiti district of HP, location (31°44' 57" and 32°59'57"N Latitudes and 76°46'29" and 78°41'34" E Longitudes). Leaves from 100 male and 100 female individual sea buckthorn plants were randomly sampled according to the probability sampling method (Taherdoost, 2018). The leaves collected were immediately transported to IIT Delhi and dried using a microwave dryer (Twin Engineers, Vadodara, India). The dried leaves were then stored at -18°C in dark bottles till further experimentations.

Chemicals

Methanol, ethanol sodium sulphate (Na_2SO_4), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), hydrochloric acid (HCl), 2-propanol, , sodium nitrite ($NaNO_2$), sodium carbonate (Na_2CO_3), Folin–Ciocalteu reagent, sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), aluminium chloride ($AlCl_3$), gallic acid, rutin, catechin, epigallocatechin, sesamin, epigallocatechin gallate, rutin, ellagic acid, quercetin hydrate, butylated hydroxytoluene (BHT), and quercetin were purchased from Sigma Aldrich, USA and they were of analytical grade.

Leave extract preparation

Cold percolation (CP) method

In this method, sea buckthorn leave extracts were prepared as described by Zu et al., (2006). Briefly, about 10 g of each male and female leaves (Fig.14) were powdered using a domestic mixer-grinder (Sujata grinder-Dynamix model). The powder was soaked in 100 mL methanol for three days and filtered with the help of Whatman filter paper. The filtrate was then collected and evaporated in a water bath overnight at 40°C. The dried extracts were stored in airtight dark bottles at 4°C.

Fig.14 Sea buckthorn leaves (male and female) sample collected from Lahaul-Spiti of Himachal Pradesh

Ultrasound assisted extraction (UAE)

The powdered sample (10±0.01 g) was weighed and added to 100 mL of methanol. It is then subjected to ultrasound pre-treatment using an ultrasound probe system (VCX750, Sonics & Materials, USA) as described by Sanwal et al. (2022). The prepared extract was centrifuged (5804R, Eppendorf, Hamburg, Germany) at 15,557 × *g* and 4°C for 10 min, and the supernatant was collected. The residues were extracted again (twice) using methanol and the extracts were combined and stored in airtight dark bottles at 4°C till further analysis.

Estimation of total flavonoid content

Total flavonoid content (TFC) was analysed as described by Zhishen et al., (1999) with slight modifications. In brief, about 100 µL leaf extract was mixed with 50 µL of 5% $NaNO_2$. 500 µL of 10% $AlCl_3$ solution added after 6 min for the formation of flavonoid-aluminium complex. After 7 min, 250 µL of 1 M NaOH was mixed and centrifuged for 10 min at 5000 rpm. The separated supernatant was studied for the absorbance at 510 nm using microplate photometer (VT 05404–0998, EPOCH, USA). The total flavonoid was calculated as mg of rutin equivalents per g dry weight of leaves.

Table 2 Coding of sea buckthorn leaves samples

	Male leaves				Female leaves			
	H. salicifolia		*H. rhamnoides*		*H. salicifolia*		*H. rhamnoides*	
	CP	UAE	CP	UAE	CP	UAE	CP	UAE
Sample code	MSC	MSU	MRC	MRU	FSC	FSU	FRC	FRU

Estimation of total phenolic content

Total phenolics content (TPC) of leaf samples were determined using Folin–Ciocalteu method adapted from Swain and Hillis (1959) with slight modifications. 100 µL each leaf extract samples were taken in test tubes and volumes were made up to 500 µL using distilled water. Then 250 µL Folin-Ciocalteau reagent was added for oxidation of the sample. After 5 min of reaction, neutralization of the mixture was achieved by adding 1.25 mL of 20% aqueous Na_2CO_3 solution. The mixtures were incubated at ambient temperature for 40 min until the development of blue colour. The mixture was centrifuged at 4000 rpm for 5 min. Absorbance was recorded at 725 nm using a microplate photometer (VT 05404–0998, EPOCH, USA). Calibration curve was prepared with gallic acid. Total phenolic content of the leaf sample was expressed as mg of gallic acid equivalent (GAE) per g of dry weight.

Determination of condensed tannin content

Condensed tannin content (CTC) was analysed according to the method described by Sun et al. (1998). In brief, 50 µL of leaf extract was mixed with 750 µL of sulfuric acid and 1.5 mL of 4% vanillin methanol solution. The mixture was kept in the dark for 15 min. Absorbance was observed at 500 nm using a microplate photometer (VT 05404–0998, EPOCH, USA). Condensed tannin was determined from the calibration curve of standard solutions and was expressed as mg catechin equivalent/g of dry weight.

Antioxidant activity

Antioxidant activity of the extract was evaluated by two different spectrophotometric assays and HPLC analysis using photodiode array detector (PDA).

Free radical scavenging activity

Free radical scavenging property were analysed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. 1.4 mM solution of DPPH was prepared in methanol. A micro titre plate was used and 10 µL of sample added to 250 µL of DPPH solution. It was then incubated for 15 mins in dark, and absorbance was recorded spectrophotometrically at 517 nm. DPPH radical scavenging activity confirmed by the decreasing absorbance.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was analysed based on the method described by (Suresh Kumar et al., 2010). 0.2 mL of the extract sample was added with 3.8 mL of FRAP reagent and mixed properly. The solution was kept in the dark for 10 min at room temperature. Spectrophotometer was used to measure absorbance at the wavelength of 593 nm. The results were expressed as mg of butylated hydroxy toluene (BHT) equivalent per g of sea buckthorn leaves.

Identification and quantification of marker antioxidant compounds

Extraction preparation

About 10 g of leaves powder i.e., *H. salicifolia* male (SM), and female (SF); *H. rhamnoides* male (RM) and female (RF) were percolated in methanol at 30º C for five days. All collected filtrates were passed from charcoal to remove chlorophyl. The filtrates were then vacuum dried using a rota evaporator (RV 10, IKA). The values of extract yield were for RM, RF, SM and RF were 2.6±0.2 g, 3.0±0.21 g, 2.5±0.18 g, and 3.5±0.21 g respectively.

Standard preparation

1 mg of each standard molecule i.e., gallic acid, epigallocatechin, sesamin, epigallocatechin gallate, rutin, ellagic acid, quercetin hydrate and quercetin were dissolved in 1 mL HPLC grade methanol to make concentration of 1 mg/mL.

Sample preparation

100 mg dried extract was hydrolysed in 1N sulfuric acid and pH neutralized samples were used for HPLC analysis. The concentration of extract samples was 100 mg/mL.

HPLC analysis

A HPLC-PDA system (Waters Milford MA, USA) was used for separation and quantification of antioxidant components. Photodiode array detector (PDA, model 996) was used for the analysis of the compounds. The separation of components was carried out in a RP Column of Sunfire C18 (4.6 mm × 250 mm, 5 μm coating; Waters, USA) and an Empower Pro-software (Waters, USA) was used for the analysis. A gradient solvent system was used at 25◦C and the mobile phases for gradient elution included solvent A (methanol, 100%), and B (water with 0.1%TFA). The solvents were mixed as follows: 80% A–20% B (0–10 min), then 40% A–60% B (10–20 min), then 50% A –50% B (20–30 min) and 80% A–20% B (30–40 min) and after that 100% A (40-50 min). The flow rate was 1 mL/min for the injection volume 20 μL. Quantification of polyphenols was made at 210-400 nm using a signal detection. Gallic acid, epigalocatechin, sesamin, epigalocatichin galate, rutin, ellagic acid, quericitine hydrate and quericitine were used for quantification of polyphenoles. The yields of each compound were expressed using Eqn. (1).

$$\text{Yield}\ \left(\frac{\text{g}}{\text{hg}}\right) = \frac{\text{Sample peak area}}{\text{Standard peak area}} * \frac{\text{Standard concentration}}{\text{Sample concentration}} \tag{1}$$

Phyto-characterization using GC-MS analysis

Phytochemical analysis of all 8 methanolic leaf extract (Table 2) was carried out using GC-MS (QP 2010 Shimadzu, Japan) containing thermal desorption system TD 20. Helium (99.99% purity) was used as carrier gas at a constant flow rate. Total flow and column flow was maintained as 16.3 mL/min and 1.21 mL/min respectively. Injector temperature was set at 200°C whereas the temperature of mass transfer line maintained at 240°C. The temperature of the oven was programmed as follows: column oven temp - 100°C and injection temp - 260°C. GC-MS was run for briefly for 50 min. Turbo mass software was used to manage the chromatograms and mass spectra. The amount of each component was calculated in the form of relative percentage by comparing the component's average area to the total area and expressed as percentage (g/hg) with peak area. The mass spectra from National Institute of Standards and Technology (NIST) database and Willey libraries were used for comparing the retention time and mass weight of the phytoconstituents with authentic samples. The obtained GC-MS data was searched and matched with the mass spectrometry library, then the significant information was chosen for selecting the pinnacle number (similarity ≥96). Each volatile substance represented by specific peak was investigated for the relative rate using peak region standardization technique. Major volatile components detected in sea buckthorn leaves sample along with their relative contents (g/hg) are listed in Table S5 to S8.

Estimation of inorganic minerals

Inorganic minerals were analysed using ICP-MS. Inductive coupled Plasma mass spectrometry (ICP-MS, 7900, Agilent) instrument at the central research facility, Indian Institute of Technology, New

Delhi, was used for analysing the mineral content in the sea buckthorn leaf extracts. The parameters used for obtaining the data are as follows: auxiliary gas- flow, nebulizer gas-flow, plasma gas-flow, and helium gas flow in reaction cell was set as 1 L/min, 1 L/min, 15 L/min and 0.2 mL/min respectively. The analysis was carried out using the reflected power and forward power of 45 W and 1500 W respectively. Furthermore, analyzer vacuum was set at 6×10^{-5}. Electron multiplier (EM) detector was used for analysing the mineral components of the leaves sample.

Statistical analysis

All experiments were conducted in triplicates and the data were expressed as their mean values (mean± std deviation). The phytochemical content, antioxidant activity and mineral profiling data obtained for all variables were subjected to analysis of variance (ANOVA) and a Multiple Range Test (Tukey's test) at a significance level of $p<0.05$ using Minitab 18 software. The GC-MS data was used to establish a relationship between constituents (classified based on their bioactivities) and factors using an ANOVA interaction plot and principal component analysis (PCA).

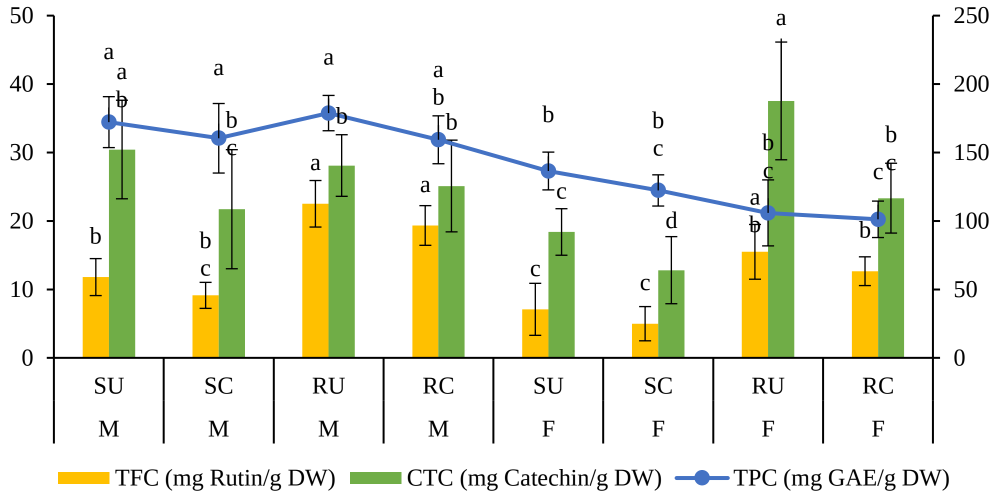
Results and Discussion

Phytochemical analysis of sea buckthorn leaves

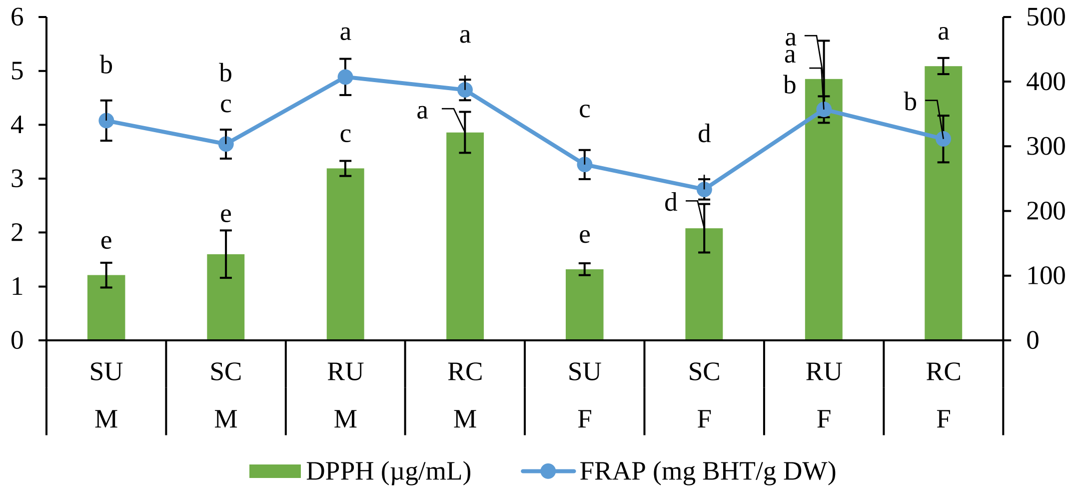
There were significant differences ($p<0.05$) in concentrations of total phenols, total flavonoids and condensed tannins among different species and gender of sea buckthorn leaves (Fig.15a and 15b). Various scientific reports reveal that the reproductive needs between the male and female of dioecious plants show differences that causes divergence in the gender physiology which simultaneously influence the production and concentration of secondary metabolites (Brandt et al., 2020; Seethapathy et al., 2018). Simpson (2013) emphasized the importance of gender-based research on phytochemical and impact on pharmacological properties of *Cannabis sativa* L., a dioecious plant species. Male and female plants from a dioecious species exhibit differences in morphological characters, growth rates, phytoconstituents and secondary metabolites (Retuerto et al., 2000).

Effect of Genders

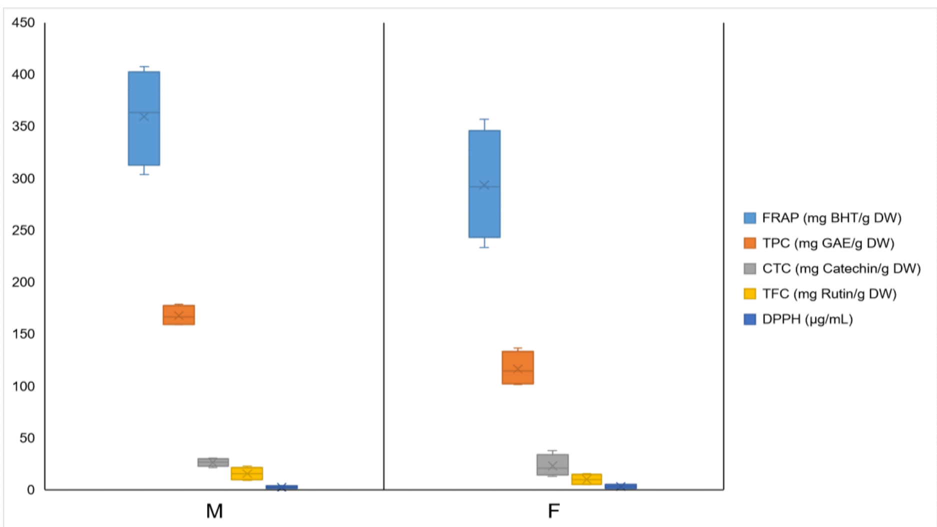
Male leaves of sea buckthorn showed significantly ($p<0.05$) high number of phytochemicals compared to female leaves (Fig.1a). The total phenolic components found in the male leaves of *H. rhamnoides* and *H. salicipholia sp.* was observed to be 178.8±12.9 and 172.2±18.6 mg GAE/g *dw* respectively, whereas the female leaves showed significantly ($p<0.05$) lower phenolic content of 105.9±24.1 and 136.5±13.8 mg GAE/g *dw* in *H. rhamnoides* and *H. salicipholia sp.* respectively as compared to the male leaves. The range of flavonoid content in the male and female leaves was found to be 9.13-22.51 and 4.99-15.49 mg Rutin/g *dw* respectively. The difference may be because of female plants may allocate nutrients and carbohydrates to reproductive function for a longer period than males (Agrawal et al., 1999).



(a)



(b)



(c)

Fig.15 Comparison between male and female leaves based on (a) phytochemical analysis, (b) antioxidant activity, and (c) ranges of different quality parameters

Furthermore, condensed tannin content of both the genders from two species was analysed to have 21.72- 30.44 mg Catechin/g *dw* and 12.81-37.54 mg Catechin/g *dw* in male and female leaves respectively. Inter-gender variation in tannins have been reported in several species (Elmqvist et al., 1991). Therefore, as observed in Fig.15a and Fig. 15c, the male leaves from both the species were found better than female leaves in total phenolic, total flavonoids and condensed tannin content. Similar studies on gender-based differences of dioecious plants revealed differences on physiological attributes and metabolites of male and female plants (Bollard et al., 2005; Kleps et al., 2007) and a significant variation ($p<0.05$) in the concentration of secondary metabolites of male and female leaves was reported for the medicinal plant *Tinospora cordifolia* (Guduchi), *Menispermaceae* (Miers) and *Cannabis sativa* (Marijuana) (Altman et al., 2021; Bajpai et al., 2016). It is not same for all kind of dioecious plants, a report on the *Tinospora cordifolia* plant revealed that in comparison with male variety, the females have higher concentration of secondary metabolites along with better antioxidant activity (Choudhary et al., 2014).

Effect of species

H. salicipholia is reported as best species of the genus *Hippophae* because of its high-quality fruit and it precisely grows in the Himalayan region at an altitude of 1500-3500 m (Ilango et al., 2013). In case of leaves, the highest range of TPC was found to be 178.8 and 172.2 mg GAE/g dw in *H. rhamnoides* and *H. salicipholia* respectively. Similarly, the ranges of TFC in *H. rhamnoides* and *H. salicipholia* species of sea buckthorn leaves was found to be 12.66- 22.51 and 4.99- 11.80 mg rutin/g *dw* respectively (Fig.1c). Also, the CTC analysis of all the leaves samples exhibited similar trend by showing the highest analysed value of 37.54 and 30.44 mg Catechin/g *dw* for *H. rhamnoides* and *H. salicipholia* respectively. Therefore, the comparison between the species revealed that the leaves from *H. rhamnoides* have better results for phytochemicals than *H. salicipholia* species (Fig.15a).

Effect of extraction method

The phytochemical analysis of the leave extracts prepared using CP and UAE methods showed significant difference ($p<0.05$) in total phenolic (TPC), total flavonoids (TFC) and condense tannin content (CTC) (Fig.15a and c). The ranges of TPC, TFC and CTC of UAE and CP extractions yielded 105.9- 178.8 mg GAE/g *dw*, 7.09-22.51 mg Rutin/g *dw*, 18.39-37.54 mg Catechin/g *dw* and 101.2- 160.4 mg GAE/g *dw*, 4.99-19.34 mg Rutin/g *dw*, 12.81-25.11 mg Catechin/g *dw* respectively. The extracts prepared using UAE method resulted in the higher yield of phytochemicals compare to CP.

Antioxidant activity of leaves samples

DPPH and FRAP assay

The analysis based on the DPPH activity exhibited potent antioxidant activity in the sea buckthorn leaves with IC_{50} value of 1.21-3.86 and 1.32-5.09 µg/mL in male and female leaves respectively. The male leaves showed significantly ($p<0.05$) higher antioxidant activity than female leaves as confirmed by

FRAP assay. Male leaves exhibited the range for ferrous reducing antioxidant power of 303.45- 407.33 mg BHT/g *dw*, while female leaves resulted the range of 233.45- 356.92 mg BHT/g *dw* (Fig.1b and c). Similar trends were observed for various dioecious species viz. Populus and Jojoba, and showed higher DPPH activity for male types (Bajpai et al., 2016; Zhang et al., 2010). Kumar et al. reported that significant correlations ($p<0.05$) between phenolic content and antioxidant assays could support the hypothesis of the contribution of these compounds to the total antioxidant capacity of leaves extracts.

Quantification of phenolic compounds, flavanol glycosides, and ellagitannins

The male and female leave extracts showed significant differences ($p<0.05$) in the yield of components like gallic acid, epigallocatechin, epigallocatechin gallate, ellagic acid, sesamin, rutin, quercetin hydrate, and quercetin during HPLC analysis (Fig.16). Male leaves from *H. rhamnoides* species exhibited a yield of gallic acid i.e., 2.642 g/hg as compared to female leaves i.e., 0.370 g/hg, whereas gallic acid which is reported to have potent antioxidant activity was not detected in both the genders from *H. salicipholia* species. The yield of 0.436 g/hg of epigallocatechin was detected only in the female leaves of *H. rhamnoides* and was absent in the male leaves, though it was found in male (0.077 g/hg) and female (0.051 g/hg) leaves of *H. salicipholia*. Epigallocatechin gallate was found only in the male leaves of both the species. Sesamin and rutin were not detected in the leaves of salicipholia species. Rutin was found to have a good yield of 1.009 g/hg in the female leaves of *H. rhamnoides.* Sesamin and qercetin hydrate was only detected in the male leaves of *H. rhamnoides* with the yield of 0.018 and 0.074 g/hg. Similar variation ($p<0.05$) in alkaloids concentration was reported for the male and female of medicinal plant *Tinospora cordifolia* (Willd) and *Menispermaceae* (Miers). In a study done by Simpson et al. (Simpson, 2013), the major constituent present in the male leaves of *Dodonaea polyandra* Merr. and L. M. Perry (*Sapindaceae*) was labdane diterpenoids, whereas the female leaves were found to be rich in clerodane diterpenoids. Moreover, intergender variation in tannins and phenol glycosides have been reported in several species of various plants. Furthermore, one more study on gender-based differences confirmed that male and females represent different metabolites (Mittelstrass et al., 2011). The authors showed differences of cell regulatory processes that may play a role in gender-specific differences in metabolites based on quantitative difference in acetylation of phenols in male and females' landraces.

Estimation of minerals in leaves samples

Sea buckthorn leaves were studied for minerals such as Mg, K, Ca, Cr, Fe, Ni, Cu, Zn, and Pb content and they revealed significant difference ($p<0.05$) between both the gender and between two species (Fig.17a & 17b). Mg and Ca were observed to be higher in the female leaves than male, whereas K was higher in the male leaves. Potassium has an important role in biochemical functioning, photosynthetic rate, conductance and stomatal opening (Bednarz & Oosterhuis, 2008). In a study done by Obeso et al. (Obeso et al., 1998) revealed that under the low light conditions, the females leaves of *Ilex aquifolium* showed higher photosynthesis efficiency than male leaves. The presence of components viz. Cd, Ni, Cr and Zn were comparable in both the genders. Fe content was higher in female (837.15 µg/g**)** than male

(741.31 µg/g) in *H. salicipholia* species, but in *H. rhamnoides* species, the content was almost double in male (1014.91 µg/g) than female (694.03 µg/g) leaves. Pb content was found in acceptable recommended amount in both the leaves. The reported ash values of *H. rhamnoides* leaves exhibit higher content than *H. salicipholia* species as reported by Ilango et al. (2013). These inorganic components are the major essential and non-essential components of teas (Milani et al., 2016).

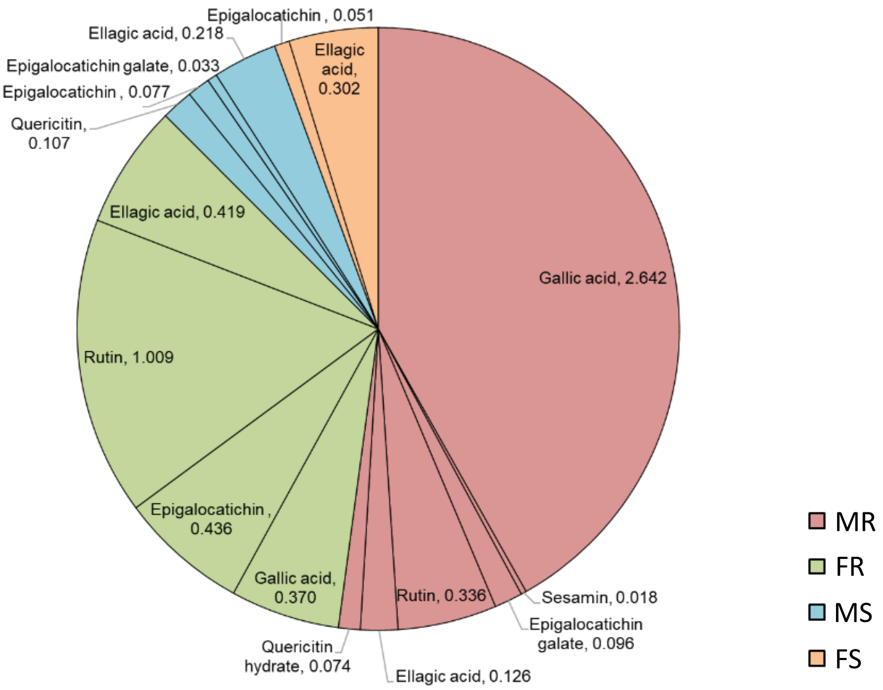
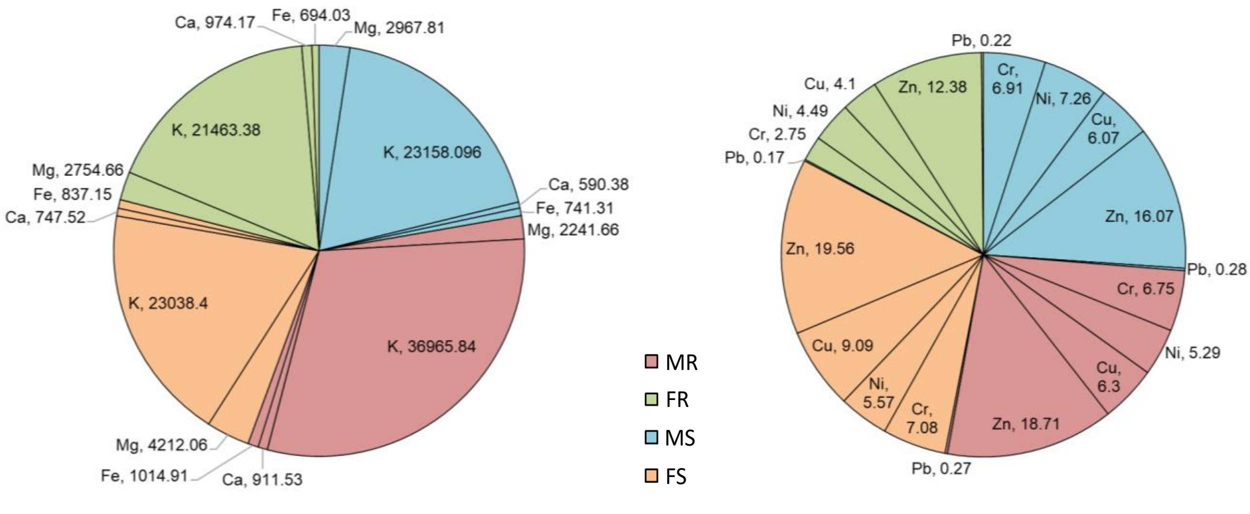


Fig.16 HPLC detected antioxidants components in sea buckthorn leave comparison of the polyphenols



(a) (b)

Fig.17 ICPMS analysis of sea buckthorn leaves indicating amount (µg/g) of (a) Mg, K, Ca, and Fe, and (b) Cu, Zn, and Pb in male and female leaves

Overall impact of variables on phytochemical constituents of Sea buckthorn leaves

Tukey's test applied for multiple comparisons to examine all pairwise contrasts, revealed the overall influence of all variables (gender, species, and extraction methods) on the phytochemical constituents of sea buckthorn leaves. The male leaves of the *H. rhamnoides* species were shown to be the best for nutraceuticals processing. Furthermore, the extraction procedures had a significant (*p<0.05*) impact on the secondary metabolites found in the leaf extracts. The phytochemical yield including, total phenolic content, total flavonoid content, condensed tannin content, antioxidant activity, and mineral profile of the leaf extract prepared from the male leaves of *H. rhamnoides* utilizing ultrasonic assisted extraction was observed to be highest of all.

Phyto-characterization of volatile components

To increase the understanding of chemical diversity between two genders, both the gender of *H rhamnoides* and *H. salicipholia* leaves were analysed using GC-MS and total of 90 (combined) constituents were identified. The chemical profiling showed relationships between the chemical composition and morphological features at the intra specific level. The GC-MS analysis of the leaf's extracts exhibited various aldehydes, arenes, acids, alcohols, ketones, esters, methoxy phenolics and other components which contribute to the development of tea flavour. Male leaves were found to be rich in citronellol (4.90 g/hg), sitosterol (3.05 g/hg), catechol (0.43 g/hg), eugenol (0.14 g/hg), squalene (2.21 g/hg) and amyrin (4.21 g/hg), whereas, female leaves were rich in hexadecenoic acid (13.26 g/hg), linalool (0.41 g/hg), levomenthol (0.61 g/hg), geraniol (0.78 g/hg) and santalool (1.21 g/hg).

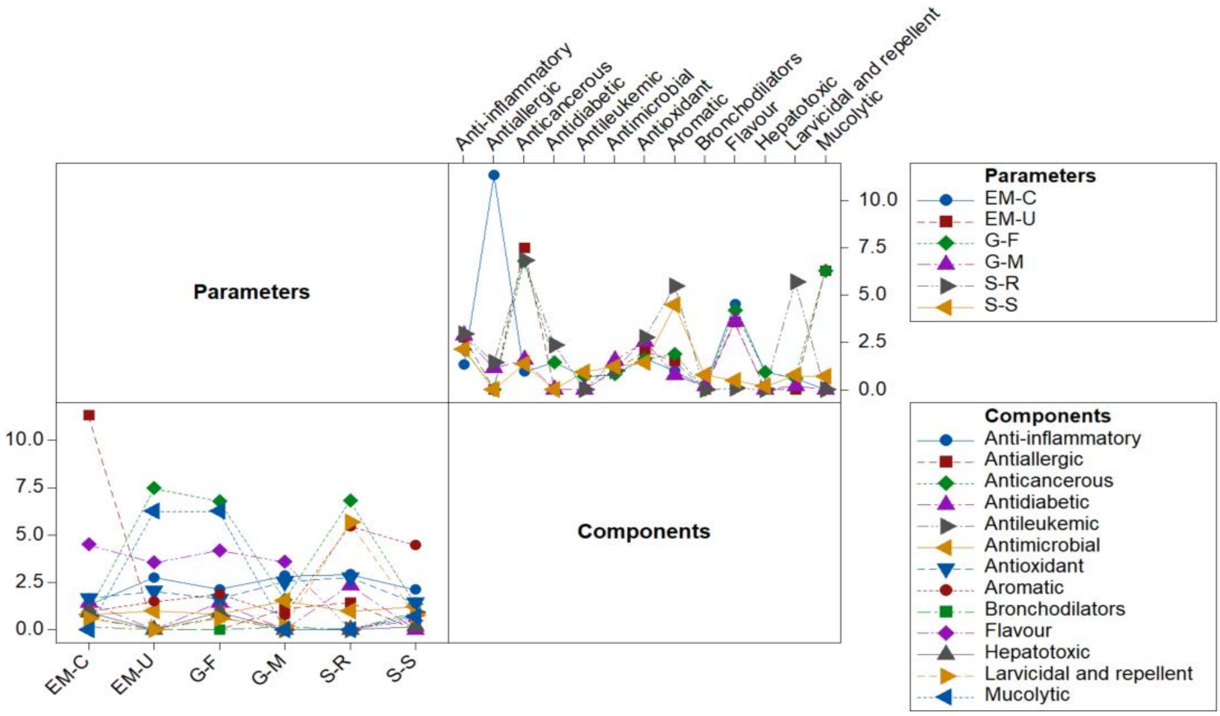


Fig.18 GC-MS analysis (a) ANOVA interaction plot based on bioactivities of components detected in the leaf's samples

Table 2 shows the components detected in the sea buckthorn leaves samples. The components were categorised based on their dominant activity as reported in the literature. Presence of components with antioxidant, antimicrobial, anti-inflammatory, anticancer, and other activities were found in the leave samples. Major flavour and aroma components like cetene, syringol, benzaldehyde, citronellol, and santalol detected in the extracts. Compounds like beta-linalool, curcumene, hydroxy methyl furfural, octadecanone, 1-momolinolein, isozonarol, beta-ionol, levomenthol, alpha-terpineol, citral, santalol, isocitronellol, amylcinnamic aldehyde, benzyl salicylate, geraniol, tonalid, gamma-sitosterol, octenol, toluene, propyl piperidine and ambrox were not detected in the male leaves of both the species (Table 2). The activity of these compounds studied through review of literature revealed that mostly aroma and flavour compounds are not detected in the male leaves unlike in female leaves. Linalool and geraniol, the major aroma causing components, were found in female leaves and not in male sea buckthorn leaves. An analysis of the aroma extracts of green mate leaves tea performed using GC-MS detected 144 volatile compounds. It is reported that the major aroma causing components in the green mate was linalool and 2-butoxyethanol (Polidoro et al., 2016). Furthermore, Kraujalyte et al.(Kraujalyte et al., 2016) studied the aroma components of black tea and found out that linalool and its oxidized derivatives are majorly responsible for the aroma of black tea. Also, in an earlier study some terpene alcohols were reported in *C. sinensis* such as geraniol, nerolidol, linalool, nerol, and alpha-terpineol (Kawakami & Yamanishi, 1983). A tea-infusion made from lemon grass was analysed using GC-MS. The outcomes of the study revealed that the major phytocomponents detected in the infused tea were citral, linalool, geranial, β pinene, myrcene and citronellol (Lasekan & Lasekan, 2012).

Some components like 4-hydroxy anisole, fucosterol, capric acid, pentadecanoic acid, octadecene, gamma-sitostenon, eugenol, methyl jasmonate, acetosyirngone, resorcinol, octadecane, hexadecanol, methyl commate, solanesol, nonanoic acid, syringol, pentaethylene glycol, benzaldehyde, megastigmatrienone, ursolic aldehyde, govanine, heptacosalnol, hexadecenoic acid, and acetovanillone were not found in female leaves but detected in the male leaves. The reported activity of these compounds reveals them mostly antimicrobial. Govanine, an anticancer component was found in male leaves but absent in the female leaves of both the species. Male leaves from *H. rhamnoides* species were established significantly ($p<0.05$) as compared to *H. salicipholia* species in components like flavonoids, tannins and rich aroma-flavour producing components while male leaves were found to have comparable antioxidant activity along with antibacterial and antihyperglycemic components. Consequently, it could be inferred that the gender-related bio-chemical diversity within a species is of considerable importance depending upon its uses. Based on the observations, suitable raw material could be selected for the specific nutraceutical processing as shown in Fig.18, through an ANOVA interaction graph prepared using Minitab software.

Table 2 GC-MS proofing of sea buckthorn leave extracts

Activity	Compound	Gender		Species		Extraction method	
		Male leaves	Female leaves	*H. salicipholia*	*H. rhamnoides*	CP	UAE
		Relative abundance (g/hg)					
Antioxidants	Vitamin E	11.87±6.18	9.57±6.71	9.39±6.41	12.10±6.52	8.52±7.79	12.09±5.07
	Neophytadiene	0.95±0.32	0.80±0.17	0.96±0.16	0.89±0.27	0.77±0.2	0.99±0.26
	4-Hydroxy anisole	0.92±0.00	ND	0.92±0.00	ND	0.92±0.00	ND
	Catechol	0.43±0.00*	0.05±0.00*	0.43±0.00**	0.05±0.00**	0.43±0.00	ND
	Pentadecanoic acid	0.47±0.00	ND	0.47±0.00	ND	0.47±0.00	ND
	1- Octadecene	0.27±0.00	ND	0.27±0.00	ND	0.27±0.00	ND
	gamma. - Sitostenon	0.14±0.00	ND	0.14±0.00	ND	0.14±0.00	ND
	Hydroxy methyl furfural	ND	0.34±0.00	0.34±0.00	ND	0.34±0.00	ND
	Curcumene	ND	0.22±0.00	0.22±0.00	ND	0.22±0.00	ND
	Glycine	5.57±0.29*	2.56±0.73*	ND	4.06±1.79	4.06±1.79	ND
	Methyl palmitate	0.66±0.00	0.65±0.00	ND	0.66±0.00	0.66±0.00	ND
	Alpha-amyrin	4.11±2.82	1.21±0.26	3.20±2.55	2.79±3.11	0.99±0.05***	4.25±2.57***
	Heptadecane	0.77±0.00*	1.38±0.00*	ND	1.08±0.43	ND	1.08±0.43
	Butylated Hydroxytoluene	1.63±0.00*	1.29±0.00*	1.46±0.24	ND	ND	1.46±0.24
	1-Tetracosanol	5.35±1.77*	1.25±1.00*	4.28±3.28	2.32±2.51	ND	3.30±2.64
	beta-Amyrin	2.43±0.68*	1.24±0.00*	1.36±0.12**	2.43±0.00**	ND	1.71±0.63
	Fucosterol	0.45±0.00	ND	0.45±0.00	ND	0.45±0.00	ND
Antimicrobial	Benzoic acid	0.52±0.23	0.5±0.43	0.30±0.16**	0.83±0.21**	0.48±0.44	0.55±0.19
	Octadecanoic acid	0.77±0.00	0.89±0.00	0.83±0.08	ND	0.83±0.08	ND
	Linolenic acid	1.36±1.41	0.36±0.22	0.86±1.01	ND	1.44±1.31	0.28±0.11
	1-Nonadecene	0.84±0.00*	0.70±0.00*	0.77±0.09	ND	0.77±0.09	ND
	Capric acid	0.15±0.00	ND	0.15±0.00	ND	0.15±0.00	ND
	1,3,4-Eugenol	0.14±0.00	ND	0.14±0.00	ND	0.14±0.00	ND
	Methyl jasmonate	0.19±0.00	ND	0.19±0.00	ND	0.19±0.00	ND
	8-Pentadecanone	0.12±0.00	ND	0.12±0.00	ND	0.12±0.00	ND
	Acetosyringone	0.13±0.00	ND	0.13±0.00	ND	0.13±0.00	ND
	Phytyl tetra decanoate	1.47±0.00*	0.94±0.00*	1.21±0.37	ND	1.47±0.00***	0.94±0.00***
	Octadecanone	ND	0.83±0.00	0.83±0.00	ND	0.83±0.00	ND
	Resorcinol	0.21±0.00	ND	ND	0.21±0.00	0.21±0.00	ND
	Gingerenone A	1.25±0.00*	1.17±0.00*	ND	1.21±0.06	1.21±0.06	ND
	Beta-sitosterol	6.18±3.61	2.72±2.21	6.43±1.76**	3.46±3.61**	2.07±0.00	5.64±3.50
	1-Monolinolein	ND	1.50±0.00	ND	1.50±0.00	1.50±0.00	ND
	Tetradecane	1.00±0.00*	0.48±0.00*	1.00±0.00**	0.48±0.00**	ND	0.74±0.37

	4-Phenyl-2-butanol	0.29±0.00	ND	0.29±0.00	ND	ND	0.29±0.00
	Heneicosane	0.54±0.00	0.50±0.00	0.52±0.03	ND	ND	0.52±0.03
	Dibutyl Phthalate	2.27±1.03*	1.71±0.00*	1.63±0.12**	2.99±0.00**	ND	2.08±0.79
	Isomenthol	4.27±2.50*	1.14±1.22*	2.25±0.35	3.16±4.07	ND	2.70±2.41
	Tetratriacontyl heptafluorobutyrate	1.50±0.00*	1.17±0.00*	1.34±0.23	ND	ND	1.34±0.23
	Pyrogallol	5.74±0.00*	0.43±0.00*	3.09±3.75	ND	3.09±3.75	ND
	Octadecane	0.72±0.00	ND	ND	0.72±0.00	ND	0.72±0.00
	Hexadecanol	0.54±0.00	ND	ND	0.54±0.00	ND	0.54±0.00
Anti-inflammatory	Alpha-linolenic acid	8.89±0.82*	3.6±3.71*	7.85±2.29	4.65±5.18	ND	6.25±3.76
	Beta-linalool	ND	0.41±0.00	0.41±0.00	ND	0.41±0.00	ND
	Phytol	0.87±0.24*	1.67±0.00*	ND	1.14±0.49	1.19±0.69	1.04±0.00
	Glycine	5.77±0.00*	2.04±0.00*	ND	4.06±1.80	4.06±1.80	ND
	Palmitic acid	4.55±2.95	1.78±1.67	4.32±3.02	2.52±2.82	1.91±1.53	4.45±3.35
	Methyl Commate	0.80±0.00	ND	0.80±0.00	ND	ND	0.80±0.00
	Iso zonarol	ND	1.53±0.00	ND	1.53±0.00	ND	1.53±0.00
	Arachidonic acid	ND	3.00±0.00	ND	3.00±0.00	ND	3.00±0.00
	Solanesol	0.33±0.00	ND	0.33±0.00	ND	0.33±0.00	ND
Flavour Components	1-Tetradecene	0.10±0.00*	0.08±0.00*	0.09±0.01	ND	0.09±0.01	ND
	Cetene	1.03±0.00*	0.08±0.00*	0.56±0.67	ND	0.56±0.67	ND
	1-Nonadecene	0.84±0.00*	0.70±0.00*	0.77±0.09	ND	0.77±0.09	ND
	Nonanoic acid	0.31±0.00	ND	0.31±0.00	ND	0.31±0.00	ND
	2-Methyl-4-vinylphenol	1.51±0.00	ND	0.51±0.00	ND	1.51±0.00	ND
	Syringol	0.08±0.00	ND	0.08±0.00	ND	0.08±0.00	ND
	Pentaethylene glycol	0.07±0.00	ND	0.07±0.00	ND	0.07±0.00	ND
	Benzaldehyde	0.42±0.00	ND	0.42±0.00	ND	0.42±0.00	ND
	Beta ionol	ND	0.46±0.00	ND	0.46±0.00	ND	0.46±0.00
	Mome Inositol	12.90±15.99*	37.66±3.91*	19.54±21.70	24.97±19.44	35.89±6.41***	14.07±18.04***
Aromatic components	Citronellol	6.85±0.00*	1.76±0.00*	4.31±3.59	ND	4.31±3.59	ND
	Megastigmatrienone	0.12±0.00	ND	0.12±0.00	ND	0.12±0.00	ND
	Levomenthol	ND	0.61±0.00	0.61±0.00	ND	0.61±0.00	ND
	Alpha-Terpineol	ND	0.63±0.00	0.63±0.00	ND	0.63±0.00	ND
	Citral	ND	0.26±0.00	0.26±0.00	ND	0.26±0.00	ND
	Santalol	ND	1.21±0.00	1.21±0.00	ND	1.21±0.00	ND
	Isocitronellol	ND	9.14±0.00	ND	9.14±0.00	ND	9.14±0.00
	Amyl cinnamic aldehyde	ND	1.83±0.00	ND	1.83±0.00	ND	1.83±0.00

	Benzyl salicylate	ND	3.16±0.00	ND	3.16±0.00	ND	3.16±0.00
	Ursolic aldehyde	0.68±0.05	ND	0.64±0.00	0.71±0.00	ND	0.68±0.05
Anticancer	Coumaran	0.49±0.00*	0.26±0.00*	0.36±0.16	ND	0.36±0.16	ND
	Govanine	0.28±0.00	ND	0.28±0.00	ND	0.28±0.00	ND
	Geraniol	ND	13.15±17.49	0.78±0.00**	25.51±0.00**	0.78±0.00***	25.51±0.00***
	Tetracontane	1.48±0.00*	1.23±0.00*	1.36±0.18	ND	ND	1.36±0.18
	Tonalid	ND	16.36±0.00	ND	16.36±0.00	ND	16.36±0.00
	Squalene	4.12±2.87	2.41±1.37	5.27±1.68	2.27±1.97	1.67±1.08	4.07±2.43
	1-Heptacosanol	2.34±0.00	ND	ND	2.34±0.00	ND	2.34±0.00
Other							
Antidiabetic	Gamma-sitosterol	ND	1.43±0.00	ND	1.43±0.00	1.43±0.00	ND
Antiallergic	Hexadecanoic acid	1.11±0.00*	11.30±0.00*	1.11±0.00*	11.30±0.00	6.21±7.21	ND
Larvicidal and repellent activity	Tetradecanoic acid	0.35±0.00	0.38±0.00	0.37±0.02	ND	0.37±0.02	ND
	Octenol	ND	0.76±0.00	0.76±0.00	ND	0.76±0.00	ND
Bronchodilators	Acetovanillone	0.15±0.00	ND	0.15±0.00	ND	0.15±0.00	ND
Hepatotoxic activity	Toluene	ND	0.91±0.00	0.91±0.00	ND	0.91±0.00	ND
Antileukemic	Propyl piperidine	ND	0.67±0.00	0.67±0.00	ND	0.67±0.00	ND
Mucolytic	Ambrox	ND	6.24±0.00	ND	6.24±0.00	ND	6.24±0.00

ND: Not Detected;

Mean (n=3) values with different superscripts ($^{*, **, ***}$) within columns are significantly different ($p < 0.05$)

Principal component analysis

For better understanding of the relationships between constituents identified using GC-MS (classified based on their bioactivities) and the factors (gender, species, and extraction methods) considered in this study, PCA based on correlation coefficients was applied to the experimental results (Fig.19). The PCA explained 84.50% of the analytical variables in two axes, PC1 and PC2. PC1 accounted for 56.33% of total variance and was prominently influenced by antimicrobial and flavour compounds. Similarly, PC2 accounted for 28.17% of the total variance and was highly influenced by antimicrobial and anti-inflammatory compounds. A significant difference could be established between the species, as compared to method of extraction and gender. The results from PCA supported the experimental results discussed in this study. Consequently, *Hippophae*, the dioecious species showed gender dimorphism in primary and secondary characteristics.

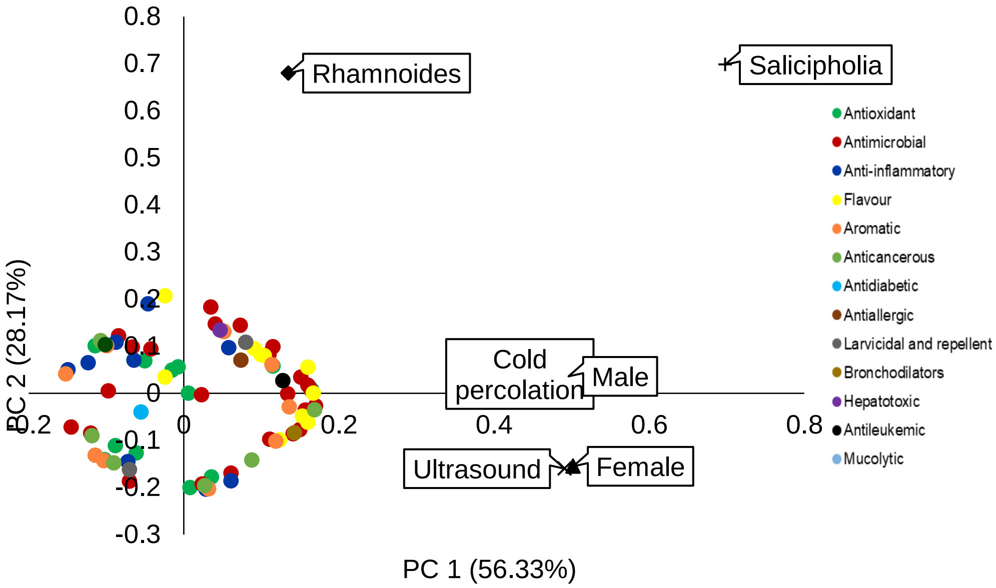


Fig.19 GC-MS analysis; PC1-PC2 loading plot based on principal component analysis

Conclusion

Male leaves were established significantly superior (*p<0.05*) in total phenols, flavonoids, and tannins while female leaves were found to have comparable antioxidant activity along with rich aroma-flavour producing components. *H. rhamnoides* species may be preferable for nutraceuticals processing, whereas for tea processing *H. salicifolia* may be recommended because of its rich flavor and aroma components. Also, the extraction methods had a great influence on the secondary metabolites and flavor extraction of the leave extracts. The extracts prepared by UAE resulted in better yield for phenolic and flavonoid components, but the volatile components were mostly destructed due to ultrasonic treatment. This comprehensive study gives an interpretation on the dioecism in *Hippophae* sp. and enhances the understanding towards the adaptation of male and female sea buckthorn plants for tea and nutraceutical processing. Based on current study we can establish the preferred gender and species for the processing of nutraceuticals and nutritionally rich sea buckthorn leaves tea.

2.2.2 Development of a Functional Tea from Sea buckthorn (*Hippophae* sp.) leaves through fermentation with kombucha consortium

Abstract: The nutrient-dense Sea buckthorn (*Hippophae sp*.) leaves contain high variety of phytochemicals with strong antioxidant and antibacterial characteristics, making an attractive raw-material for kombucha fermentation. The kombucha drink is primarily prepared from black tea (1–2%), sucrose (5–10%) and pellicle called SCOBY (2.5–5.0%) and incubated at 25–37 °C for 7–14 days. A kombucha consortium of bacteria and yeast was employed to develop a unique functional beverage by fermenting sea buckthorn leaves for 14 days at 25 °C. Kombucha's physicochemical, antioxidant, nutraceutical, and flavour components were investigated and compared to sea buckthorn leaf infusion. Analytical techniques such as HPLC, ICP-MS, GC-MS, and spectrophotometric approaches utilised for characterisation. Tea kombucha exhibited higher amounts of total phenols (40.83 mg GAE/g), flavonoid (70.02 mg CE/g) and improved DPPH scavenging ability (IC50 value- 0.048 µg/mL). Also, enhanced content of ascorbic acid (10.33 to 164.73 mg/100 mL), acetic acid (50.36 to 181.54 mg/100 mL) and citric acid (684.14 to 1602.52 mg/100 mL) with high titratable acidity, and lower pH value was observed. Consequently, the sourness of high organic acid content influenced sensory preference. Hence, Sea buckthorn leaves offer a lot of potential as a raw material for fermenting non-alcoholic functional beverages for its flavour enhancement and valorisation.

Introduction

Sea buckthorn leaves (SBL) have recently attracted scientific attention. Anti-inflammatory, antimicrobial, antioxidant, antitumor, immunomodulatory, adaptogenic, radioprotective, and tissue regeneration are some of the pharmacological activities that have been discovered in the SBL. Furthermore, it has been shown that after oral administration of SBL, there is no cytotoxicity or deleterious effect (Chawla et al., 2007, Ganju et al., 2005, Geetha et al., 2005; Gupta et al., 2005; Gupta et al., 2008; Saggu et al., 2007, Upadhyay et al., 2009). Despite this, the unpleasant flavour of SBL is unappealing, being bland and low in sourness. Sea buckthorn leaves are rich in flavonol glycosides (FGs) (Pop et al., 2013). FGs are of interest because of their health benefits, such as significant antioxidant activity and anti-inflammatory activity along with low sensory thresholds for astringent sensations (Xiao et al. 2016; Scharbert & Hofmann, 2005). In addition to FGs, notable contents of ellagitannins (ETs) are also found in SB leaves, and the total content of ETs reaches more than 100 mg/g DW (Suvanto et al., 2018). ETs also possess a wide range of biological activities, such as antioxidative functions, anti-inflammatory activities, and prebiotic effects (Landete, 2011). Factors such as cultivar, harvesting time, and leaf position in the plant as well as processing technologies are known to affect significantly the content and composition of phenolic compounds (Bakkalbaşi et al., 2008, Mäkilä et al., 2017, Yang et al., 2015). In recent years, the interest and consumption of herbal infusions (commonly called teas or tisanes) from a great diversity of edible plants has increased considerably due to their potential health benefits and attractive flavor and taste. Similarly, there has been an increasing interest in utilization of SB leaves and converting them into tea-type beverage products. However, most studies have focused on the fresh SBL, and the emphases have been on organic solvent extractions aiming to identify new compounds or maximize the yields of phenolics (Pop et al., 2013, Suvanto et al., 2018, Tian et al., 2017). In order to increase the consumption and application in the food industry, the solution might be to enhance the bland taste of sea buckthorn leaves through kombucha fermentation.

Kombucha is one of the latest "healthy trends" that has emerged among consumers all over the world. It is a non-alcoholic beverage that contains plenty of vitamins, amino acids, and other nutrients that have been linked to a variety of health benefits. The global kombucha market was worth USD 1.84 billion in 2019 and is expected to grow to USD 10.45 billion by 2027, with a CAGR of 23.2 percent throughout that time. Kombucha is a sweetened, fermented tea beverage that is typically brewed using black or green tea. Promising results in *in vitro* and *in vivo* studies have prompted research groups from around the world to search for alternative raw materials for tea fungus fermentation. Fermentation occurs rapidly after adding tea fungus i.e., symbiotic culture of bacteria and yeast (SCOBY) to the sweetened tea. The relationship between yeast and bacteria in tea fungus consortia is complex. Substances secreted extracellularly by microbes may stimulate or inhibit the growth of accompanying microflora. To comprehend this phenomenon of coexistence and intimate dependency of diverse microorganisms in one ecological system, extensive analysis of their interactions is required (Villarreal-Soto et al. 2018).

From our best knowledge, there is no report about the use of sea buckthorn leaves and kombucha consortium in combination as of yet. Also, the behaviour of polyphenolic profile and sugar and organic acids in the kombucha analogues from sea buckthorn leaves is unknown. The main objective of the present work is to characterize fermentation process of sea buckthorn leaves infusions by kombucha and studying possible chemical changes in the present polyphenol compounds.

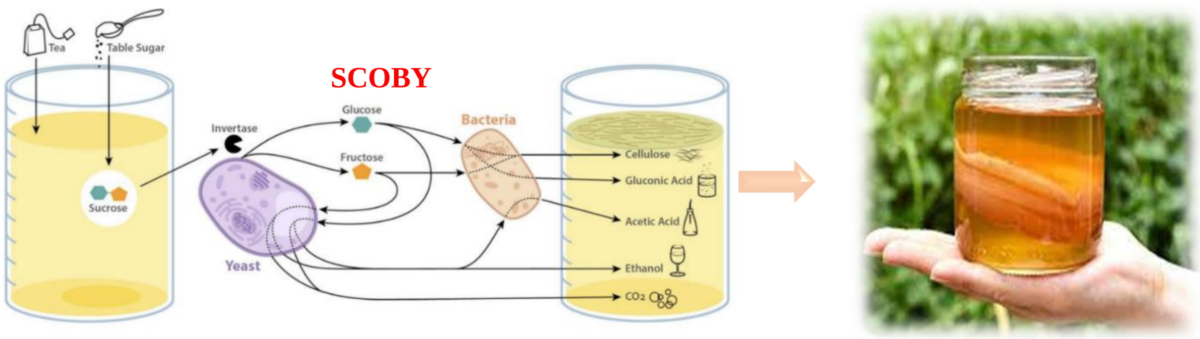


Fig.20 Mechanism of kombucha fermentation

In this study, we investigated the physicochemical properties (like pH values, total acidity, changes of microbial population, tea polyphenols compounds and catechin categories) and analyse systematically biochemical dynamics of kombucha fermented for different periods of time. Such an exploration may eventually allow the correlation of biochemical dynamics to the fermentation process, thus, improving the quality of sea buckthorn leaves tea.

Material and Methods

Raw Material

Sea buckthorn leaves of two species *Hippophae salicifolia* and *Hippophae rhamnoides* were supplied by G.B. Pant National Institute of Himalayan Environment & Sustainable Development, Kullu, Himanchal Pradesh (HP), India. The leaves were harvested from Lahaul- Spiti district of HP, location (31°44' 57" and 32°59'57"N Latitudes and 76°46'29" and 78°41'34" E Longitudes). The leaves collected were immediately transported to IIT Delhi and dried using a microwave dryer (Twin Engineers, Vadodara, India). The dried leaves were then stored at -18°C in dark bottles till further experimentations.

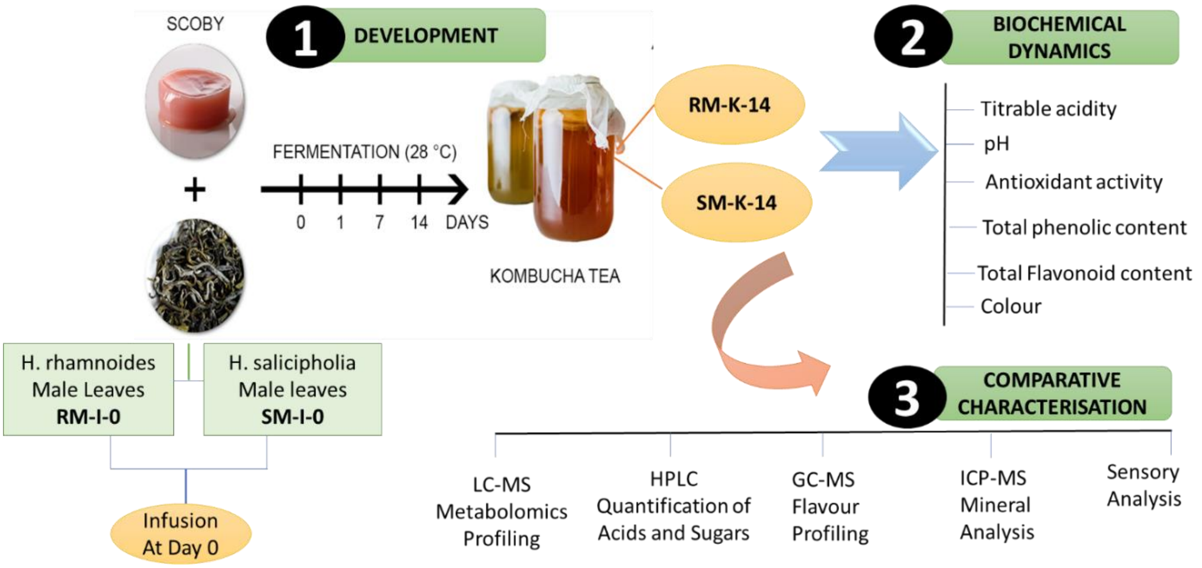


Fig. 21 Methodology used for the novel tea processing

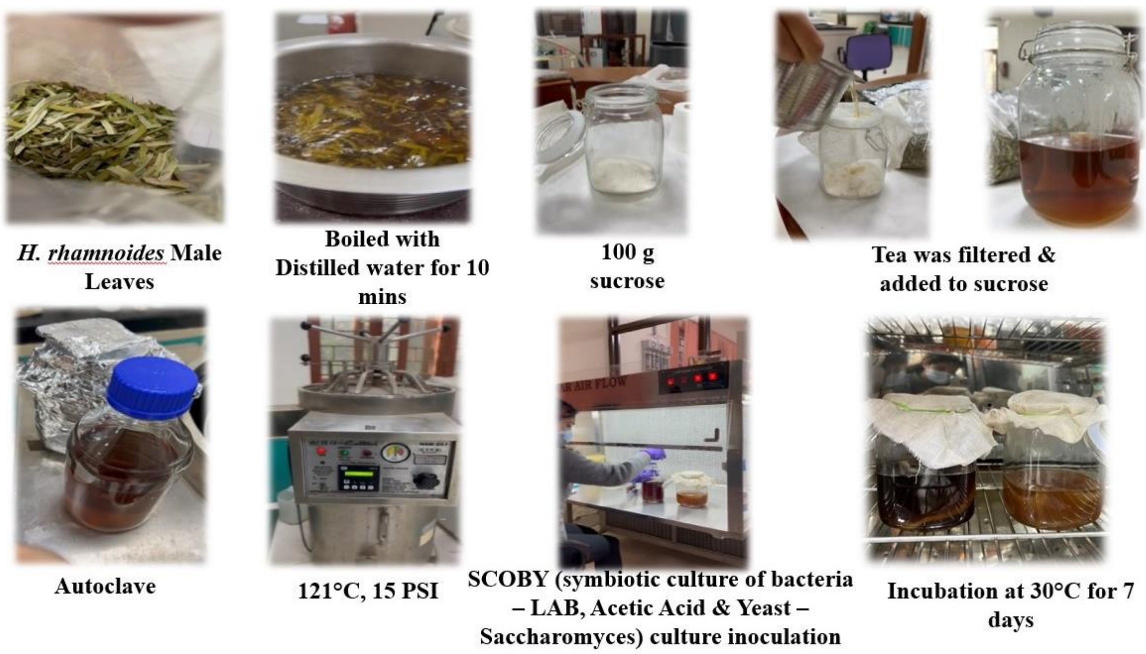


Fig. 22 Development of novel kombucha tea through fermentation

Infusion preparation and Kombucha Fermentation

Decoctions were obtained by putting 2% (*w*/*v*) of dry sea buckthorn leaves into water at 80°C for 10 min. Afterwards, obtained infusion was filtered using muslin cloth. The starting kombucha consortium was maintained in sweetened (sucrose 10% *w*/*v*) black tea at 30°C. Freshly cultured kombucha was used for further subcultures or fresher fermentation batches. Fermentation conditions were time 14 day, with 10% (*w*/*v*) of sucrose, inoculum (2.5% *w*/*v*, Kombucha consortium) and incubation temperature of 30°C. Total volume of fermented sea buckthorn leaves were 0.5 L.

Bioactive components

Sample (0.5 mL) were mixed with Folin-Ciocalteu reagent (0.5 mL), and sodium carbonate (7.5%, 1.5 mL) was added to the reaction mixture. After incubation at room temperature (around 25 ± 2 ◦C) for 30 min, the optical absorbance was measured at 765 nm using a UV- spectrophotometer. Gallic acid was used as a standard, and results are expressed as mg gallic acid equivalents (mg GAE) per 100 mL of the kombucha samples.

Total flavonoid contents of tea and sea buckthorn kombucha were determined according to the method proposed by Aadil et al. (2013). Samples (0.5 mL) and 5% sodium nitrite solution (70 µL) were mixed for 5 min at room temperature, then, 150 µL 10% aluminium chloride was added. After incubating for 5 min at room temperature, 0.5 mL of 1 M sodium hydroxide and 1.3 mL of distilled water were added, and absorbance was measured at 415 nm using a UV spectrophotometer. Quercetin was used as a standard, and results are expressed as mg quercetin equivalents (mg QE) per 100 mL of kombucha.

Antioxidant activities

Free radical scavenging property were analysed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. 1.4 mM solution of DPPH was prepared in methanol. A micro titre plate was used and 10 µL of sample added to 250 µL of DPPH solution. It was then incubated for 15 mins in dark, and absorbance was recorded spectrophotometrically at 517 nm. DPPH radical scavenging activity confirmed by decreasing the absorbance.

Ferric reducing antioxidant power (FRAP) was analysed based on the method described by Kumar et al., (2010). 0.2 mL of the extract sample was added with 3.8 mL of FRAP reagent and mixed properly. The solution was kept in the dark for 10 min at room temperature. Further, absorbance was measured at the wavelength of 593 nm. The results were expressed as mg of butylated hydroxy toluene (BHT) equivalent per 100 mL of Kombucha.

Organic acid content

Organic acid content was determined in accordance with the method proposed by Jayabalan, Marimuthu, and Swaminathan (2007) with minor modifications. Samples from days 0 and 14 of tea and sea buckthorn kombucha were collected and centrifuged at 6767 × g within 10 min at 8 °C. Then, the extracts were filtered through nylon syringe filters (0.45 µm). After preparing the samples, assays were performed on a High-performance liquid chromatography (HPLC) instrument (Agilent 1200) equipped with a Sep-Pak C1 cartridge and SUPELCOGEL'MC- 610H column (300×7.8 mm, Supelco, Bellefonte, PA, USA). The separated organic acids were detected at UV 210 nm on a VWD-300 variable wavelength detector.

Sugar content

The samples were centrifuged, and the supernatant was analysed for sugars (glucose, fructose, sucrose) using a HPLC (Agilent 1200), equipped with a refractive index detector (RID) and ion exchange Hi-Plex H-column (300x7.7mm). 1µL of standard solutions of sucrose, fructose, glucose (concentration 1mg/mL) and aliquot of sample (filtered through 0.22 µm PTFE filter) was injected with an autoinjector (Dixit et al., 2021). 10 µL of volume was injected to obtain the retention time for each compound. The column and detector temperatures were kept constant at 70°C and 50°C respectively. The flow rate applied was 0.5 mL min^{-1} with a run time of 20 min. The phase was 4.0mML^{-1} H_2SO_4 in ultrapure water (Coelho et al., 2018).

GC MS based Flavour Profiling

The Kombucha and control samples were analysed for the alcoholic profile using GC-MS method. A GC-MS device (Perkin Elmer Clarus, 680 model) conditions were set up as: capillary SUPELCOWAX™ 10 columns (30m x 0.25mm id and 0.25µm film thickness), temperature programming maintained at 50°C for 3min and the heated at a rate of 3°C min^{-1} to 150°C followed by a heating rate of 20°C min^{-1} to 240°Cand kept at this temperature for 20 min. The mass scan range was m/z 50-300 and the electric potential was set to EI 70 eV (Baldermann et al., 2014).

ICP MS based Mineral Profiling

Inorganic minerals were analysed using an Inductive coupled Plasma mass spectrometer (ICP-MS, 7900, Agilent). The parameters used for obtaining the data are as follows: auxiliary gas- flow, nebulizer gas-flow, plasma gas-flow, and helium gas flow in reaction cell was set as 1 L/min, 1 L/min, 15 L/min and 0.2 mL/min respectively. The analysis was carried out using reflected and forward power of 45 W and 1500 W respectively. Furthermore, the vacuum was set at 6×10-5. An electron multiplier (EM) detector was used for the analysis.

LC-MS based non-targeted metabolomic profiling

The metabolomics analysis of tea samples was conducted with the help of an instrument manufactured by Thermo Scientific, USA (Model name: Q Exactive Orbitrap) using an Acquity I-class ultra-high-performance liquid chromatography (UPLC) system coupled to a quadrupole time-of-flight mass spectrometer (Waters, USA). The chromatographic separation of chemical compounds was carried out using a BEH C18 column (100 mm×2.1 mm, 1.7 µm; Waters, USA). The mobile phase consisted of mobile phase A [H2Ocontaining 0.1% (v/v) formic acid] and mobile phase B [acetonitrile containing 0.1% (*v*/*v*) formic acid], and the gradient elution was as follows: 0−3 min, 5% B; 3−10 min, 20% B; 10−15 min, 100% B; 15−20 min, 95% B. Samples (1μL) were eluted at 0.4 mL/min, and thecolumn oven was kept at 45°C. The detection wavelengths were 280,350, and 520 nm. The mass spectrometer was operated in the negative ionization mode over a full scan range of 50−1000m/z with the following settings: gas temperature, 450°C; gasflow, 15 L/min;source temperature, 115°C; capillary voltage, 2 kV; cone voltage,40 V; and collision energy, 6 eV. The raw data acquired from LC-MS was initially processed by Waters UNIFI qualitative analysis software (Waters, U.S.A.) to generate a peak table that included information on retention time (Rt), mass-to-charge ratio (m/z), and MS intensity of each feature. The Rt tolerance and m/z tolerance for the peak alignment were set to 0.01 min and 0.1 mDa, respectively. Tandem mass spectrometry (MS/MS) of these features was manually matched with MS2 fragments against online database Dictionary of Natural Products (DNP, http://dnp.chemnetbase.com/). The screened features were further filtered by a specific tea metabolome database enrolling all of the phytochemicals in tea previously reported in the literature.

Colour analysis

The colour values (L, a and b) of fermented beverage were recorded for 5days at specified time intervals using a colorimeter (Konica Minolta, Inc., Japan Model CR-20 (8°: de; ø8 mm; D65; Standard deviation within ΔE*ab 0.1) described by (Maskan, 2001). In the colorimeter $L*$ value denotes the colour brightness ranging from 0 (darkness) to 100 (whiteness); $a*$ value represents the greenness or redness of sample and $b*$ indicates the yellowness and blueness of sample (Teichert et al., 2020). The values of $L*$, $a*$ and $b*$ were converted into $\Delta E*$ (total colour difference) and ΔC (chroma) using the equations (1) and (2) given below (Costa et al., 2013):

$$\Delta E = \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2} \quad (1)$$

$$\Delta C = \sqrt{(a *)^2 + (b *)^2} \quad (2)$$

Results and Discussion

This study was carried out to explore the suitability of sea buckthorn leaves as raw material for Kombucha fermentation. Previous studies have proven that the major organic acids produced during fermentation are acetic acid, gluconic acid and glucuronic acid (Jayabalan et al. 2014). In fact, in the kombucha system, yeasts convert glucose and fructose into ethanol via glycolysis (Jayabalan et al. 2014). Acetic acid bacteria use glucose to produce gluconic acid and ethanol to produce acetic acid, with the latter being the predominant organic acid produced by acetic acid bacteria (Jayabalan et al. 2010). Glucuronic acid is considered to be one of the important components found in Kombucha beverage due to its detoxifying action through conjugation to the xenobiotics (Vīna et al. 2014). Jayabalan et al. (2007) established that maximum D-glucuronic acid concentration was obtained after 12 days of fermentation on sweetened black tea. Yavari et al. (2010, 2011) found that the highest level of glucuronic acid produced on cherry juice and grape juice was achieved after 14 days of fermentation and that grape juice was the best substrate for glucuronic acid production by kombucha culture.

Phenolic components

The health-promoting activities of Kombucha beverage are also attributed to their phenolic content and their ability to act as antioxidants. The observed changes in total phenolic content (Fig 23) during fermentation are comparable with those reported previously in investigations on the traditional Kombucha beverage of black tea (Chu and Chen 2006; Jayabalan et al. 2007). Total phenol content increased linearly during fermentation and explained this increase by both enzyme synthesis by the Kombucha consortium and by acid hydrolysis.

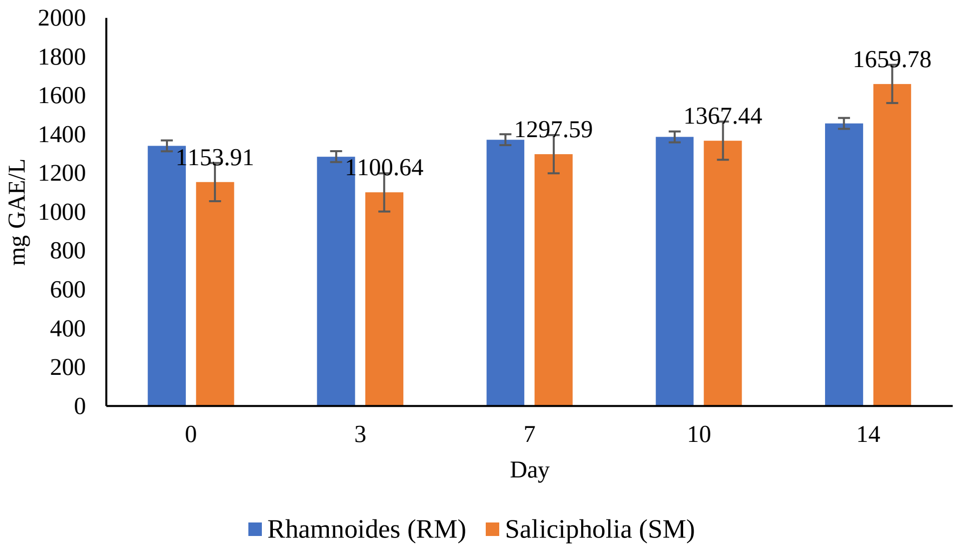


Fig. 23 Changes in total phenolic content during fermentation

Total flavonoid content of tea kombucha was like that of control sample at the starting point, then increased during fermentation (Fig. 24). The remarkable increase in total flavonoid contents during fermentation reveals the impact of kombucha consortium. The enhancement in flavonoid content is related to changes in acid levels in this fermentation environment, which can release the combined flavonoid compounds (Tu et al., 2019). They also reported that another reason for higher flavonoid abundance is due to microorganisms which can secrete enzymes such as β-glucosidase during fermentation, thereby releasing more flavonoid compounds.

Antioxidant activity

The health benefits of Kombucha are also attributed to its antioxidant activity. Antioxidants are known to prevent many disorders and metabolic diseases caused by free radicals (Jayabalan et al. 2014). In this study free radical scavenging activity (DPPH) was used to determine the antioxidant activity of the kombucha tea during fermentation. The results of the assay have been shown in Fig.25. Similar trends have been reported by many authors (Chu and Chen 2006; Jayabalan et al. 2008; Malbaša et al. 2011). Malbaša et al. (2011) and Ayed and Hamdi (2015) observed a significant increase in DPPH radical scavenging activity of tea and cactus pear juice during fermentation, with average values of 48.7 and 18.1% respectively. The extent of the antioxidant activity in the Kombucha beverage would appear to depend on many factors, such as fermentation time, type of substrate, and the normal microbiota of the Kombucha culture, which in turn determines the nature of the secondary metabolites which develop during the fermentation process (Jayabalan et al. 2014).

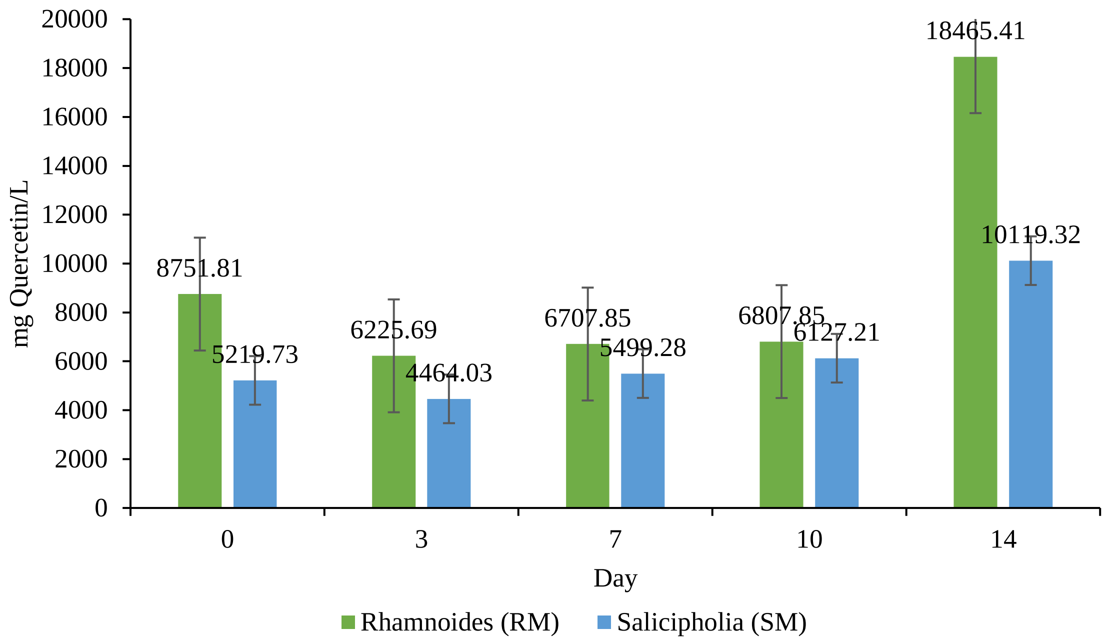


Fig. 24 Changes in total flavonoid content during fermentation

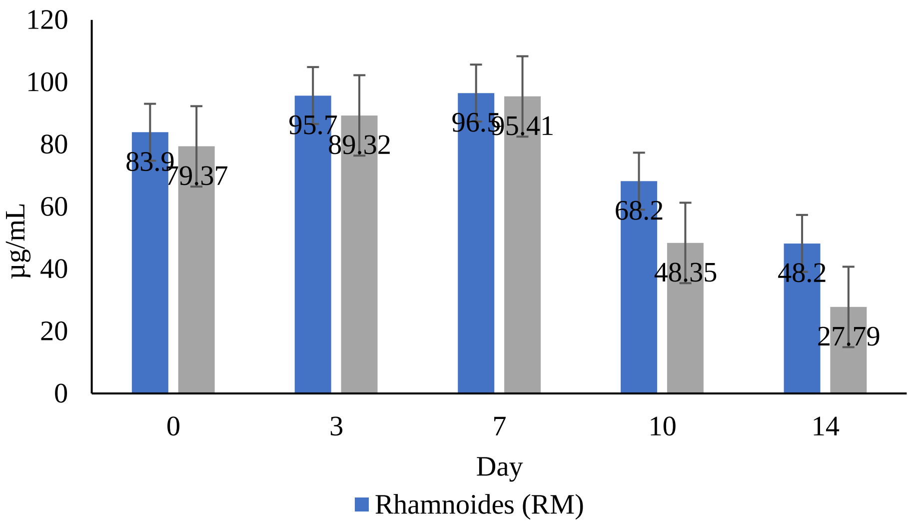


Fig. 25 Changes in the antioxidant activity during fermentation time

Colour Analysis

The results of colour analysis using colorimeter for kombucha tea during fermentation is represented in the Table 3. The fermented beverage with time was found to be imparting dark brown colour as compared to the non-fermented beverage. This colour difference between the fermented and non-fermented beverage is indicated by the lower values of *L** for fermented beverage. This is indicative that the fermented beverage showed more saturated yellow colour compared to the non-fermented juice (Costa et al., 2013). No substantial browning was observed during fermentation which is suggestive from the low negative values of *a**. The colour of the fermented drink showed a slight reduction in the yellow colour intensity indicated by the reduction in values of *b** and Chroma on fermentation. The change in the colour showed a significant visual difference during kombucha fermentation due to the substantial difference in Δ*E** values (Martelli, 2020).

Table 3 Colour analysis using Colour Reader CR-10 Plus

Sample	Fermentation time (day)	L*	a*	b*	Chrome (ΔC)	ΔE*
Rhamnoides Kombucha (RM)	0	31.2 ± 0.90	1.7 ± 0.26	7.6 ± 0.334	7.78781	
	3	31.9 ± 1.05	2.6 ± 0.01	9.0 ± 0.278	9.36803	1.80555
	7	32.3 ± 0.73	3.2 ± 0.12	9.4 ± 0.34	9.92975	2.58844
	10	32.1± 0.99	1.5± 0.89	6.8± 0.71	6.96348	1.22066
	14	30.4 ± 0.345	3.9 ± 0.15	8.0 ± 0.22	8.9	2.37487
Salicipholia Kombucha (SM)	0	27.2 ± 0.325	2.0 ± 0.049	4.5 ± 0.212	4.92443	
	3	27.9± 0.424	1.8 ± 0.06	4.0 ± 0.275	4.38634	0.88318
	7	30.4 ± 0.532	2.3± 0.88	9.0± 0.54	9.28924	5.52992
	10	33.8± 0.642	2.5± 0.136	5.9± 0.98	6.40781	6.76535
	14	29.1± 0.442	3.8± 0.08	5.7± 0.331	6.85055	2.87924

Comparative characterisation

Organic acids

Organic acids are vital components which affect sensory, chemical and microbiological stability of foodstuffs and beverages (Vitas et al.,2018). Organic acid content in sea buckthorn leaves infusion at day 0 (RM- I0 S& M- I0) and kombucha samples at day 14 (RM- K14 & SM-K14) were studied (Fig 26). Different types of organic acids (malic, acetic, succinic, gluconic, glutaric, citric, malonic, lactic acid etc) from the initial day (0 d) of fermentation, and the end of fermentation (14 d) were analysed. The dominant organic acid found in the kombucha samples was gluconic acid, succinic acid and acetic acid which is produced by acetic acid bacteria. High acetic acid content might have affected the sensory attributes of the final drink as evidenced by its lowest score in sourness compared to the control sample. Succinic acid, which gives the sour with a salty-bitter taste, evolved during fermentation due to yeast metabolism, and it is also a fermentation end product (Chidi, Bauer, & Rossouw, 2018). Higher succinic acid contents were achieved in sea buckthorn tea kombucha samples.

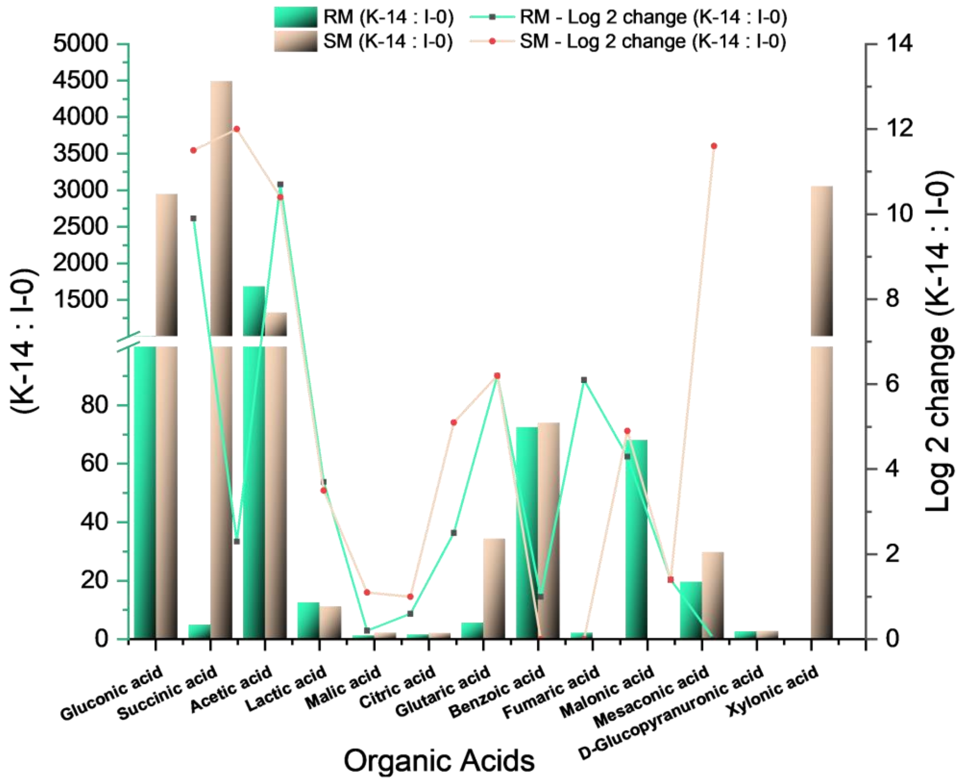


Fig. 26 Changes in organic acids during kombucha fermentation

Phenolic acids

Dynamics of phenolic acids during fermentation process is plotted in Fig.27. The increase in the concentration of catechins and gallic acid can be related to the hydrolysis of catechin gallate and catechin 5-O-gallate. The increase in catechin content during the production of kombucha tea might be related to an increase in the concentration of its precursors, eriodictyol and cinnamic acid, which are associated with the degradation of naringenin and p-coumaric acid, respectively. The reduction in the naringenin concentration might be associated with the degradation of nepetin and hesperidin–flavonoids found only on day 0–and p-coumaric acid. Some important phenolics, such as gallic acid, rosmarinic acid, cinnamic acid, 5-O-galloylquinic acid, and catechol, may have been formed from the degradation of p-coumaric acid during fermentation. Another consequence of the increase in cinnamic acid was the increase in the concentration of two other phenolic acids, 4-hydroxybenzoic acid and gentisic acid.

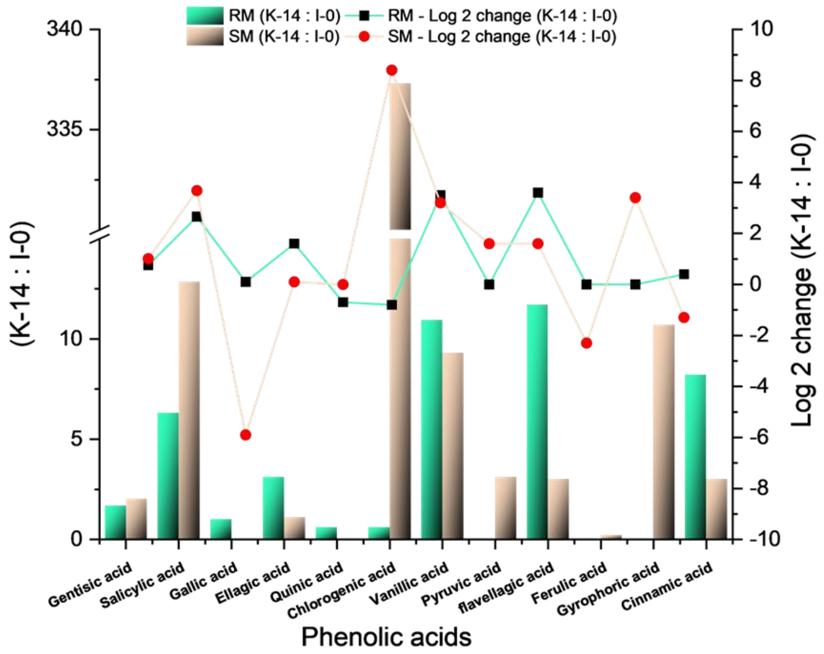


Fig.27 Changes in phenolic acids during kombucha fermentation

Polyphenols

Gluconobacter has been identified as the key bacterial strain which enhances the bioaccessibility of polyphenols and the antioxidant activity of the beverages (Dufresne and Farnworth 2000). However, we observed a slight decrease in some polyphenols in the study. This phenomenon can be explained by the polymerization of some of the phenolic compounds to molecules of higher molecular weight, leading to the detection of lower polyphenol content. This observed decrease in phenolic content is in agreement with the results reported by Chu and Chen (2006). The health-promoting activities of Kombucha beverage are also attributed to their polyphenolic content and their ability to act as antioxidants. The changes in the polyphenol components during fermentation is shown in Fig 28. Some polyphenols increased linearly during fermentation and explained this increase by both enzyme synthesis by the Kombucha consortium and by acid hydrolysis. The total content of polyphenols and monomeric anthocyanins was positively correlated to the antioxidant activity of the beverage before and after fermentation. A high polyphenol content is generally associated with high antioxidant activity (Sun et al. 2015). However, Dani et al. (2009) suggested that the antioxidant capacity of phenolics has a concentration saturation limit, and that above this limit the activity cannot increase further with phenolic concentration.

Sugars

Sugar content in all samples changed significantly by fermentation (Fig.29). At the end of the fermentation, sucrose decreased significantly in both the samples. Additionally, glucose was detected in the kombucha samples at 14th day. The depletion of sucrose could be associated with the glucose and fructose content at 14 d since sucrose could be hydrolysed into reducing sugars and produce ethanol. Subsequently, the Acetobacter bacteria strain prefer glucose to transform gluconic acid and ethanol into acetic acid (Neffe-Skocinska et al., 2017). This correlation between sucrose and reducing sugar content could explain the amount of organic acid produced.

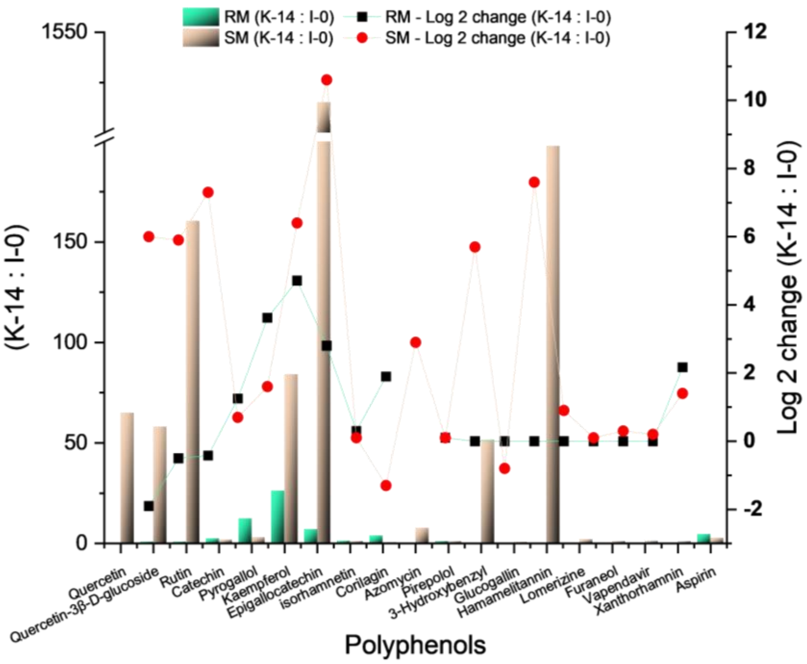


Fig. 28 Changes in polyphenols during kombucha fermentation

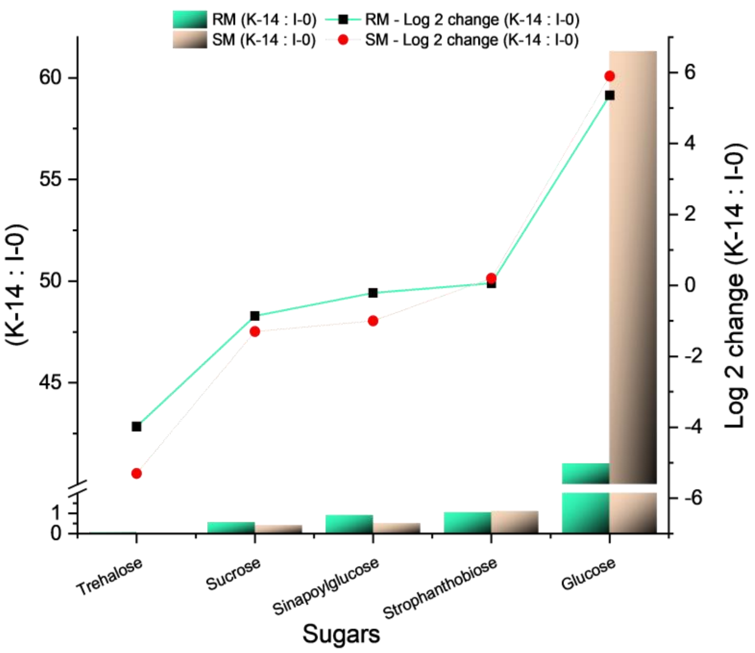


Fig. 29 Changes in sugars during kombucha fermentation

Vitamins

Untargeted metabolomic profiling of the control and fermented kombucha samples revealed that vitamins are stable during the processing. The dynamics of vitamins during the kombucha fermentation process is plotted in the Fig 30. The drastic increment in the folic acid content of *salicipholia* kombucha sample can be seen in the study. According to the results of the present study, in the presence of enzymes secreted by the kombucha microbial consortium, including invertase, polyphenolic compounds were decomposed and consequently the antioxidant activity enhanced; The results of the present study are consistent with the findings of other researchers in this field (Srihari and Satyanarayana., 2012; Jayabalan et al., 2008). High contents of group B vitamins, plus vitamin C minerals make the Kombucha drink an excellent remedy for improving the health of human beings.

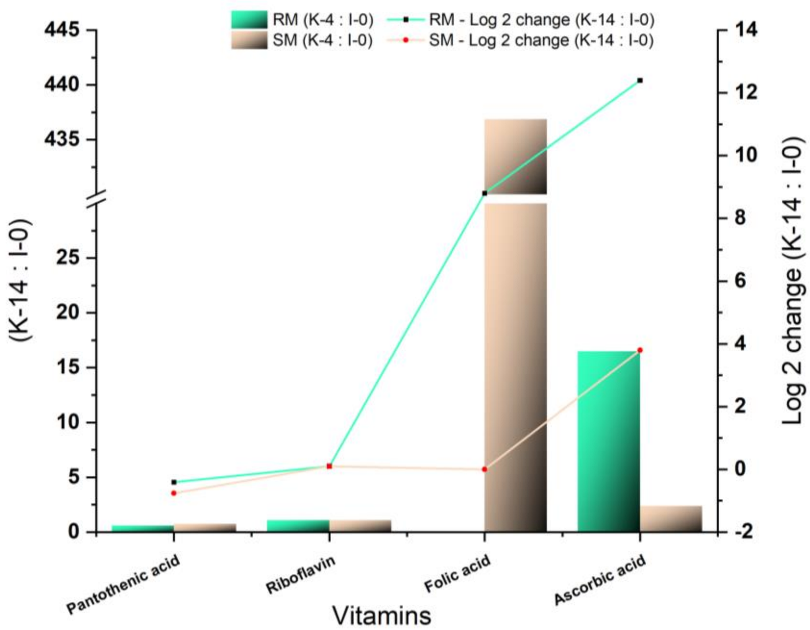


Fig.30 Changes in vitamins during kombucha fermentation

Minerals

The contents of minor and trace elements: magnesium, potassium, nickel, copper, zinc, lead, arsenic and cadmium in the Kombucha tea sample and control tea infusion are presented in Fig. 31. Mineral content of the essential elements (Cu, Mg, Ca, K, Ni and Zn) for normal physiological processes in the organism increased as a result of the Kombucha cultivation in the sea buckthorn tea infusion. The copper content did not increase in kombucha beverage. Cadmium was not found in the investigated samples. Decreased lead concentration in the drink proved the kombucha was able to detoxify beverages, possibly by its high glucuronic acid content.

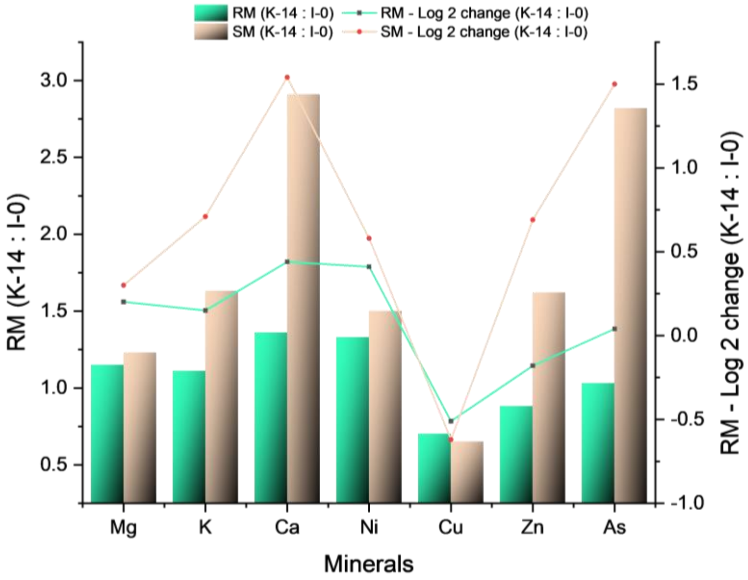


Fig.31 Changes in minerals during kombucha fermentation

Conclusion

Tea kombucha exhibited higher amounts of total phenols, flavonoid and improved DPPH scavenging ability. Also, enhanced content of ascorbic acid, acetic acid and citric acid with high titratable acidity, and lower pH value was observed. Consequently, the sourness of high organic acid content influenced sensory preference. Hence, Sea buckthorn leaves offer a lot of potential as a raw material for fermenting non-alcoholic functional beverages for its flavour enhancement and valorisation. Such an exploration may eventually allow the correlation of biochemical dynamics to the fermentation process, thus, improving quality of sea buckthorn leaves tea.

2.2.3 Development of a Low-Cost Quick Technology for Extraction of Sea Buckthorn Oil

Abstract: Effects of ultrasound-assisted extraction (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 (*Hippophae salicipholia*) seed 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 could achieve a maximum oil recovery of 6.87 g. $hg^{-1}$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 ratio10:1 (mL: g). The SBSO samples revealed high carotenoid content (237.04±3.86 mg per 100 g β-carotene equivalent), potent free radical scavenging ability (1.42±0.01 IC50 μg per mL), and oxidative stability (totox value = 8.04±0.02) upon the UAE treatment. GC-MS profiling of the oil sample extracted at optimum UAE conditions exhibited better retention of fatty acids.

Introduction

Sea buckthorn (*Hippophae salicipholia*) is an important medicinal plant gaining popularity in mass-scale cultivation in various parts of the globe because of its diverse functional and biological properties. This plant species is widely grown in the Indian Himalayan regions. According to a report published in the “Down to Earth” (December 2017), this species covers about 17,000 hectares across the Indian Himalayan Regions(Venkatesh, 2017). The sea buckthorn has even been coined as ‘God sent medicine’ or ‘Liquid Gold’ in the Tibetan, Chinese, and Mongolian culture. Each part of sea buckthorn plants, i.e., leaves, bark, pulp, and seeds, are reported as a rich source of various bioactive components such as vitamins, essential amino acids, carotenoids, organic acids, unsaturated fatty acids, tannins, phytosterols, and flavonoid(Kaushal & Sharma, 2011; Olas, 2018; Zheng et al., 2017). During the processing of sea buckthorn berries, seeds are considered as waste; however, they contain a unique cocktail of fatty acid composition including fat-soluble vitamins and minerals for industrial applications.

Sea buckthorn seed contains about 0.26-15 g.hg^{-1}oil, which varies according to subspecies, origin, package of practice, harvesting time of the berries, and the extraction methods (Bal et al., 2011). Oil has enormous demand in the cosmeceutical and pharmaceutical industries for the development of nutraceuticals and natural medicines. Sea buckthorn seed oil (SBSO) contains two highly essential fatty acids, i.e., omega-3 fatty acid linolenic (C18:3n-3) and omega-6 fatty acid linoleic (C18:2n-6), which account in the range of 20 to 35 g. hg^{-1}, and 30 to 40 g. hg^{-1} respectively. Omega-3 fatty acid linolenic (C18:3n-3) is well recognized to have chronic, and heart disease preventive properties, and omega-6 fatty acid linoleic (C18:2n-6)is an essential ingredient for the human diet (Fan et al., 2007). Studies showed that SBSO possesses unique properties to prevent UV-induced impairments along with lipid metabolism disorders; hence it is widely used in skin photo-protection cosmetics (Gęgotek et al., 2018; Li et al., 2007). Furthermore, SBSO helps recover skin diseases such as eczema, wounds, and burns. Therefore, it is utilized as a promising therapeutic agent in dermatitis treatment (Vinita et al., 2017; Zeb, Alam, 2009; Zeb & Khan, 2008).SBSO has been reported as a potent antioxidant in all *in-vitro* model systems (Chauhan et al., 2007). Carotenoids in SBSO revealed stimulating collagen synthesis and antioxidant activity (Kumari, 2017). Therefore, while extracting SBSO, it is essential to maintain the antioxidant activities of the oil along with other bioactive compounds for better and effective applications in the pharmaceutical and cosmeceutical industries.

Ultrasound-assisted extraction (UAE) is an emerging technology in food and bioprocess engineering in recent times. The compression and expansion propagated by mechanical sound waves due to ultrasound produce pressure, which, when surpasses the liquid's tensile strength, forms vapor bubbles. The subsidence of these bubbles produces perturbation in microporous particles of biological material, creating high-velocity interparticle collisions and macro turbulence. Consequently, matrices breakdown and erosion occur due to encroachment of the microjets. This enables intensification of extraction from a biological mass because of the internal diffusion mechanism and increment in mass transfer by eddy (Shirsath et al., 2012). Compared to conventional extraction techniques, the application of UAE produces higher oil yield with better retention of bioactive in bio-matrices and reduces extraction time while utilizing a lower volume of solvent to feed ratio, which has acknowledged UAE apt for low-cost extraction (Chemat et al., 2011; Dzah et al., 2020; Lukić et al., 2019). Applications of UAE have been successfully studied for extracting many oil-bearing compounds from different natural plant matrices (Vilkhu et al., 2008). Isopencu et al. (2019) optimized microwave and ultrasound-assisted extraction of SBSO based on extraction yield only. However, no study so far has been reported on the effects of UAE variables on antioxidant activity, carotenoid content, FFA value, peroxide value, anisidine value, totox value, and moreover, stability of SBSO while enhancing the extraction efficiency. Therefore, it becomes imperative to optimize the UAE process variables for achieving the desirable physicochemical and functional attributes of SBSO. This study aims to investigate the effect of ultrasound power, time, temperature, and solvent to sample ratio on extraction yield, antioxidant activity, total carotenoid content, and physicochemical properties of SBSO, and to optimize the desirable UAE conditions to achieve improved oil quality and stability. The quality of oil samples was evaluated by measuring their antioxidant activity, total carotenoid, anisidine, peroxide, totox, and free fatty acid values.

Materials and Methods

Raw materials

Dried sea buckthorn berries (*Hippophae salicipholia*) were obtained from Lahaul district of Himachal Pradesh (31°44' 57" and 32°59'57"N Latitudes and 76°46'29" and 78°41'34" E Longitudes), India. The seeds were removed from berries manually, cleaned in distilled water, and dried in a hot air oven (Jaisbo, Delhi) at 60˚C to a constant weight. The proximate composition (wet basis) of the dried sea buckthorn seeds was moisture: 5.78±0.64 g. hg^{-1}, protein: 39.52±1.54 g. hg^{-1}, and ash: 2.08±0.02 g. hg^{-1}. The dried sea buckthorn seeds were ground and sieved (0.5 mm) to obtain a powder with consistent particle size. The powder was stored at 4˚C for further investigation.

Fig.32 Sea buckthorn berries after separation of seed

Fig.33 Extracted Sea buckthorn seed oil

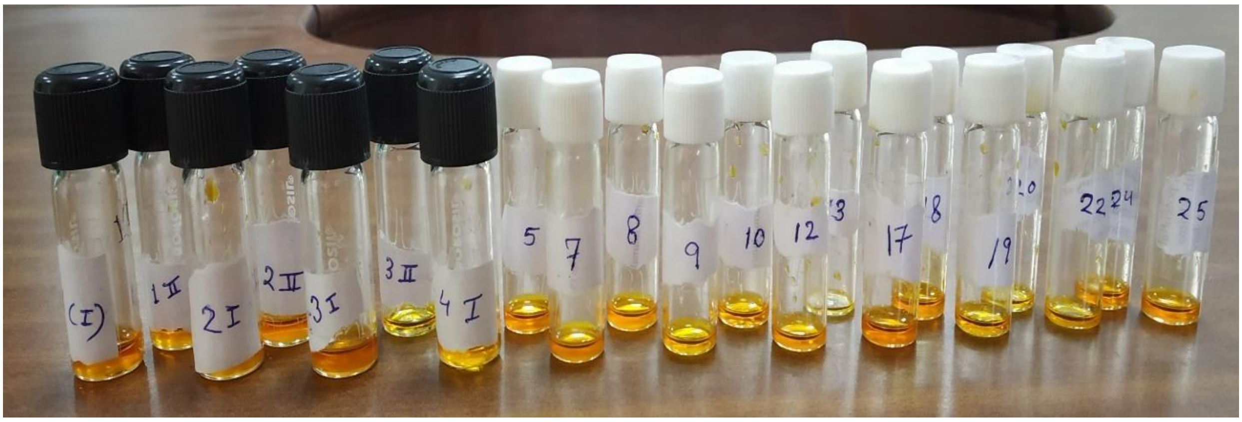


Fig.34 Treated seed oil extract by varying parameters for the optimization of best quality oil extract

Chemicals

N-hexane, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), chloroform, acetic acid, potassium iodide, ethanol (96%), sodium thiosulfate, sodium dichloride, β-carotene, potassium hydroxide, starch solution, petroleum ether, sodium methoxide, and phenolphthalein indicator, boron trifluoride-methanol (BF_3-MeOH) reagent, sodium chloride (all reagent grade) were procured from Sigma-Aldrich, USA.

Ultra-sound pre-treatment and extraction

An ultrasonic system (VCX750, Sonics & Materials, USA) with a probe-type tip (1 cm diameter) was used for the pre-treatment of sea buckthorn seed powder during the extraction. Variables used for the UAE were: extraction time (θ): 5 to 30 min, temperature (*T*): 25 to 50˚C, ultrasound power (*P*): 235 to 700 W, and solvent to sample ratio (*R*): 6:1 to 10:1 (mL: g). The ranges of power level and treatment time were selected based on the preliminary runs supported by literature (Isopencu et al., 2019; Kuriakose, 2015; Samaram et al., 2015). A recirculating type of hot water bath (ESCY IC201, Bandi Technology, New Delhi) coupled with a temperature controller was used to maintain the desired temperatures during the extraction. N-Hexane was used as the solvent medium for the extraction.

Experimental design and statistical analysis

Effects of treatment time (θ), temperature (*T*), ultrasound power (*P*), and solvent to sample ratio (*R*)on extraction yield (*Y*), antioxidant activity (*AA*), total carotenoid content (*TC*), and peroxide (*PV*), anisidine (*AV*), totox (*TV*) and free fatty acid (*FFA*) values of SBSO samples were studied. The extraction was conducted according to a 2-level full factorial (2^4) experimental design with 5 replicates at the centre point, generating a total of 21 experiments. The independent variables were coded between-1and +1. The experimental data were modulated to second-order regression equations and analyzed using one-way analysis of variance (ANOVA) to determine the significant difference among 21 experiment treatments. A probability of $p<0.05$ or the highest *F*-ratio was considered significant. The terms which were non-significant($p>0.05$) were deleted in the regression equations. The model efficiency was determined by calculating the values of coefficient of determination(R^2), significance($p<0.05$) of lack of fit, pure error, and significance ($p<0.05$) of the regression equation. The numerical optimization method was used to generate optimum conditions of the UAE to achieve the most desirable properties. Experimental design and data analysis were conducted in Minitab software v.16 (Minitab Inc., State College, USA). *Design Expert* (Version 7.0) and *Origin Pro* (2021b) were used for graphical analysis.

Measurement of oil yield

A 50 g seed powder was taken in a 500 mL beaker, and n-hexane was mixed according to solvent to sample ratio of6:1 to 10:1 (mL: g). The samples were subjected to ultrasound treatments with power levels 235 to 700W and treatment time 5 to 30min. The temperature was maintained and recorded continuously using a thermometer. After the extraction, Whatman No.1 filter paper was used for the filtration of the extracts. Hexane was evaporated at 50˚C using a rotary evaporator (RV 10, IKA India). The weight of oil was measured using a weighing balance (Aczet CY 513, India, accuracy: 0.0001), and the extraction yield (*Y*) was calculated using Eqn.1 (Dzah et al., 2020).

$$Extraction\ yield\ (Y) = \frac{Weight\ of\ oil\ (g)}{Weight\ of\ the\ initial\ sample\ (g)} x\ 100 \qquad (1)$$

Measurement of total carotenoids

Total carotenoid was assayed according to Ranjith et al. (2006). In brief, 1 mg of extracted oil sample was mixed with 0.5 mL of 5 mg. hg^{-1} NaCl aqueous solution and vortexed for the 30s. The mixture was then centrifuged (REMI R-8C) for 10 min at a relative centrifugal force (RCF) of 2058 x g, and the supernatant was collected. The absorbance was recorded using a microplate photometer (VT 05404-0998, EPOCH, USA) at 460 nm. In the measurement, β-carotene was used as the standard for calculation (β-carotene equivalent) of total carotenoid content in oil samples.

Measurement of DPPH antioxidant activity

Antioxidant activity of SBSO samples was analysed using on 2,2-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity assay (Samaram et al., 2015).A 1.4mmol. L^{-1} solution of DPPH was prepared in hexane. A 10 µL sample was taken in a microtiter plate, and a 250 µL DPPH solution was mixed. The mixture was then kept for 15 min in a dark room. The absorbance of the cross was then recorded at 517 nm using a microplate photometer (VT 05404-0998, EPOCH, USA). The scavenging activity of DPPH was confirmed by the declining absorbance of the DPPH solution. The scavenging of free radicals was estimated by absorbing the blank solution and optical density (OD) of the sample showing the sample's absorbance. The scavenging activity was calculated using Eqn.2. The concentration (µg. mL^{-1}) of the oil sample imparting 50% inhibition (IC_{50}) was analysed by plotting sample concentrations against inhibition percentages.

$$Scavenging\ activity\ \ (\%) = \frac{\text{Absorbance of blank}-\text{Absorbance of sample}}{\text{Absorbance of blank}} x100 \qquad (2)$$

Physicochemical analysis of SBSO

The oxidative stability of SBSO samples was analysed in terms of peroxide (*PV*), anisidine (*AV*), totox (*TV*), and free fatty acid (*FFA*) values. *PV* and *AV* were determined using AOCS official methods (Cd 8-53, 2003; Cd 18-90, 2009). FFA (% oleic acid) was measured using ISO Standard No. 660 (ISO, 2009). Total oxidation value (*TV*) was evaluated using *PV* and *AV* values according to Eqn. (3).

$$TV = 2PV + AV \qquad (3)$$

GC-MS analysis of SBSO

The SBSO obtained at the optimized UAE condition was analysed for the fatty acid profile using the GC-MS method. Fatty acid methyl esters (FAMEs) of the oil sample were prepared by using the boron trifluoride-methanol (BF_3-MeOH) method. A 20 mg of oil sample was mixed with 2 mL 0.5 mol. L^{-1} NaOH-methanol solutions and heated for 7 min at 100˚C. The mixture was then cooled, and 3 mL of 14% BF_3-MeOH reagent was added. The vessel was then sealed and heated for 5 min at 100˚C. The mixture was cooled, and 7 mL of saturated NaCl along with 2 mL of hexane was added. The mixture was shaken thoroughly to form a hexane layer which was used for GC-MS analysis.

Analysis of FAMEs was conducted using a GC-MS (QP 2010 Shimadzu, Japan) equipped with thermal desorption system TD 20. The carrier gas used was 99.99% Helium at a total flow of 16.3 mL.min^{-1} and a constant flow of 1.21 mL.min^{-1}. The temperature setting for the injector and mass transfer line was 230°C and 270°C, respectively. The temperature of the oven was programmed as Column oven temperature: 140°C and Injection temperature: 260°C. The total running time of GC-MS was 50 min using Rxi$^{(R)}$-5Sil capillary column (30m x 0.25 mm x 0.25 mm). Turbo mass software was adopted to handle the mass spectra and chromatograms. The average peak area of the compounds was compared with the internal standard (methyl decanoate) for calculating the relative abundance of the compound in the sample. The constituents were determined by comparing their relative retention indices (KI), fragmentation patterns, and molecular mass. The mass spectra of the peak in the sample are finally compared with the mass spectra data available from the Willey libraries and the National Institute of Standards and Technology (NIST) library.

Results and Discussion

Yield Efficiency

Table 1 shows the experimental values of yield (*Y)*, antioxidant activity (*AA)*, total carotenoid content (*TC)*, peroxide value (*PV)*, anisidine value (*AV)*, totox value (*TV)*, and free fatty acid value (*FFA)* of SBSO due to ultrasound pre-treatment at different combinations of θ, *T*, *P*, and *R*. The extraction yield varied from 1.28 to 6.87 g. hg^{-1} at different UAE conditions. The highest oil yield (6.87 g. hg^{-1}) was obtained after 5 min treatment time at 50°C and solvent to sample ratio of 10:1 (mL: g) with ultrasound power 700 W. The UAE produced better oil yield (*Y*) at high temperatures with an increase in *R* values. Higher temperature and reduced time favoured the extraction yield. The *Y* values increased with an increase in *P* levels and a decrease in importance. The maximum extraction yield was observed at the highest level of *P* and *T* values. The UAE variables had a significant effect (p<0.05) on the extraction yield as determined by a higher R^2value (97.22%) (Table 4). The interaction effect between θ and *R* had the least significance(p<0.05) (Table 5).

The results well explained the influence of ultrasound intensity on the extraction of plant materials which are also described by Dhanani et al. (2017), Kuriakose (2015), Rathod (2015), and Maran et al. (2017). Attrition, particle disruption, and cell breakdown are the major governing factors caused due to cavitation phenomenon upon ultrasound treatment. They form the critical factors for the extraction augmentation by enhancing mass diffusivity and mass transfer during a UAE process (Khadhraoui et al., 2018). Further, the application of ultrasound generates mechanical vibration resulting in the broader interaction surface area between a solvent and sample, facilitating the leisurely invasion of liquid into a solid matrix and thereby improving extraction efficiency of the desired component (Pan et al., 2012). Kaushal and Sharma (2011) and Yue et al. (2017) reported that the yield of SBSO from *H. rhamnoides* and *H. salicipholia* extracted using the Soxhlet extraction method ranged from 4.75 to 5.25 g. hg^{-1}, and 4.3 to 4.9 g. $hg^{-1,}$ respectively, which was significantly lower than the present values. This indicated that higher ultrasound power *P* generated excessive free radicals resulting in the degradation of polyphenols and hampered its biological activity. This is because as ultrasound power increases, it enhances the hydrodynamic force, which improves the extraction efficiency of a solvent (Knorr, Ade-Omowaye, and Heinz, 2002). Further, the extraction of oil at the elevated temperature with a high level of solvent to sample ratio yielded higher oil recovery from sea buckthorn seed. At elevated temperatures, ultrasound waves get thrust to diffuse oil from a sample matrix into a solvent (Dzah et al., 2020). Many studies supported the fact that elevated temperatures during solvent extraction increased the yield of phenolic components from plant matrices (Altemimi et al., 2016; He et al., 2016; Toma et al., 2001). However, it should be taken into consideration that high temperatures may impinge on oil quality and stability, and thermal shock could affect the bioactive components available in oil.

Furthermore, while analysing the effect of solvent to sample ratio on the extraction of SBSO, it revealed that the yield of the oil was enhanced from 1.79 to 6.87 g. hg^{-1} significantly (Table 2). Zhang et al. (2009), in a study, the observed improvement in extraction efficiency when high level of solvent to feed ratio was applied during the extraction of oil from almond powder. This is because high-level solvent ratio provides enough capacity for oil extraction and boost oil dispersion from vegetal matrix (Zou et al., 2014). The interaction effects of selective ultrasound extraction variables on the yield are shown in Fig.1 through response contour graphs which illustrates the influence of different parameters on extraction efficiency of SBSO.

DPPH Antioxidant activity

The DPPH activity of SBSO represented by IC_{50} values varied from 1.42 to 4.73 µg. mL^{-1}, which was significantly affected by treatment time, exposed temperature, ultrasound power, and solvent to sample ratio (Table 4). These IC_{50}values were lower than the value (7.8 µg. mL^{-1}) reported by Chauhan et al. (2007) for aqueous seed extract *H. rhamnoides* species. This revealed that the ultrasound pre-treatment induced more potent antioxidant activity in the SBSO samples. The reason may be because conventional solvent extraction methods generally cause damage to bioactive compounds due to elevated temperature and elongated treatment time, whereas UAE is reported to be very effective for extracting thermosensitive and unstable compounds due to the application of shorter extraction time at a comparatively lower temperature (Muñiz-Márquez et al., 2013). Approximately two-fold increment in antioxidant capacities of defatted canola, hemp, and flaxseed oils are reported using the UAE process compared to the conventional extraction methods. These ultrasound-assisted hemp, flax, and canola oil extracts exhibited 17.18%, 13.62%, and 34.17% DPPH inhibition, respectively (Teh and Birch, 2014).

Table 4 Experimental design of independent variables and measured responses

Run	*θ* (min)	*T* (℃)	*P* (W)	*R* (mL: g)	*Y* (g. hg^{-1})	*AA* (IC_{50} µg. mL^{-1})	*TC* (mg/100g β-carotene equivalent)	*PV* (mmol.kg^{-1})	*AV* (mg KOH.g^{-1} oil)	*TV* (*AV*+2*PV*)	*FFA* (% oleic acid)
1	17.5	37.5	465	8	5.20±0.02	2.629±0.04	105.496±2.12	0.48±0.00	6.2±0.00	7.16±0.00	2.19±0.00
2	30.0	50.0	700	10	5.09±0.04	2.271±0.01	99.052±1.09	0.41±0.00	6.6±0.00	7.42±0.00	4.29±0.00
3	30.0	25.0	235	10	3.87±0.11	3.975±0.00	103.447±1.21	0.69±0.00	5.9±0.01	7.28±0.01	2.33±0.01
4	30.0	25.0	235	6	5.15±0.06	3.869±0.01	110.799±2.13	0.60±0.00	6.3±0.00	7.5±0.00	2.88±0.00
5	30.0	25.0	700	10	2.96±0.13	2.765±0.02	180.638±3.32	0.44±0.00	6.5±0.00	7.38±0.00	3.51±0.02
6	5.0	50.0	700	6	1.79±0.09	4.231±0.00	206.310±2.08	0.78±0.00	5.8±0.01	7.36±0.01	2.04±0.00
7	5.0	50.0	700	10	6.87±0.05	4.732±0.03	214.319±1.97	0.81±0.00	5.3±0.00	6.92±0.00	2.94±0.01
8	30.0	50.0	700	6	1.28±0.10	2.876±0.02	115.340±3.44	0.81±0.00	5.8±0.00	7.42±0.00	3.04±0.00
9	5.0	25.0	235	10	5.72±0.01	4.411±0.00	191.372±2.37	0.71±0.00	5.4±0.00	6.82±0.00	2.76±0.00
10	30.0	50.0	235	10	3.39±0.04	3.011±0.00	135.113±2.46	0.65±0.00	6±0.01	7.3±0.01	3.47±0.03
11	5.0	50.0	235	10	5.56±0.01	4.172±0.00	178.545±3.03	0.50±0.00	5.1±0.02	6.1±0.01	2.31±0.00
12	5.0	25.0	235	6	3.29±0.03	3.863±0.01	111.052±1.12	0.77±0.00	5.5±0.00	7.04±0.00	3.01±0.00
13	5.0	25.0	700	6	5.11±0.07	2.994±0.04	113.843±2.39	0.47±0.00	6.1±0.01	7.04±0.00	3.28±0.00
14	5.0	50.0	235	6	6.66±0.01	4.639±0.00	237.042±3.86	0.78±0.00	5.6±0.00	7.16±0.00	2.49±0.01
15	30.0	50.0	235	6	1.19±0.12	1.989±0.05	107.381±2.77	0.70±0.00	5.4±0.03	6.8±0.02	3.66±0.01
16	5.0	25.0	700	10	2.44±0.07	2.341±0.01	101.078±1.92	0.49±0.00	5.9±0.01	6.88±0.01	2.05±0.00
17	30.0	25.0	700	6	3.19±0.00	1.423±0.01	110.034±1.49	0.72±0.00	6.6±0.02	8.04±0.02	3.72±0.02

θ: treatment time, *T*: temperature, *P*: ultrasound power, *R*: solvent to sample ratio, *Y*: yield, *AA*: antioxidant activity, *TC*: total carotenoid content, *PV*: peroxide value, *AV*: anisidine value, *TV*: totox value, and *FFA*: free fatty acid

Values are presented as means ± standard deviation (*n=3*)

Table 5 Statistics of the fitted second order regression equations

Regression coefficient	Y (g. hg^{-1})	AA (IC_{50}) µg/mL	TC (mg/100g β-carotene equivalent)	PV (mmol/kg)	AV (mg KOH/g oil)	TV (AV+2PV)	FFA (% oleic acid)
Constant	34.40	3.930	22.680	1.800	3.680	7.790	0.940
b_1	1.767	0.562	1.435	-0.101	0.185	0.005	0.020
b_2	1.061	0.234	0.923	0.025	0.006	0.116	0.047
b_3	0.6556	0.111	0.363	-0.024	0.020	-0.021	0.096
b_4	4.495	1.117	4.423	0.092	0.147	0.384	0.390
b_{12}	-0.048	-0.017	-0.040	-	-	-0.003	0.008
b_{13}	-0.024	-0.010	-0.019	0.002	-	-0.003	-
b_{14}	-0.192	-0.061	-0.200	0.006	-0.017	-0.007	-0.017
b_{23}	-0.0184	-	-0.009	-	-	-	-
b_{24}	-0.116	-0.030	-0.110	-0.007	-0.061	-0.021	-0.017
b_{34}	-0.079	-0.020	-0.056	-	-	-	-
b_{123}	0.0006	-	-	-	-	-	-0.011
b_{124}	0.005	-	-	-	-	-	-0.014
b_{134}	0.003	-	-	-	-	-	-
b_{234}	0.002	-	-	-	-	-	-
b_{1234}	-	-	-	-	-	-	-
R^2	97.22	88.82	85.80	77.26	99.93	94.69	72.160
p-value	0	0	0	0	0	0	0

Significant at p<0.05, Y: yield, AA: antioxidant activity, TC: total carotenoid content, PV: peroxide value, AV: anisidine value, TV: totox value, FFA: free fatty acid, b_1, b_2, b_3 and b_4 are regression coefficients of main single effect of time, temperature, ultrasound power and solvent to sample ratio, respectively, b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34}, b_{123}, b_{124}, b_{134}, b_{234} and b_{1234} are estimated regression coefficient for the interaction effects

In this study, a significant (p<0.05) effect of UAE variables on IC_{50} values was observed with an R^2 value of 88.82% (Table 5). The treatment time, temperature, ultrasound power, and solvent to feed ratio, and their interaction terms showed a significant effect on the IC_{50} values (Table 6). The most potent antioxidant activity was obtained with the oil extracted using low R values (Table 4). Moreover, high ultrasound power resulted in substantial antioxidant activities even during the application of a low level of temperature. This could be because of the warm solvent and additional turbulence created due to ultrasound treatment which improved the revival of fat-soluble antioxidants resulting in the enhancement of antioxidant capacity (Wang & Weller, 2006). A lower level of R depicted better antioxidant of the oil along with moderate temperature application.

Furthermore, the shorter extraction time with moderate ultrasound power recovered oil with lesser IC_{50} values, i.e., more potent antioxidant activity. The study well explained the significant dependence of UAE variables on the antioxidant capacity of SBSO. Significance ($p<0.05$) of interaction effects of extraction time, temperature, ultrasound power, and solvent to sample ratio on the IC_{50} values were represented using contour graphs in Fig 36.

Total carotenoid content

The range of total carotenoid content (*TC)* values was observed from 99.05 to 237.04 mg per 100g *β*-carotene equivalent in the oil samples (Table 4). Analysis of the regression model (Table 5) indicated that the main effects of treatment time, temperature, ultrasound power level, and solvent to feed ratio were significant for the higher recovery of carotenoids. Moreover, the interaction effect of time and solvent to sample ratio had a lower effect ($p=0.05$) on the total carotenoid content (Table 6). The carotenoid content was observed to increase with a shorter extraction time. Also, higher temperature and higher ultrasound power resulted in the desired carotenoid content in the oil. For instance, carotenoid content was enhanced with increasing solvent to sample ratios. Beveridge (1999, 2003) reported that carotenoids content ranged between 50 and 85 mg/100g oil for the Caucasus variety of sea buckthorn seeds. The author also reported the carotenoid content of 41.1 mg/100 g oil for *H. rhamnoides* species. Carotenoid content in SBSO exhibited considerable variations by various authors when extracted using different extraction techniques like a solvent (22.2 mg/100 g oil), supercritical CO_2 (6.2 to 11.7 mg/100 g oil), and screw press (15.3 mg/100 g oil). Beveridge (2003) reported a broad range of total carotenoids varying from 330 to 1000 mg/100 g in sea buckthorn pulp oil depending on plant subspecies or cultivars. The presence of zeaxanthin and lycopene was also reported in lesser concentrations in SBSO samples (Teleszko et al., 2015; Yang & Kallio, 2002). Fig.3 depicts the trend of variations in the total carotenoid in SBSO as a function of UAE variables which predicts that elevated temperature and reduced treatment time resulted in a higher yield of carotenoid content.

Physicochemical analysis

The peroxide values of SBSO samples ranged from 0.41 to 0.81 $mmol.kg^{-1}$ depending on UAE conditions. The results revealed that ultrasound pre-treatment provided a stable oil (*PV* of fresh oil > 5 $mmol.kg^{-1}$) with reliable stability (*PV*< 0.8 $mmol.kg^{-1}$). This suggested that there was no major peroxidation reaction occurred during the extraction process. It can be explained by the fact that SBSO samples contained a high amount of natural antioxidants such as vitamin E and carotenoids, and therefore, protect against peroxidation (Zielińska & Nowak, 2017). Shorter treatment time and lower temperature could be the reasons for the better stability of the oil, as reported by Isopencu et al. (2019).

Table 6 *p*-value and *F*-ratio of extraction variables in final reduced models

Responses		Main effects				Interaction effects										
		θ	*T*	*P*	*R*	*θT*	*θP*	*θR*	*TP*	*TR*	*PR*	*θTP*	*θTR*	*θPR*	*TPR*	*θTPR*
Y	*p*-value	0.008	0.009	0.007	0.025	0.023	0.019	0.800	0.36	0.006	0.22	0.050	0.27	0.55	0.006	0.01
	F-ratio	18.43	16.44	15.30	10.01	10.45	2.19	0.07	1.00	20.24	1.97	6.52	1.53	0.39	20.17	16.52
AA	*p*-value	0.01	0.022	0.006	0.033	0.007	0.069	0.003	-	0.018	0.011	-	-	-	-	-
	F-ratio	27.52	16.90	12.98	10.50	11.28	17.22	15.44		4.84	10.23					
TC	*p*-value	0.009	0.049	0.042	0.035	0.013	0.03	0.031	0.022	0.050	0.046	-	-	-	-	-
	F-ratio	10.83	5.17	3.30	4.17	9.50	6.61	4.21	9.22	5.04	5.90					
PV	*p*-value	0.00	0.001	0.00	0.00	-	0.00	0.00	-	0.00	-	-	-	-	-	-
	F-ratio	236.83	41.54	405.00	279.05		253.30	77.66		198.08						
AV	*p*-value	0.00	0.00	0.00	0.007	-	-	0.007	-	0.00	-	-	-	-	-	-
	F-ratio	2420.00	845.00	1445.00	20.0			20.0		180.00						
TV	*p*-value	0.030	0.048	0.050	0.021	0.024	0.008	0.008		0.030	-	-	-	-	-	-
	F-ratio	9.04	4.98	5.41	11.01	10.13	16.69	17.82		9.01	-	-	-	-	-	-
FFA	*p*-value	0.00	0.16	0.006	0.001	0.001	-	0.002	-	0.050	-	0.038	0.00	-	-	-
	F-ratio	196.62	2.66	20.84	35.78	60.52		33.37		6.10		7.81	87.68			

Insignificant at $p>0.05$, *Y*: yield, *AA*: antioxidant activity, *TC*: total carotenoid content, *PV*: peroxide value, *AV*: anisidine value, *TV*: totox value, and *FFA*: free fatty acid; *θ*, *T*, *P*, and *R* represent the single effect of variables, *θT*, *θP*, *θR*, *TP*, *TR*, *PR*, *θTP*, *θTR*, *θPR*, *TPR* and *θTPR* represent the interactions between variables

The levels of treatment time, temperature, ultrasound power, and solvent to sample ratio had a significant effect (p<0.05) on peroxide values (Table 5). The interaction terms showed a significant impact on *PV*, which indicated the formation of peroxide and hydroperoxide compounds at different stages of the extraction. It was observed that extended extraction time resulted in lower peroxide values because a longer treatment time could degrade peroxide and hydroperoxide compounds in the oil. The *AV* of oil samples ranged from 5.1 to 6.6% (Table 4). A relatively high R^2 (99.93%) value suggested that the *AV* was significantly influenced by UAE variables (Table 5). Interaction terms showed negligible *p*-value and hence had a significant effect on the *AV* (Table 6). θ and T negatively affected the values of *AV*.

Additionally, when the extraction process was extended to 30 min, the *AV* was observed to increase while decreased when extraction time was reduced (Table 4). According to Guillén & Cabo (2002), high peroxide values, i.e., high production of hydroperoxide, does not always imply the high production of secondary oxidation products, i.e., low AV values. Moreover, frying oils generally have low *PV* values, but *AV* values were found effective for evaluating the quality of oil (Labrinea et al., 2001).

TV of SBSO samples ranged from 6.10 to 8.04 (Table 4). A significant regression with a relatively higher R^2 value (94.70%) was adequately fitted to the stability data (Table 5). Higher temperature with longer time resulted in increased *TV* of the oil. When extraction was carried out at low ultrasound power for short time lower *TV* i.e., achieving stable oil. Due to elevated temperature, antioxidants of oil undergo thermal degradation resulting in poor oil stability or high *TV*. Lower *P* level with high R produced the stable oil with the lowest *TV*. Tables 2 and 3 indicate the main and interaction effects of the treatment time, temperature, ultrasound power level, and solvent to feed ratio on *TV* of SBSO samples. *FFA* values of oil samples at various UAE variables were observed to vary from 2.04 to 6.6 units (Table 4). A second-order regression with R^2 (72.60%) was effectively fitted to the stability data. Fig. 36 shows variations in totox (*AV*+2*PV*) values of SBSO samples as a function of UAE variables.

Optimisation and validation UAE extraction

Overlaying of all 3D plots were conducted to determine multiple graphical optimizations to attain the most suitable extraction conditions with maximum extraction yield, antioxidant value, total carotenoid content, and physicochemical properties of the oil. The optimum values of all UAE variables were predicted by applying various multiple numerical optimizations. The optimum values numerically predicted were T= 50˚C, θ= 8.28 min, P= 552 W, and R=10:1 (mL: g). At this condition, the predicted yield, antioxidant activity, total carotenoid, peroxide, anisidine, and free fatty acid values were 6.4 g. hg^{-1}, 4.1 µg. mL^{-1}, 181.6 mg/100g β-carotene eq, 0.65 $mmol.kg^{-1}$, 5.5 mg KOH/g oil, and 2.7% oleic acid, respectively. The validation study supported the extraction yield (5.8±0.5 g. hg^{-1}) obtained by the optimum extraction conditions. The experimental measurement of oil resulted in antioxidant activity = 2.26±0.9 µg. mL^{-1}, total carotenoid content = 188.34±0.8 mg/100g β-carotene eq, *PV*=0.51±0.03 $mmol.kg^{-1}$, *AV* = 6.1±0.05 mg KOH/g oil, and *FFA* = 2.49±0.04% oleic acid. Linear regressions were fitted between predicted and experimental values to validate the precision of the reduced model fitted to target responses. Higher R^2 values (72.16 to 99.93%) revealed the fitness of the predicted and experimental values.

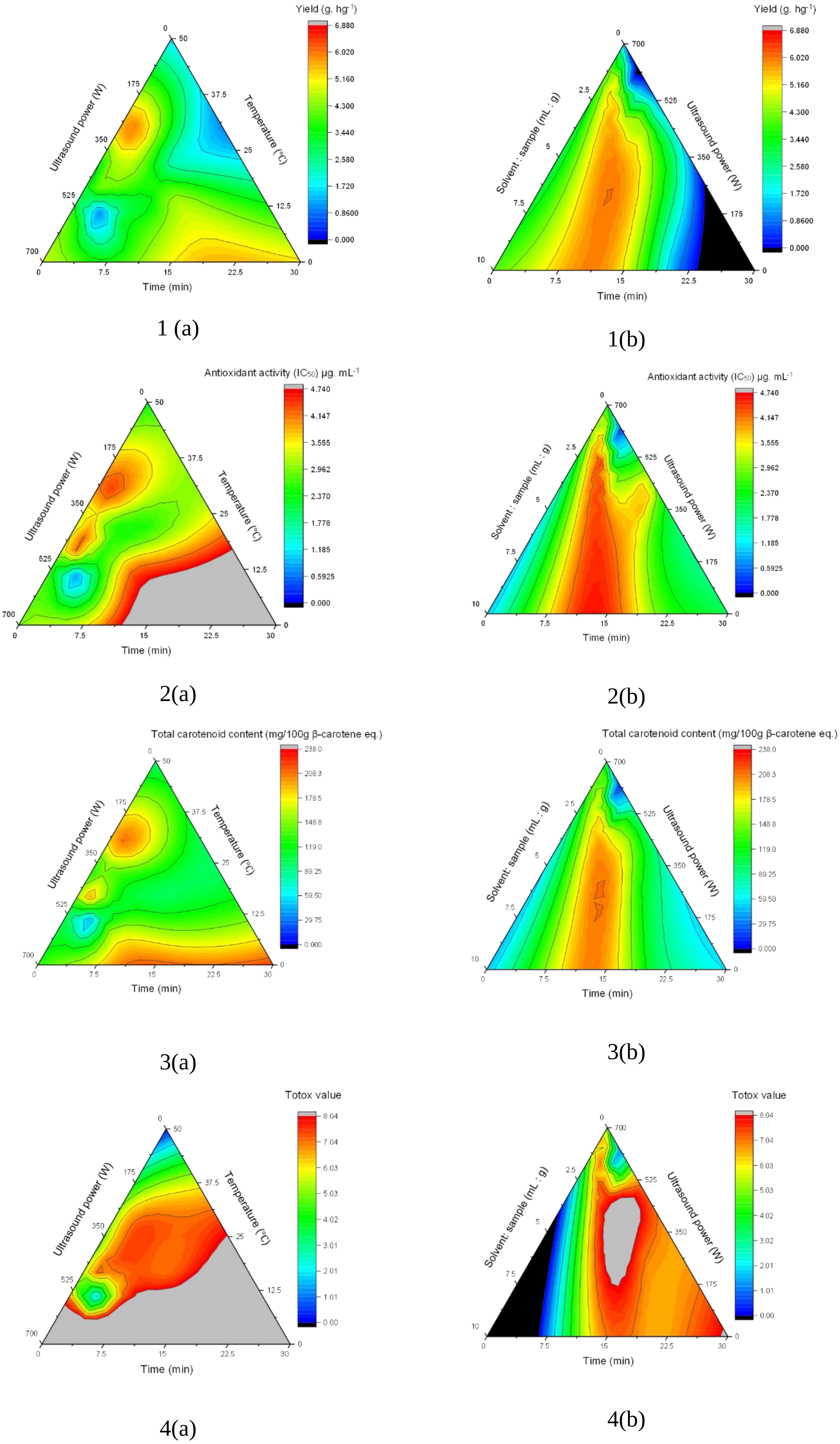


Fig .36 Interaction effects of ultrasound extraction

Fatty acid profile of optimally extracted SBSO

The fatty acid composition of the extracted SBSO was determined using GC-MS, and the results are presented in Table 7. The analysis confirmed the identification of fatty acid methyl esters upon ultrasound extraction, thereby confirming quantitative measures (Fig 37). SBSO was found out to be rich in linoleic acid (18:2 n-6, 32.55 g. hg^{-1}). According to literature fatty acids in SBSO are commonly linoleic (18:2 n-6, 30–40 g. hg^{-1}), linolenic (18:3 n-3, 20–35 g. hg^{-1}), oleic (18:1 n-9, 13–30 g. hg^{-1}), palmitic (16:0, 15–20 g. hg^{-1}), stearic (18:0, 2–5 g. hg^{-1}), and vaccenic (18:1 n-7, 2–4 g. hg^{-1}) acids (Yang & Kallio, 2002, 2001). Eleven fatty acids were reported in SBSO samples extracted by subcritical and supercritical methods, which included myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 (n9)), vaccenic acid (C18:1 (n7)), linoleic acid (C18:2), γ-linolenic acid (C18:3), eicosaenoic acid (C20:1) and eicosatrienoic acid (C20:3) (Zheng et al., 2017). Fatty acid profiling of UAE oil in this study revealed additional fatty acids such as ricinoleic acid (1.14 g. hg^{-1}), docosanoic acid (1.00 g. hg^{-1}), hexadecatrienoic acid (0.23 g. hg^{-1}). α-linolenic acid (0.54 g. hg^{-1}), docosatetraenoic acid (0.11 g. hg^{-1}), eicosadienoic acid (0.37 g. hg^{-1}), heneicosanoic acid (0.20 g. hg^{-1}), and Docosanoic acid (1.00 g. hg^{-1}) along with the above reported fatty acids. Ricinoleic acid found in SBSO has been reported as primary constituent of castor oil having antimicrobial, antinociceptive and anti-inflammatory properties. Furthermore, reports suggest that ultrasound-assisted extraction also improved the extraction of all fatty acids in flaxseed oil, chia seeds, and papaya seeds (de Mello et al., 2017; Gutte et al., 2015; Senrayan & Venkatachalam, 2019). Along with polyunsaturated acid (PUFA), monounsaturated fatty acid (MUFA), and saturated fatty acid (SFA), phytosterols like beta-Sitosterol (2.35 g. hg^{-1}) and Stigmasterol (0.24 g. hg^{-1}), and compounds like citronellol (0.60 g. hg^{-1}), 1-heptatriacontanol (1.04 g. hg^{-1}), isocarvomenthol, 1-hydroxy (0.35 g. hg^{-1}), and 8-hydroxylinalool (0.49 g. hg^{-1}) were also detected in the SBSO sample.

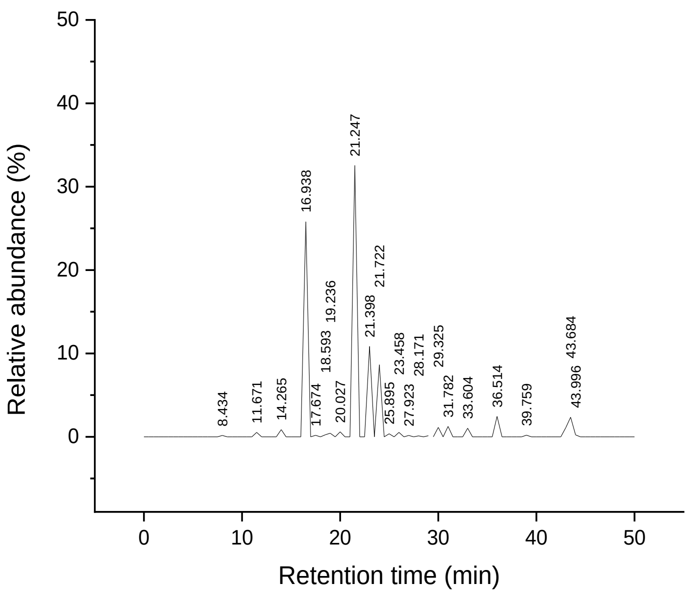


Fig. 37 GC-MS Chromatogram for volatile components of SBSO

Table 7 GC-MS analysis of optimally extracted sea buckthorn seed oil

Compound	Type	Molecular formula	Molecular weight	Relative quantity (g. hg^{-1}) ± SD (n=3)	Relative retention index (KI)
Methyl octanoate	SFA	$C_{11}H_{22}O_4$	218	0.16±0.01	-
Citronellol	Monoterpenoid	$C_{15}H_{28}O_2$	240	0.60±0.02	1228
8-Hydroxylinalool	Monoterpenoid	$C_{10}H_{18}O_2$	170	0.49±0.01	-
1-Hydroxy-Isocarvomenthol	Monoterpenoid	$C_{10}H_{20}O_2$	172	0.35±0.00	-
□-Decalactone	Lactone	$C_{10}H_{18}O_2$	170	0.19±0.01	1467
Hexacosane	Alkane	$C_{26}H_{54}$	366	0.24±0.01	1600
Methyl Hexadecatrienoate	PUFA, omega 3	$C_{17}H_{28}O_2$	264	0.23±0.01	-
Methyl myristate	SFA	$C_{15}H_{30}O_2$	242	0.54±0.00	1707
Methyl Palmitoleate	MUFA, omega-7	$C_{17}H_{34}O_2$	270	0.43±0.00	-
Methyl Palmitate	SFA	$C_{17}H_{32}O_2$	268	25.85±0.01	1927
Methyl Linoleate	PUFA, omega-6	$C_{19}H_{34}O_2$	294	32.55±0.01	2092
Methyl Linolenate	PUFA, omega 6	$C_{19}H_{32}O_2$	292	0.17±0.01	2096
Methyl Oleate	MUFA, omega-9	$C_{19}H_{36}O_2$	296	10.85±0.00	2104
Methyl Stearate	SFA	$C_{19}H_{38}O_2$	298	8.64±0.01	2128
Methyl Eicosatrienoate	PUFA, omega 3	$C_{21}H_{36}O_2$	320	0.13±0.01	-
Ricinoleic acid	MUFA, omega 9	$C_{19}H_{36}O_3$	312	1.14±0.01	2299
Methyl Arachidate	SFA	$C_{21}H_{42}O_2$	326	2.45±0.01	2309
Methyl Heneicosanoate	SFA	$C_{22}H_{44}O_2$	340	0.20±0.01	-
Methyl Docosatetraenoate	PUFA, omega 6	$C_{23}H_{38}O_2$	346	0.11±0.00	-
Methyl Docosanoate	SFA	$C_{23}H_{46}O_2$	354	1.00±0.01	2510
Methyl Tricosanoate	SFA	$C_{24}H_{48}O_2$	368	0.20±0.00	-
Methyl Tetracosanoate	SFA	$C_{25}H_{50}O_2$	382	0.32±0.00	2710
Stigmasterol	Phytosterol	$C_{32}H_{56}O$	484	0.24±0.02	-
Beta-Sitosterol	Phytosterol	$C_{32}H_{58}O$	486	2.35±0.00	-

Conclusion

The study showed that the UAE variables had a significant effect on the extraction efficiency and quality of SBSO. The UAE processing of SBSO considerably reduced the treatment time (8.28 min) while using a lower quantity of solvent and preserving bioactive components with better extraction efficiency. At optimum extraction condition (temperature: 50˚C; time: 8.28 min; ultrasound power: 552 W; solvent to sample ratio: 10:1 mL: g), the predicted values of yield, antioxidant activity (IC_{50}), total carotenoid, peroxide, anisidine, and free fatty acid were 6.4 g. hg^{-1}, 4.1 µg. mL^{-1}, 181.6 mg/100g β-carotene eq, 0.65 mmol.kg^{-1}, 5.5 mg KOH/g oil, and 2.7 g. hg^{-1} oleic acid, respectively. The use of ultrasound in an extraction process saves treatment time and solvent. Therefore, small and large industrial implementation of the process would be an effective technique in the extraction of SBSO, which has diverse applications in the cosmetics and pharmaceutical industries.

2.2.4 Development of a Hybrid-Multi Mode Microwave Vibro-Fluidized Hybrid Dryer for Sea buckthorn (*Hippophae Sp.*) Berries and Large Cardamom

Drying is one of the oldest methods of food preservation and it is a difficult food processing operation mainly because of undesirable changes in quality of the dried product. High temperatures and long drying times, required to remove the water from the sugar containing fruit material in conventional air drying, may cause serious damage to the flavour, colour, nutrients, reduction in bulk density and rehydration capacity of the dried product (Lin, Durance & Scaman, 1998; Drouzas, Tsami & Saravacos, 1999). Major disadvantages of hot air drying of foods are low energy efficiency and lengthy drying time during the falling rate period. Because of the low thermal conductivity of food materials in this period, heat transfer to the inner sections of foods during conventional heating is limited (Adu & Otten, 1996; Feng & Tang, 1998). The desire to eliminate this problem, prevent significant quality loss, and achieve fast and effective thermal processing has resulted in the increasing use of microwaves for food drying. Microwave-assisted drying is considered as one of the fastest drying techniques because of its inherent characteristics to shorten drying time and retain product quality (Datta and Anantheswaran, 2001). It is well established that application of microwaves along with other conventional drying methods is useful in food processing (Contreras et al., 2008; Cui et al., 2003; Sharma and Prasad, 2006). Microwave assisted drying is considered as one of the fastest drying techniques because of its potential characteristics to shorten drying time and retain product quality. In addition, utilization solar energy and agro-waste for on-farm processing of agricultural produce is getting momentum in present scenario. The UV treatment further help in reduction of the spores of fungi hence reducing aflatoxin content.

Hot air-drying method employed for Cardamom drying involved exposure of product to hot air for longer time which affected the quality viz. extraction yield and colour of the product. The drying time can be reduced considerably, and quality product can be achieved by adopting modern drying techniques. In recent years, microwave drying which is one of the quick drying methods, has gained popularity as an alternative drying method for a wide variety of food and agricultural products. However, it is important to know the retention of colour and aroma after a drying time. Based on above studies, the following drying system (Fig.13) is designed which can be used to dry large cardamom and Sea buckthorn berry.

The drying system mainly consists of two chambers; one chamber is for pre-treatment of microwave and the second one is hot air convection drying where the material will flow in a belt conveyor system. The product will be pre-treated by microwave till its latent of vaporisation. The idea is to bring down the temperature of product to end point of sensible heat.

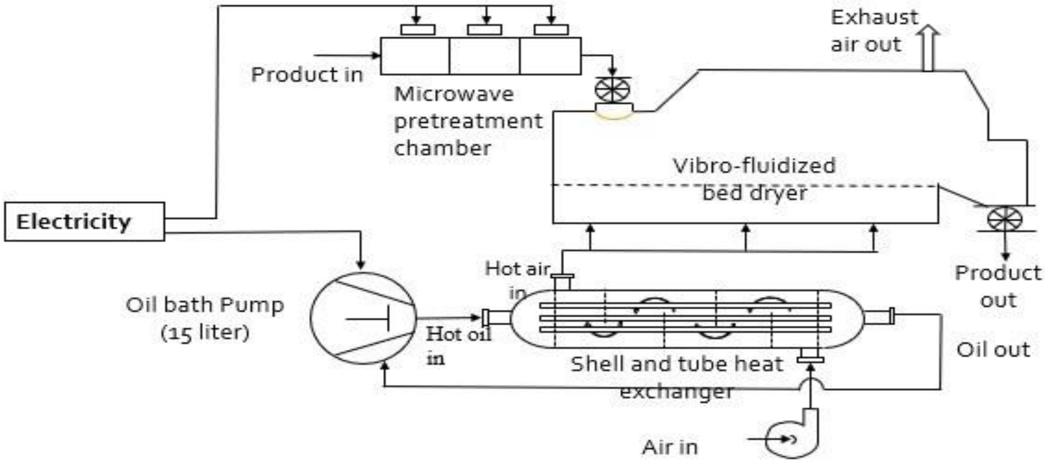


Fig.38 Schematic view of the hybrid dryer

The hot air to the second chamber will be provided from shell and tube heat exchanger where air will be heated by heated oil. The oil is being heated in an oil bath and can be supplied to the heat exchanger by an oil pump. As it is expected that the exhaust air from drying unit contains heat energy; the same can be re-utilised in the heat exchanger. And the relative humidity of the exhaust air or saturated air will be controlled by a humidity controller. The whole system will be operated by solar energy through solar panel and battery. During off-sunshine hour the system will be operated by biomass energy by a biomass combustion device.

Procurement of an oil bath and pump, biomass combustion chamber with pre-heater, oil-air heat exchanger with variable frequency drive and 3 KW off-grid solar system have been completed. The detail specification are as follows (design was done based on total drying energy requirement of about 3000 kJ).

- Oil bath and pump: oil bath of capacity 15 L which increases ambient temperature to 160°C and oil pump of flow rate of 16 L/min
- Biomass combustion chamber of 55,000 to 60,000 kcal/h and is consist of preheater made of shell and tube heat exchanger where oil will be heated and recirculated with a flow rate of 4000 L/h
- Oil-air heat exchanger: Flow rate of air 210 m^3/h to heat from 15°C to 60°C by heating media of hydraulic oil available at 130°C and flow rate is 3 m^3/h which is coupled to a centrifugal type blower (variable frequency drive) having free air delivery of 150 CFM
- 3 KW off-grid solar system: 9 solar panels 315 W each, 150 Ah-48V solar battery, 3kVA inverter to convert DC to AC.

The main purpose of the dryer system is to ensure the drying process conducted in a controlled temperature, shorter drying time, meet the specific moisture content and less human intervention. The development covers both, hardware and software development of the system followed by system validation. Summary of the overall research methodology of this project is shown in figure below.

Novel Multi-Mode Vibro-Fluidized Hybrid Dryer

Microwave assisted drying is considered as one of the fastest drying techniques because of its potential characteristics to shorten drying time and retain product quality. In addition, utilization solar energy and agro-waste for on-farm processing of agricultural produce is getting momentum in present scenario. The UV treatment further help in reduction of the spores of fungi hence reducing aflatoxin content.

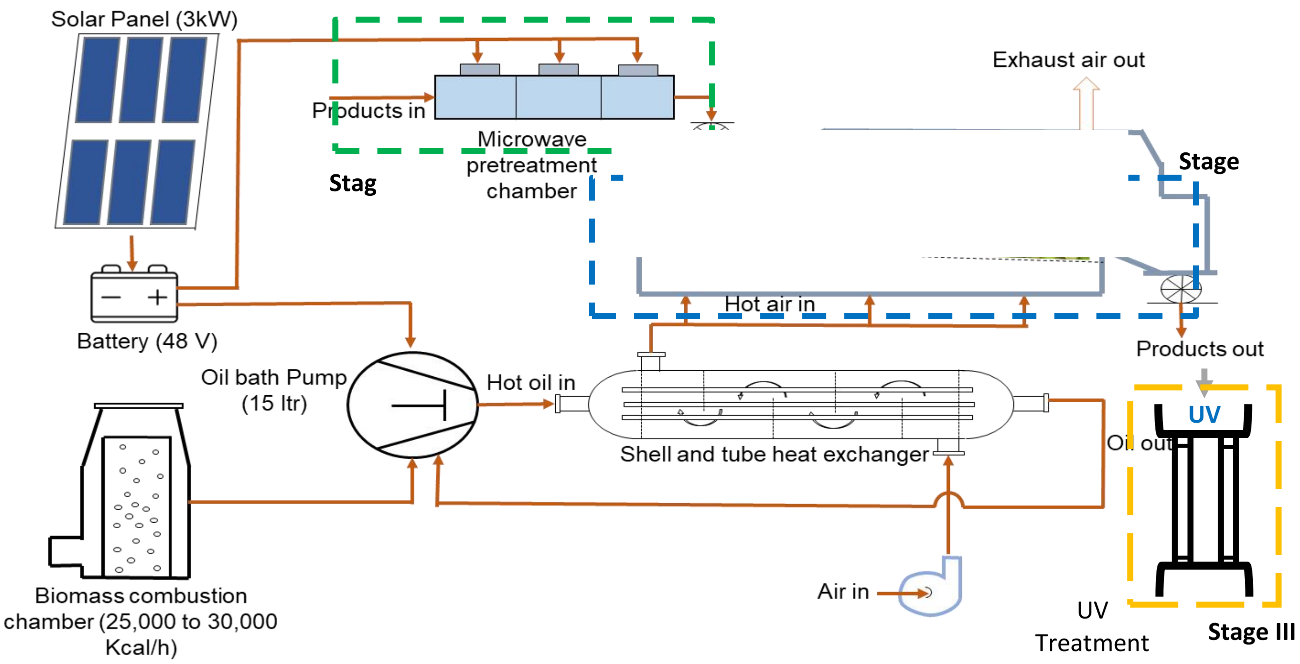


Fig. 39 Line diagram of the microwave assisted vibro fluidized dryer

2.3 Objective 3

Establishment of replicable community models in the selected project sites through rural transformative technologies and participatory rural action research for sustainable utilization of the natural product resources into various high value commercial products in collaboration with local grass root organizations

2.3.1 Setup of Technology Incubation Centre at Kirting, Lahaul & Spiti

Technology Incubation Center was established in Kirting village of tribal district of Lahaul & Spiti, Himachal Pradesh. Seabuckthorn is locally known by different names like *Charma, Salla, Tarbu, tses-ta-lulu,* etc. in the different regions of Himachal Pradesh. It is mainly available in the Tribal district of Lahaul & Spiti and some parts of Kinnaur and Chamba district of Himachal Pradesh. Detailed study was carried out in the year 2018 and 2019 on the species accessing its population in the lahaul valley and its traditional usage and existing value chain in the region. Traditionally, people residing in the cold desert area of the region are using the species for various indigenous uses like fuelwood, small timber, agriculture implements and also used by the '*Amchi/Larje' (*traditional healers*)* for treating various ailments and diseases. In order to create awareness and build the capacity of locals on the species around 200 stakeholders were trained on harvesting, post harvesting and marketing of the sea buckthorn through various interactive meetings and seminars. Through the interaction and study in the region it was observed that main intervention needed in the value chain management of sea buckthorn is primary processing at local level as the sea buckthorn berries are highly perishable and needs processing within the same day of its harvesting. Besides, berries juice diversification in terms of its products such as processing of leaves, seeds and dry hull of the berries can give a boost to the sea buckthorn-based enterprise while ensuring its environmental sustainability and economic benefit to the locals. Marketing is another aspect for which locals needs interventions or help. But looking at various ends it was found that for marketing agencies they need the products or raw material with quality processed. Realizing these aspects, a technology Incubation center was established in the region to harness the maximum potential of the species and easy and quality processing of the produce by locals. In year 2020 despite of COVID pandemic the technology Incubation was set up and operationalize in the valley. In 2020, around 300 kgs of the berries were processed in the unit and at the same time around 100 kgs of leaves were also processed for making leaves tea. Procurement of these raw material were done from the locals such as leaves @ Rs 35 per kg (wet) and Rs 100 per kgs for berries. In total around 60 beneficiaries were benefitted economically through collection which is very tedious process. These technologies made the processing easy through quality control which can fetch locals a good market return also sea buckthorn being a niche product of the region. Other than these locals were also given training on the value addition techniques of the produce starting from harvesting of leaves and berries to product development such as leaves tea, pulp juice, jam making, etc. Farmers who collected small number of leaves and berries for their own consumption were also facilitated through the center. For implementation of the work MoU has been done with Yangley Yuva Mandal of the region who is currently looking after the center. Also, for marketing the products developed in the center has been done through Entrepreneurship Cell of the Institute and Thapasu Foods, a startup of Himachal Pradesh. At present fresh seabuckthorn berries are sold in market at INR 100 per kg and its value-added products such as dry leaves upto INR 1000 per kg, pulp Juice at INR. 800 per litre, squash at INR 250 per litre bottle, dry berries at INR. 1500 per kgs, seeds at INR 400 per kgs, dry hull at INR 300 per kgs, jam at INR 300 for 250 gm bottle, and seed oil upto INR 20000 per litre. This one-year technological intervention in the region opened up the new dimension in the seabuckthorn based enterprise development with maximum benefit to the locals.

Following equipment have been established in TIC.

1. **Steam Jacketed Kettle:** This kettle is used for cooking of pulp or juices or making sugar syrup / brine solution. The kettle is 2/3rd jacketed for uniform distribution of the heat.
2. **Semi- Automatic Liquid Filling Machine:** Semi-automatic volumetric liquid filling machines used to fill variety of liquids. It can be used for different types of glass, Plastic, Metal containers.
3. **Semi- Automatic Capping Machine:** Screw on capper is compact & versatile model for sealing of various plastic caps.
4. **Corking Machine:** Machine is suitable to pack Tomato Ketchup, sauces, Vinegar or any other liquid in Glass Bottles.
5. **Single Chamber Vacuum Packaging Machine:** Single chamber vacuum packaging machines can finish the whole programs of vacuum extraction, gas flushing (optional), sealing, cooling and exhaust automatically.
6. **Sieve Shaker:** For separation and size determination of particles. A typical sieve shaker separates particle by passing them through a series of chambers with mesh filters and agitating the sample to obtain complete separation.
7. **Refrigerated Circulator: S**uitable for temperature application to external systems with simultaneous operation inside the bath tank. Circulator working temperatures are from - 1 °C to 100 °C

Fig. 40 Technology incubation centre at Kirting, District Lahaul and Spiti, Himachal Pradesh




Fig.42 Microwave dryer system at TIC, Kirting, Lahaul valley

Fig. 41 Sea buckthorn Processing in TIC

Fig.43 Bottle filling machine

Fig. 44 Vacuum ppackaging machine

Fig.45 Steam jacketed kettle

Fig 46. Pictorial view of the bottle capping machine

Fig 47. Corking machine

2.3.2 National Seminar on Value chain creation of SBT

One Day National Seminar/Webinar was organized on “Creation of Seabuckthorn Value Chain in Trans Himalaya” by GBP ‘NIHE’, HRC, Mohal, Kullu, Himachal Pradesh. The Seminar was sponsored by National Bank for Agriculture and Rural Development (NABARD), Shimla and National Mission on Himalayan Studies (NMHS), Ministry of Environment, Forest & Climate Change, Government of India. A total of 57 stakeholders (mainly farmers) participated from the Leh, Lahaul and Spiti Valley of Ladakh UT and Himachal Pradesh.

Speakers were of diverse background who were directly or indirectly involved in the Sea buckthorn value chain or can contribute towards the value chain creation. All the speakers emphasized on the collaboration of farmers groups with scientific institutions for scientifically validated value-added products that can fetch maximum benefit to the farmers. It was also suggested to tame the river side areas, community land, marginal land for sea buckthorn cultivation as the land holding of the farmers in all these regions are very low. Use of traditional knowledge system for low volume high yield crop such as sea buckthorn. Locally available cost-effective techniques such as for berries harvester, leaves and berries drying methods, seed oil extraction, harvesting and post harvesting techniques such as quality control, packaging, labelling and marketing issues were also discussed for maximum benefit with zero wastage. Diversification of value-added products of sea buckthorn besides traditional use of berries in juice to leaves tea, dried seeds, seed oils, other healthcare foods, cosmetics and medicines were also agreed upon. Plantation of sea buckthorn plants in land slide prone areas were also suggested being a species soil binder and also improves soil fertility through improving soil carbon and nitrogen.

Fig.48 Glimpses of National Seminar on value chain creation of Sea buckthorn

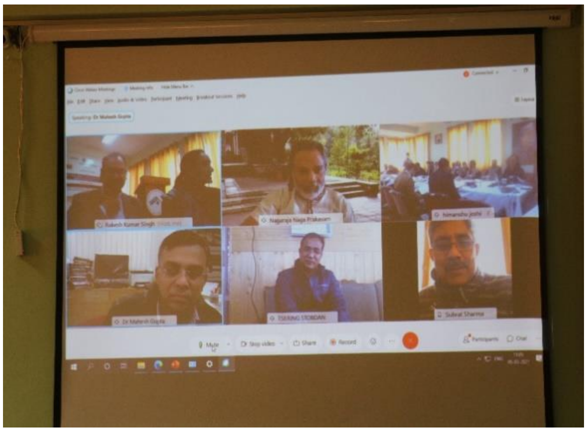


Fig.48 Glimpses of National Seminar on value chain creation of Sea buckthorn

2.3.3 Outreach activities in the local communities of Sikkim

In order to promote improved technologies and large cardamom harvesting knife, 98 number of improved large cardamom harvesting knives were procured from CAEPHT centre of All India Coordinated Research Project on Ergonomics and Safety in Agriculture and distributed among farmers (Fig.). In addition, project team also participated in number of Technology Demonstration Mela organized by CAEPHT Ranipool.

Table 8 Details of programme in Sikkim

S. No.	Details of the programmes	Number of participated
1.	Technology and Machinery Demonstration Mela-2019 organized by CAEPHT Ranipool(Fig. 4) on 15th Feb. 2019	200
2.	Technology and Machinery Demonstration Mela-2020 organized by CAEPHT Ranipool on 15th Feb. 2020 (Fig. 5)	250
3.	Technology and Machinery Demonstration Mela-2021 organized by CAEPHT Ranipool (Fig. 6) on 13/03/2021	95
4.	Demonstration on use of large cardamom harvesting knives (Fig.7)	50

Fig.49 Glimpses of programmes organized for Distribution of improved large cardamom harvesting knives

Fig.50 Glimpses of Technology and Machinery Demonstration Mela-2019 organized at CAEPHT Ranipool on 15/02/2019

Fig.51 Glimpses of Technology and Machinery Demonstration Mela-2020 organized at CAEPHT Ranipool on 14/02/2020

Fig.52 Glimpses of Technology and Machinery Demonstration Mela-2021 organized at CAEPHT Ranipool on 13/03/2022

Fig.53 Glimpses of demonstration of Large Cardamom Harvesting knives organized

Publications

1. Book

Sanwal, N., Satheeshkanth, S. S. M., Sahu, J. K. (2022). Aromatic, Medicinal and Agricultural Plants in Indo Trans Himalayan Region - A compilation, New Delhi Publishers. (ISBN: 978-93-92513-39-8)

2. International Journal

1. Sanwal, N., Mishra, S., Sahu, J. K., & Naik, S. N. (2022). Effect of ultrasound-assisted extraction on efficiency, antioxidant activity, and physicochemical properties of sea buckthorn (*Hippophae salicipholia*) seed oil. LWT, 153, 112386. (Impact Factor 6.056).
2. Mishra, S., Sahu, J. K., Sanwal, N., & Sharma, N. (2021). Hot air convective drying of small cardamom (Elettaria cardamomum Maton): Evaluation of drying, color, and aroma kinetics. Journal of Food Process Engineering, 44(4), e13649. (Impact Factor 2.356)
3. Mishra, S., Sanwal, N., Sharma, N., & Sahu, J. K. (2022). Multivariate analysis of chemometric based aroma dynamics in small cardamom (Elettaria cardamomum Maton) during drying. Journal of Food Science and Technology, 59(12), 4761-4771. (Impact factor 2.701)
4. Bangar, S. P., Sharma, N., Sanwal, N., Lorenzo, J. M., & Sahu, J. K. (2022). Bioactive potential of beetroot (Beta vulgaris). Food Research International, 111556. (Impact factor 7.425)
5. Nikita Sanwal, N., Mishra, S., Sharma N., Sahu, J.K., & Naik, S. N. (2022). Evaluation of the Volatile Components, Phytochemicals and Antioxidants of Sea buckthorn (Hippophae sp.) Leaves using GC-MS analysis and HPLC with PDA -A gender-based comprehensive metabolic profiling"- *JFDS-2022-0829*. (Impact factor 3.693)- *Under review*

3. National Journal

1. Sanwal, N., Sahu, J. K., Naik, S. N. 2021. "Study on a low-cost, quick technique for the extraction of sea buckthorn (Hippophae sp.) seed oil from Indian Himalayan region of Himachal Pradesh". Environmental Issues, challenges and mitigation measures in Himachal Pradesh. Book Chapter GBPNIHE 11-22. (ISBN: 978- 81-94783-73-2)
2. Sanwal, N., Sahu, J. K., Naik, S. N. 2021. "Integrated processing of sea buckthorn (Hippophae sp.)- A systematic approach". Creation of sea buckthorn value chain in trans Himalaya. Review article GBPNIHE 1-14.

4. Conferences/Seminar

1. Nikita Sanwal and Jatindra K. Sahu. 2019. "Gender based comparative investigation of the phytochemical characteristics of Sea buckthorn leaves from Lahaul-Spiti valley of Indian Himalayan Region", presented at 33rd EFFoST International Conference, 12-14 November 2019, Rotterdam, Netherlands.

2. Nikita Sanwal, Jatindra K Sahu, S N Naik. 2021. ‘Microbial production of cellulose using SCOBY fermentation of Sea buckthorn (Hippophae sp.) leaves during the development of process technology for kombucha tea manufacturing,’ 2nd International Conference on Advances in Biopolymers (ICABP 2021) University of Kashmir, Srinagar, India on 8th-9th November, 2021.
3. Nikita Sanwal, Jatindra K Sahu, S. N. Naik. 2021. A Comparison between Ultrasound extraction and conventional Soxhlet extraction of oil from Sea buckthorn (Hippophae sp.) seed” at 27th international conference of international academy of physical sciences on "Advances in food science and technology" on October 26-28, 2021
4. Nikita Sanwal and Jatindra K. Sahu. 2021. “Comparative investigation of the volatile composition of sea buckthorn leaves using GC MS analyses” presented at ‘National Seminar on Creation of Sea buckthorn Value Chain in Trans Himalaya on 5th March, 2021 organized at GBPNIHE, Himachal Regional Centre, Mohal- Kullu, Himachal Pradesh.
5. Nikita Sanwal, Jatindra K. Sahu, S N. Naik. 2020. “Ultrasound Assisted Extraction of Bioactive Compounds from Sea buckthorn (Hippophae sp.) Leaves.” 4th Amifost 2020, International virtual conference, Amity University, 21st December 2020.
6. Nikita Sanwal, Jatindra K. Sahu, S N. Naik. 2020. “Comparative investigation of the volatile composition of sea buckthorn leaves (male and female) using GC MS analyses’ presented at ‘National Seminar on Creation of Sea buckthorn Value Chain in Trans Himalaya on 5th March, 2021 organized at GBPNIHE, Himachal Regional Centre, Mohal- Kullu, Himachal Pradesh.
7. International webinar on the topic ‘Advances in Food Processing for the development of Functional Foods’ by the department of Food Engineering and Technology, SLIET, Longowal – 148 106 (Pb) India on July 27, 2020.

5. Awards

1. Research Excellence Travel Award (RETA)
   Awarded Rs. 1,50,000/- for presenting research paper at International Conference.

2. Research Scholar Travel Award (RSTA)
   Awarded Rs. 1,50,000/- for presenting research paper at 33rd EFFoST International Conference, 12-14 November 2019, Rotterdam, Netherlands.

3. Second prize winner in oral paper presentation
   Awarded at 2nd International Conference on Advances in Biopolymers (ICABP 2021) University of Kashmir, Srinagar, India on 8th-9th November, 2021.

4. Third prize winner in poster paper presentation
   Awarded with third prize winner in poster presentation at 27th international conference of international academy of physical sciences on "Advances in food science and technology" on October 26-28, 2021

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of ultrasound-assisted extraction on efficiency, antioxidant activity, and physicochemical properties of sea buckthorn (*Hippophae salicipholia*) seed oil

Nikita Sanwal, Sushreesmita Mishra, Jatindra K. Sahu *, S.N. Naik

Food Customization Research Lab, Centre for Rural Development and Technology, Indian Institute of Technology Delhi, New Delhi, 110016, India

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ORIGINAL ARTICLE

Journal of Food Process Engineering

WILEY

Hot air convective drying of small cardamom (*Elettaria cardamomum* Maton): Evaluation of drying, color, and aroma kinetics

Sushreesmita Mishra | Jatindra K. Sahu | Nikita Sanwal | Nitya Sharma

J Food Sci Technol (December 2022) 59(12):4761–4771
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ORIGINAL ARTICLE

Multivariate analysis of chemometric based aroma dynamics in small cardamom (*Elettaria cardamomum* Maton) during drying

Sushreesmita Mishra[1] · Nikita Sanwal[1] · Nitya Sharma[1] · Jatindra K Sahu[1]

Food Research International 158 (2022) 111556

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Bioactive potential of beetroot (*Beta vulgaris*)

Sneh Punia Bangar [a,*], Nitya Sharma [b], Nikita Sanwal [b], Jose M. Lorenzo [c,d,*], J.K. Sahu [b]

[a] *Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson 29634, USA*

Chapter | Private full-text

Fermented Foods in Health and Disease Prevention

August 2022

In book: Microbes in the Food Industry · Publisher: Scrivener Publishing LLC

Lab: Vivek Kumar's Lab

Monalisa Sahoo · Pramod Aradwad · Nikita Sanwal · Show all 6 authors · Satya NARAYAN Naik

Receiving Best Oral Presentation award for Sea buckthorn leaves tea processing

Media Coverage

खुशखबरी

केलांग में आयोजित एक दिवसीय संगोष्ठी में वैज्ञानिकों का खुलासा, कीर्तिंग में इकट्ठी होगी खेप

लाहुल को मालामाल करेगा छरमा

दिव्य हिमाचल ब्यूरो— केलांग

बताया कि इस कार्यशाला के आयोजन से घाटी के किसानों को सीबकथॉर्न (छरमा) की खेती से जुड़ी सारी जानकारी देना मुख्यतः उद्देश्य है।

उन्होंने बताया कि संस्था घाटी के किसानों को एक विपणन मंच प्रदान करने के साथ छरमा की खेती के लिए उच्च किस्म के पौध के बारे में जानकारी देगी, जिससे उन्हें इसके अच्छे दाम प्राप्त होंगे। डा.सरला ने बताया कि संस्थान जिला में एक एकत्रीकरण केंद्र कीर्तिंग में स्थापित कर दिया गया है, जहां किसानों को सीबकथॉर्न (छरमा) के उत्पाद बनाने, पैकिंग व मूल्य वृद्धि व मारकिटिंग संबंधी सभी जानकारी उपलब्ध करवाई जाएगी, ताकि किसानों का इसकी खेती की ओर रूझान बढ़े और किसान अधिक मुनाफा कमा सकें। कार्यशाला में विभिन्न संस्थानों जैसे कि चौधरी सरवन कुमार कृषि विश्वविद्यालय पालमपुर, हिमालयी जैवसंपदा प्रौद्योगिकी संस्थान पालमपुर, भारतीय तकनीकी संस्थान, नई दिल्ली तथा गोविंद बल्लभ पंत संस्थान के वैज्ञानिक सीबकथॉर्न के विभिन्न चरणों में आने वाली परेशानियों और उनके समाधान के बारे में जानकारी दी।

Fig.55 Published in a local newspaper (Divya Himanchal; Kullu district; 03-06-2019)

Kullu | Main | 03 June 2019 | 06

अमर उजाला | कुल्लू | amarujala.com

जाहलमा में सीबकथोर्न के बारे में बताया

उदयपुर (लाहौल-स्पीति)। जीबी पंत राष्ट्रीय सतत विकास संस्थान क्षेत्रीय केंद्र मौहल, भारतीय औद्योगिकी संस्थान दिल्ली की ओर से लाहौल के जाहलमा में सीबकथोर्न पर कार्यशाला का आयोजन किया गया। वैज्ञानिक सरला शाशनी ने सीबकथोर्न से संबंधित परेशानियों को जाना। आईआईटी दिल्ली से आए प्रोफेसर जतिंद्र कुमार साहू ने छरमा प्रोसेसिंग से संबंधित नई तकनीक बताई। ब्यूरो

Fig.56 Cutting of the local newspaper (Amar Ujala; Kullu district; 03-06-2019)

छरमा से होगा किसानों की आमदनी में इजाफा

केलांग में हुआ एकदिवसीय संगोष्ठी का आयोजन

किसानों को दी परेशानियों और उनके समाधान की जानकारी

डा. सरला ने बताया कि संस्थान जिले में एक एकत्रिकरण केन्द्र कीर्तिंग में स्थापित कर दिया गया है जहां किसानों को सीबकथोर्न छरमा के उत्पाद बनाने, पैकिंग व मूल्य वृद्धि व मार्केटिंग संबंधी सभी जानकारी उपलब्ध करवाई जाएगी, ताकि किसानों का इसकी खेती की ओर रुझान बढ़े और किसान अधिक मुनाफा कमा सकें। कार्यशाला में विभिन्न संस्थानों जैसे कि चौधरी सरवन कुमार कृषि विश्वविद्यालय पालमपुर, हिमालयी जैव संपदा प्रौद्योगिकी संस्थान पालमपुर, भारतीय तकनीकी संस्थान, नई दिल्ली तथा गोविंद वल्लभ पंत संस्थान के वैज्ञानिक सीबकथोर्न के विभिन्न चरणों में आने वाली परेशानियों और उनके समाधान के बारे में जानकारी दी। इस संगोष्ठी में घाटी के किसानों ने भाग लिया और सीबकथोर्न छरमा की खेती के बारे में वैज्ञानिकों से संपूर्ण जानकारी प्राप्त की।

किए गए शोध व तकनीकों का भरपूर फायदा उठाएं। इस अवसर पर सीबकथोर्न छरमा की खेती पर किए गए शोध एवं संदर्भ पर प्रकाशित पुस्तिका का विमोचन भी किया गया।

इससे पूर्व गोविंद वल्लभ पंत संस्थान के वैज्ञानिक एवं कार्यशाला के आयोजक डा. सरला शाशनी ने मुख्यातिथि का स्वागत किया और बताया कि इस कार्यशाला के आयोजन से घाटी के किसानों को सीबकथोर्न छरमा की खेती से जुड़ी सारी जानकारी देना मुख्यतः उद्देश्य है। उन्होंने बताया कि संस्था घाटी के किसानों को एक विपणन मंच प्रदान करने के साथ छरमा की खेती के लिए उच्च किस्म के पौध के बारे में जानकारी देगी, जिससे उन्हें इसके अच्छे दाम प्राप्त होंगे।

पंजाब केसरी ई-पेपर Sun, 07 July 2019

Fig.57 Newspaper cutting (Punjab Kesari; Kullu district; 07-07-2019)

वैज्ञानिक विधि से करें छरमा का पौधरोपण

केलांग में छरमा का मूल्य शृंखला प्रबंधन शीर्षक पर संगोष्ठी में दी जानकारी

अमर उजाला ब्यूरो

केलांग (लाहौल-स्पीति)। जिला के मुख्यालय केलांग में छरमा का मूल्य शृंखला प्रबंधन पर संगोष्ठी का आयोजन किया गया।

गोविंद बल्लभ पंत राष्ट्रीय हिमालयी पर्यावरण एवं विकास संस्थान मौहल कुल्लू, भारतीय तकनीकी संस्थान नई दिल्ली के संयुक्त तत्वाधान में कार्यक्रम का आयोजन किया। कार्यक्रम की अध्यक्षता एसडीएम अमर नेगी ने की। कहा कि वैज्ञानिक विधि से पौधरोपण करें, जिससे एक ओर जहां पर्यावरण संरक्षण को बल मिलेगा। लोगों के स्वास्थ्य के लिए छरमा से बने उत्पाद लाभकारी सिद्ध होंगे। छरमा की खेती पर किए गए शोध पर एक पुस्तिका का विमोचन भी किया। इसके चलते छरमा के उपयोग के बारे में भी गहनता से बताया गया। इसके अलावा कार्यशाला की आयोजक डॉ. मालती शतरानी ने मुख्य अतिथि का स्वागत किया और छरमा की खेती से जुड़ी जानकारी दी।

Fig.58 Cutting of the local newspaper (Amar Ujala; Kullu district; 07-07-2019)

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Team at IIT Delhi