NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	GBPI/NMHS/HF/RA/2015-16 /dated 30-03-2016

Date of Submission:	1	9	1	1	2	0	2	2
	d	D	m	m	y	y	y	y

FELLOWSHIP TITLE (IN CAPITAL)

Exploring anti-microbial potential of important medicinal and aromatic plants of Himachal Pradesh, synthesis of silver nanoparticles of the plant extracts

Sanctioned Fellowship Duration: *from* (**01.04.2016**) *to* (**31.03.2019**).

Extended Fellowship Duration: *from* (01.04.2019) *to* (30.09.2019).

***Submitted to*:**

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DSL: *Date of Sanction Letter*

3	0	0	3	2	0	1	6
d	d	m	m	y	y	y	y

DFC: *Date of Fellowship Completion*

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

Part A: CUMULATIVE SUMMARY REPORT

(to be submitted by the Coordinating Institute/Coordinator)

1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	**GBPI/NMHS/HF/RA/2015-16/dated 30-03-2016**
Name of the Institution/ University:	Himachal Pradesh University
Name of the Coordinating PI:	Professor (Dr.) Arvind Kumar Bhatt
Point of Contacts (Contact Details, Ph. No., E-mail):	bhtarvind@yahoo.com +91 9418450009

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	Exploring anti-microbial potential of important medicinal and aromatic plants of Himachal Pradesh, synthesis of silver nanoparticles of the plant extracts					
ii.	IHR State(s) in which Fellowship was implemented:	Himachal Pradesh					
iv.	Scale of Fellowship Operation	Local:		Regional:	✓	Pan-Himalayan:	
iii.	Study Sites covered (*site/location maps to be attached*)	All districts of Himachal Pradesh					
v.	Total Budget Outlay (Crore) :	130,64,040 INR					

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration		
			From	To
Research associates	2	Rajesh Kumar Shandil	01-09-2016	19-09-2019
		Shikha Devi	15-09-2016	31-09-2019
Sr. Research Fellow	4	Mamta Devi	19-08-2016	31-09-2019
		Vishal Ahuja	19-08-2016	30-11-2018
		Nidhi Rana	01-12-2018	31-03-2019
		Ranju Kumari Rathour	01-04-2019	31-09-2019
Jr. Research Fellow	1	Rajeshwer	19-08-2016	31-07-2018
Project Fellows	3	Deeksha Kumari	04-08-2018	31-09-2019
		Kalpana Thakur	19-08-2016	31-09-2019
		Vaishali Sharma	19-08-2016	31-09-2019

2. Research Outcomes

2.1. Abstract:

Background: Medicinal plants and microorganisms represent the rich diversity of Indian Himalayan regions. Plants have been used for thousands of years by several communities to flavor, preserve food, treat health disorders and prevent various diseases including epidemics. Microorganisms are known to degrade and convert waste biomass into certain value added products. Thus both in one hand will help in production of cost effective products beneficial for mankind and on the other hand helps in preservation as well as conservation of environment in an eco-friendly manner.

Objectives/ Aim: 1. Exploring anti-microbial potential of important medicinal and aromatic plants of Himachal Pradesh, synthesis of silver nanoparticles of plant extracts.

2. Microbial utilization of industrial and other wastes for production of commercially viable and useful by-products.

Methodologies: 1. (a) Designing of questionnaire (b) Selection of different plant materials and their extraction by cold percolation method (c) Antimicrobial and MIC analysis using standard protocol (b) Synthesis of nanoparticles from hyper active plant extracts and their characterization (e) Purification of bioactive compounds using standard protocol.

2. (a) Isolation and screening of lignolytic, cellulolytic and xylanolytic microorganisms from the samples collected from various sites of Lahaul Spiti, Kullu, Shimla and Kinnaur districts of Himachal Pradesh, India (b) Optimization of process parameters to enhance the production of extracellular enzymes (i.e. LiP, cellulase and xylanase) (c) Alkali and microbial pretreatment of various types of waste biomass (d)

Isolation of C5 and C6 fermenting microbes (e) Fermentation of hydrolysate to bioethanol using microbes under optimized conditions.

Approaches: 1. Nanoparticles were characterized by XRD, AFM and UV-Visible spectroscopy.

2. Bioactive compounds were analyzed by FTIR and TLC.

3. Ethanol production was analyzed by Gas Chromatography.

Results: In the present study, an attempt has been made to collect ethnobotanical information through several visits, open interviews and group discussions with local people. More than 559 Vaidyas/ herbal practitioners of Himachal Pradesh, found famous for different kinds of traditional healing practices were documented. Based on traditional knowledge and literature study more than 100 medicinally important plants were screened and among these 33 plants as shown in comprehensive report were extracted with 5 different solvents to determine their antimicrobial potential against selected pathogens. Among these 33 plants methanolic extract of *Rhododendron arboretum, Rhododendron campantalum, Terminalia arjuna, Acyranthes aspera, Eucalyptus globules* and acetone extract of *Bombax cieba* were found to be more effective and further used for synthesis of silver and iron nanoparticles. Nanoparticles were characterized by XRD, AFM and UV-Visible spectroscopy. The synthesized nanoparticles then again evaluated for MIC and antimicrobial activity and were found more effective as compared to crude extract. Of these bioactive components of acetone extract of *B. cieba* and methanolic extract of *T. arjuna* were purified by column chromatography. The most effective fraction was than characterized by FTIR, stretching of C=C, C=O, C-H and H-O bond confirmed the presence of alkaloid, flavonoids and terpenoids the extracts.

For fulfillment of 2nd objective, 167 bacterial isolates were isolated from different environmental niches of Himachal Pradesh and screened for extracellular enzymes. Among these, 28 isolates were found positive for LiP production, 27 for xylanase and 80 for cellulase. Among the screened isolates, three isolates i.e. *Bacillus subtilus* L2, *Bacillus safensis* S7 and *Bacillus subtilus* CPS66 were found best producers of LiP, xylanase and cellulase, respectively and further used for saccharification of various types of waste biomass. For the fermentation process, nine C5 and thirteen C6 fermenting microbes were also isolated from rotten fruits as well as local brewery samples. Among these, EFS21 (*Wickerhamomyces anomalus*) and XFS7 *(Candida tropicalis*) emerged out to be best C6 and C5 fermenting strains. Hydrolysate fermented by *W. anomalus* EFS21 and *Saccharomyces cerevisiae* individually produced 7.7 g/l and 10.6 g/l ethanol, respectively. On the other hand, hydrolysate fermented by *C. tropicalis* XFS7 produce 8.73 g/L ethanol from wheat straw with 10% total solid load.

Conclusion: The present study deals with documentation of various herbal practitioners and medicinally important plants of Himachal Pradesh and also leads to scientific validation of herbal decoctions. The efforts made in the present work will positively help to find out methods to increase the effectiveness of plant extract to combat health disorders. The effectiveness of these plant extracts confirmed the medicinal value of these plants. Selected plants are being explored by the herbal practitioners/ local

Vaidyas in rural areas and also mentioned in ancient literature like Ayurveda, Siddha, Homeopathy and others. The usefulness of these plant extracts can be enhanced by various scientific and technological inputs for their future applications. During 2nd objective, different microoragnisms were isolated for the production of lignolytic, cellulolytic and xylanolytic enzymes. The action of these enzymes on lignocellulosic biomass was analyzed by pretreatment. The pretreated biomass was then fermented to ethanol by ethanol fermenting microbes. Ethanol is an attractive renewable source of energy. Conversion of biomass into ethanol by using microbial enzyme is a cost effective process. Thus the ethanol production from waste will help to solve two critical issues of present scenario, the energy crises and environment pollution.

Recommendations: 1. The indigenous extraction and drug formation practices recorded in the present study should be tested and standardized on scientific scale.

2. The commercial harvesting of threatened medicinal plants should be banned, strictly. Most importantly, the native communities need to be sensitized to the sustainable use and conservation of these species.

3. In the present study, C5 and C6 sugars were fermented into ethanol while lignin remained untouched so further there is a need to utilize lignin for the production of value added products in eco-friendly manner.

4. Llignocellulosic based ethanol production is difficult but application of biotechnological interventions and advancements using genetic engineering can make it more useful and easier.

2.2. Objective-wise Major Achievements

S. No.	Cumulative Objectives	Major achievements
	Promotion of traditional/ Indigenous herbal based healthcare practices with emphasis on medicinal and aromatic plants and other non-agriculture resources to improve rural economy in Himachal Pradesh. Antimicrobial potential and the MIC of phytochemicals from selected plants against the certified pathogenic strains. Green synthesis of nano-particles (Ag and Fe) and their characterization using UV- visible spectroscopy, X-ray diffraction, Atomic force microscopy, FTIR analysis, SEM/TEM, etc. Phytochemical analysis,	• Information regarding the uses of medicinal plants, herbal preparations etc. was collected from 559 famous local health practitioners of Himachal Pradesh, India. It was also observed that people inhabiting in Himachal Pradesh have a great respect for local health practitioners and faith in ayurvedic system. • During field trips it was observed that these practices are declining due to insufficiency of income sources as proper licenses are not provided to herbal practitioners by the government. So they feel insecure in adopting these practices as soul income source and feel hesitant in transferring their knowledge to their next generations. • It was observed that most plants were used in combinations and only few were used as single herbal preparations. Mostly preparations were prescribed for oral consumption because most of the formulations were in powder and decoction

	purification of bioactive components from extract.	forms. • Different plant samples *i.e. Bombax ceiba* (bark), *Eucalyptus globules* (leaves), *Berberis aristata* (stem), *Roylea cinerea* (leaves), *Bohemeria macrophylla* (leaves), *Casia fistula* (fruit), *B. variegata* (bark) and *Psidium guajava* (leaves), *Oxalis corniculata* (whole plant), *Terminalia bellirica* (leaves), *Vitex negundo* (leaves), *Acyranthes aspera* (whole plant), *Terminalia arjuna* (leaves), *Woodfordia fruitocsa* (flowers), *Lantana* camara (leaves and flower) *Aegle marmelos* (leaves), Abies pindrow (bark) *Ajuga integrifolia* (leaves), *Adhatoda vasica* (leaves), *Rhododendron arboreum* (leaves), *Cedrus deodara* (bark), *Rhododendron campanulatum* (leaves), *Bryophyllum pinnatum* (leaves), *Allium ursinum* (whole plant) and *Mentha arvensis* (leaves) were screened. • Antimicrobial potential of collected plant samples was determined using agar well diffusion method and it was found that acetone extract of *B. ceiba* was most effective against *Bacillus cereus* (17.9 mm), methanol extract of *E. globules* was most effective against *Staphylococcus aureus* 15.3 mm (40 ul), methanol extracts of *T. arjuna and A. aspera* was found highly effective against *S. aureus* (18 mm) and *Escherichia coli* (18.22 mm) respectively, methanol extract of *L. camara* was found highly effective against *Pseudomonas aeruginosa* i.e. 19 mm, methanol extract of *T. bellirica* was effective against *S. aureus* (16 mm), *A. vasica* showed 19.33 mm against *S. aureus,* acetone extract of *A. integrifolia* showed 21.33 mm zone of inhibition against *P. aeruginosa, m*ethanolic extract of *R. arboreum* showed a zone of 23.33 mm against *E. coli*, chloroform extract of C. *deodara* showed zone of 23.33 mm against *Yersinia pestis, a*cetone and methanol extract of *R. campanulatum* showed activity against *S. aureus* and *P. aeruginosa*, methanol extract of *B. pinnatum* showed activity against *S. aureus* and *P. aeruginosa*, methanol extract of *A. ursinum* showed activity against *E. coli*, chloroform extract of *M. arvensis* showed high level of activity against *E. coli* and *Salmonella typhi*, acetone extract of *R. cinerea* showed activity against *E. coli*, methanol extract against *E. coli* and *Shigella sp*. Acetone and methanolic extracts of *A. catechu* were highly

		effective against *S. aureus* with same zone of inhibition i.e. 12.5 mm. In case of *R. arboreum* methanol extract was highly effective against *S. aureus* (13.5 mm), Both acetone and methanolic extracts of *R. australe were found to be equally effective against E. coli* and *B. cereus with* ZOI of 10 mm*,* aqueous extract of *T. cordifolia* showed highest activity of 12 mm against *P. aeruginosa, A. adenophora* acetone extract was found highly effective against *B. cereus* (15.3 mm), *L. camara* leaves acetone extract was found highly effective against *S. aureus* (18.8 mm) and methanolic extract of *L. camara* flowers exhibited good antimicrobial activity against all the pathogenic isolates used against *E. coli* (15.7 mm), followed by *Shigella sp.*14.9 mm. • Minimum inhibitory conc. of 0.019 mg/100µl was required for the inhibition of *S. typhi* (acetone extract of *B. cieba*), minimum conc. of 0.039 mg/100µl (methanol extract of *E. globules*) was required for the inhibition of *S. aureus* and minimum conc. of methanolic extract of *R. arboreum* i.e. 0.094 mg/100µl was required against *Candida albicans,* minimum conc. of 0.039 mg/100 µl and 0.078 mg/100 µl of methanolic and acetone extract of *A. catechu* and *R. arboreum* respectively were required for the inhibition of *S. typhi* and lowest concentration of acetone extract of *L. camara* leaves required to inhibit the growth of *S. aureus* and *E. coli* was 0.078 mg/100µl and 0.156 mg/100µl respectively. • Phytochemical analysis of the plants *E. globules*, *B. cieba, T. arjuna, A. aspera, A. adenophora* and *L. camara* confirmed the presence of various phytoconstituents i.e. alkaloids, carbohydrates, flavonoids, saponins, steroids, soluble starch, terpenoids and tannins. • TLC was performed for the detection of alkaloids in the solvent extracts of all the plant species i.e. *L. camara* (leaves and flowers) and *A. adenophora* leaves. • AgNp's were synthesized from the acetone extract of *B. cieba* and methanolic extracts of *E. globules*, *T. arjuna*, *A. aspera* and *R. campanulatum* and their characterization was done by UV- Visible spectroscopy, XRD and atomic force microscopy. • Column chromatography eluted fractions of *T.*

		arjuna and *B. ceiba* showed very good antimicrobial activity against various certified pathogenic strains and characterized by UV-Visible spectroscopy and FTIR. • GC-MS study of acetone extract of *L. camara* leaves revealed the presence of total twenty compounds. These compounds were found to have good antimicrobial and antioxidant activities. • The significant activity exhibited for extracts of selected medicinal plants tends to support their traditional use as anti-infective agents. • It was found that the antimicrobial activity was high in solvent extract as compared to aqueous extract. • The results indicated the presence of potent antibacterial compounds in bark, leaves, roots and bulbs extracts. • These can serve a source of new therapeutic agents to control the various pathogenic bacteria.
	Microbial utilization of industrial and other wastes for the production of commercially viable and useful by products like bio-fuels, nutraceuticals etc. Efforts to develop an integrated process of waste utilization.	• Survey of the study area (i.e. Lahaul-Spiti, Kullu, Shimla and Kinnaur) was done to collect the water, dung and soil samples. • Total 167 bacterial isolates were isolated and screened for lignolytic, xylanolytic and cellulolytic activities. • Potential isolate L2 was optimized for LiP, isolate S7 for xylanase and isolate CPS66 for CMCase and FPase activities. • Collection and screening of different types of waste (fruit industrial waste and agro-residues) was carried out for the production of value added products. • Among various substrates, wheat bran resulted in production of maximum cellulase activity and after statistical optimization, 1.7 and 1.3 fold increase in CMCase activity (7.32 U/ml) and FPase activity (2.84 U/ml) was observed, respectively. • Best substrate (i.e. combination of wheat bran and wheat straw) was evaluated for cost effective production of xylanase. • Optimization of xylanase production using cost effective and easily available agro-industrial waste was carried out & after optimization of fermentation and reaction parameters, 19.8 fold improvement in the xylanase activity (141.28 U/ml) was achieved. • On the basis of morphological characterstics,

		biochemical tests and 16S rRNA gene analysis, potential isolates L2, S7 and CPS66 were identified as *B. subtilis* L2, *B. safensis* XPS7 and *B. subtilis* CPS66, respectively. • Pretreatment of various lignocellulosic wastes through physical, chemical and enzymatic treatments. Cellulase from *B. subtilis* was further utilized for the saccharification of various wastes. • Various types of waste biomasses were firstly treated with LiP in order to remove lignin followed by xylanase treatment. Among various biomasses wheat bran followed by wheat straw emerged as potential source of sugars after LiP pretreatment and xylanase treatment. • After saccharification, sugar released from the biomass was estimated by dinitrosalicylic acid (DNS) and HPLC method. • Characterization of treated substrates was done using X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Fourier Transformed Infrared Spectroscopy (FTIR). • For the fermentation process, C-5 and C-6 fermenting microorganisms were also isolated from rotten fruits as well as local brewery samples. EFS21 and XFS7 emerged out to be best fermenting isolate for the fermentation of glucose and xylose, respectively. • The best ethanol fermenting isolates EFS21 and XFS7 were optimized for the production conditions. • Based on ITS1 and ITS4 primer sequencing, potential isolates XFS7 and EFS- 21 were identified as *C. tropicalis* and *W. anomalus,* respectively. • Hydrolysate from best optimized substrate was checked for the ethanol production at flask scale fermentation using *C. tropicalis* XFS7. • Hydrolysate fermented by *W. anomalus* EFS21 and *S. cerevisiae* individually produced 7.7 g/l and 10.6 g/l ethanol respectively. • Production of ethanol was also scaled upto the fermenter level. • Bench scale production in 3 L fermenter (final volume 1.5 L) resulted in 13.01 g ethanol production from the wheat straw hydrolysate containing 96.38 g of total reducing sugars. • Apart from bioethanol production, cost effective xylanases produced from agro industrial waste was

		used for clarification of fruit juices and maximum amount of fruit juice clarification of pineapple (59.68%), apple (58.26%), mausambi (52.04%) and grape (47.12%) was seen under optimized conditions of temperature (45^0C and 50^0C) time (32 h) and enzyme conc. (8%). • In order to evaluate the commercial utilization of CMCase in detergent industry, the compatibility of purified cellulase and commercial detergents was checked and it was found that CMCase was quite stable in different detergents with maximum relative activity retained in case of ariel brand of detergent followed by tide detergent. • Purified CMCase was also used for juice clarification and maximum clarification was achieved in case of pineapple juice followed by mausambi juice and orange juice with relative clarification percentage of 54.2%, 64.4%, 76.5% respectively after 6 hr of incubation.

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, and Reason there of:
1	Formulation of strategy, herbal alternate to tackle pathogens. Selection and collection of plant materials, drying and making stock solution with different solvents. Determination of antimicrobial activity and MIC of selected medicinal plants. Green Synthesis of nanoparticles of the plant extracts. Systematic participatory	Conducted field survey of different areas of Himachal Pradesh. Enlisted local herbal practitioners and important medicinal plants. Scientific validation of traditionally used important medicinal plants. Green synthesis of nanoparticles of hyper active plant materials.	Interacted with more than 559 Vaidyas/ herbal practitioners of Himachal Pradesh and recorded more than 100 most popular medicinal plants (used by these herbal practitioners). **Annexure-A** 33 plants were collected and analysed for their antimicrobial potential. **(5 plants HRA-01 7 plants-HSPF-01 5 plants-HSRF-02**	Over the time period due to modernization traditional health practices have declined. Therefore there is urgent need for its documentation

	documentation of traditional herbal remedies, local vaidyas, medicinal formulations and their scientific relevance in modern day healthcare. Preparation of Inventory of medicinal plants duly protected from misuse. Rapid assessment of selected health practices, prioritized by local communities, identification of constrains and opportunities and linking and integrating small collectors and growers to domestic and international markets. Performing phytochemical screening and purification of bioactive compounds present in plant extracts. Compilation and analysis of data, finalization of results and reports.	Purification of bioactive compounds of most effective plant extracts and their antimicrobial potential.	**8 plants-HSPF-03** **9 plants-HSRF-04**) Silver and iron nanoparticles of hyperactive plant extracts was synthesized, characterized and again analysed for their antimicrobial potential. **Silver nanoparticles (AgNP's)-** ***Rhododendron campanulatum*** **(HRA-01)** ***Eucalyptus globules, Bombax ceiba*** **(HSPF-03)** ***Acyranthes aspera Terminelia arjuna*** **(HSRF-04)** ***Rhododendron arboreum*** **(HSRF-02)** **Iron nanoparticles (FeNP's)-** ***Acyranthes aspera Terminalia arjuna*** **(HSRF-04)** Bioactive compounds were purified by column chromatography from two most efficient plant extracts. **(Bombax ceiba (HSPF-03) and Terminalia arjuna (HSRF-04))**	
2	Isolation of useful	Various samples	167 bacterial isolates	Prototype

	microbes for the utilization of the waste. Model prototype for utilization of waste at lab scale. Scale up of waste utilization and production process	were collected from different environmental niches of Lahaul-Spiti, Kullu, Shimla and Kinnaur districts of Himachal Pradesh, India. Saccharification of waste biomass to bioethanol.	were isolated from different niches and screened for production of extracellular lignocellulosic enzymes and further used for saccharification of various types of waste biomasses. **(HRA-02 ; HSPF-05)** Isolate EFS21 and Isolate XFS7 emerged out to be best C6 and C5 fermenting strains**. (Isolate XFS7-HRA-02; Isolate EFS21 HSPF-05)**	developed for eco-friendly conversion of biomass to biofuel. Because it will help in protection of environment, cost effective process and a viable clean green alternate to non-renewable energy.

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

S. No.	Particulars	Number/ Brief Details	Remarks/ Enclosures
1.	New Methodology developed:	NA	
2.	New Models/ Process/ Strategy developed:	NA	

S. No.	Particulars	Number/ Brief Details	Remarks/ Enclosures
3.	New Species identified:	Total 09 new species were identified i.e. *Bacillus subtilus* L2; *Bacillus safensis* S7; *Pseudomonas fluorescens* LiPRL5 ; *Bacillus subtilis*-CPS66; *Bacillus pumilus* XRL5; *Wickerhamomyces anomalus* EFS21; *Saccharomyces cerevisiae* XFM3; *Candida tropicalis* XFS7.	Of 9 newly identified strains 2 were LiP producer, 3 were observed to produce xylanase, 1 cellulase producer and 3 ethanol fermenting strain.
4.	New Database established:	03 types of new databases were generated	List of 559 LHPs were documented **(Annexure A).** List of Important and rare medicinal plants was prepared **(Annexure B).** List of micro flora identified that utilize and saccharifies lignocellulolic waste **(Annexure C).**
5.	New Patent, if any:	NA	
	I. Filed (Indian/ International)		
	II. Granted (Indian/ International)		
	III. Technology Transfer (if any)		
6.	Others, if any:		

3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology	NA	
2.	Diffusion of High-end Technology in the region	NA	
3.	Induction of New Technology in the region	NA	
4.	Publication of Technological / Process Manuals	NA	
	Others (if any)		

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Existing Baseline	Additionally and Utilisation of New data (*attach supplementary documents*)
1.	**Generation of database of Herbal practitioners**	Herbal practitioners were found to use information regarding the use of medicinal plants and conventional tools for preparation of traditional medicines. A traditional health practitioner possesses an extensive knowledge of curative herbs, natural treatments and specializes in the use of herbal and other medicinal preparations for treating different diseases. But there is no theoretical data of these herbal practitioners.	In order to provide the written evidences in favour of herbal practises and practitioners a database is generated. The new data contains specialization, age, village, experience and gender of LHPs of Himachal Pradesh.
2.	**Database of important and less explored medicinal plant species**	Medicinal plants have been a valuable source of natural products for maintaining human health. Plants are good source of important compounds such as nitrogen containing compounds, phenolic compounds, vitamins and minerals which have anti-	A database is generated containing almost all important and rare uncultivable medicinal plants along with their specificity which already find their uses in Ayurveda, Siddha, Homeopathy and Unani.

		oxidant, anti-tumour, anti-mutagenic, anti-carcinogenic and diuretic activities. Microbial infections pose serious health problems all over the world, and various important plants have been found as a potential source of antimicrobial agents.	
3.	**Database of Lignocellulosic degrading microorganisms**	Biomass refers to renewable organic materials, including agricultural products or agricultural residues, animal waste, municipal waste and fruit industrial waste etc. and can be used as a raw material for the production of bio based products such as fuels, chemicals and materials. The components of biomass mainly include cellulose, hemicellulose and lignin. Next to cellulose, hemicellulose and lignin are the most plentiful renewable biopolymers found in nature and touted as a sustainable source of energy as well as biomaterials.	A new database in tabulated form was created by identifying bacterial strains able to degrade lignin, cellulose and hemicellulose.

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)			
2.	Climate Change/INDC targets			
3.	International Commitments			
4.	National Policies			
5.	Others collaborations			

6. Financial Summary (Cumulative)*

S. No.	Financial Position/Budget Head	Funds Received	Expenditure/ Utilized	% of Total Cost
I.	Salaries/ Fellowships:	7999200	7999200	0
II.	Travel	1981289	1981283	99.999
III.	Contingencies			
IV.	Activities & Other Fellowship Cost:	--	--	--
V.	Institutional Charges:	855927	855927	0
	Total	10836416	10836410	99.999
	Interest earned	298278		
	Grand Total	11134694		

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *Year-wise* Statement of Expenditure (SE)** separately, *ref*. **Annexure I.**

7. Quantification of Overall Research Progress

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	1	
2.	Fellowship Site/ LTEM Plots developed:		
3.	New Methods/ Model Developed:		
4.	New Database generated:	3	
5.	Types of Database generated:	**3**	
6.	No. of Species Collected:	**267**	
7.	New Species identified:	09	
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	10	
9.	No. of SC Himalayan Researchers benefited:	01	
10.	No. of ST Himalayan Researchers benefited:		
11.	No. of Women Himalayan Researchers empowered:	07	
12.	No. of Knowledge Products developed:		
13.	No. of Workshops participated:	03	
14.	No. of Trainings participated:		
15.	Technical/ Training Manuals prepared:	2	Annexure- E
	Others (if any):		

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)	15	15	65.92	Details at supplementary file-1(A)
2.	Book Chapter(s)/ Books:	1	1		Details at supplementary file-1(B)

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
3.	Technical Reports/ Popular Articles				
4.	Training Manual (Skill Development/ Capacity Building)				
5.	Papers presented in Conferences/ Seminars	19	16		Details at supplementary file-1(C)
6.	Policy Drafts (if any)				
7.	Others (specify)				

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

**Please provide supporting copies of the published documents.

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	*Research Questions Addressed*	*Succinct Answers (within 150–200 words)*
1.	Isolation of potential microbe for waste utilization.	Potential bacterial strains i.e., *Bacillus subtilis* L2, *Bacillus safensis* XPS7 and *Bacillus subtilis* CPS66 were isolated from Himalayan regions which carry high lignolytic, xylanolytic and cellulolytic activities, respectively.
2.	Waste management	Eco-friendly process developed for cost effective production of enzymes. It contributes towards the saccharification of lignocellulosic waste to fermentable sugars.
3.	Conversion of waste to useful by-products.	Ethanol fermenting strains were isolated from the Himalayan regions for conversion of fermentable sugars to ethanol.
4.	Conservation of valuable microbial diversity	In this study, different microoragnisms (viz., *Pseudomonas fluorescens* LiPRL 5, *Bacillus subtilis* L2, *Bacillus safensis* XPS7, *Bacillus pumilus* XRL5, *Bacillus subtilis* CPS66, *Pseudomonas sp.* HPUVXlt-16, *Wickerhamomyces anomalus* EFS21, *Saccharomyces cerevisiae* XFM3 and *Candida tropicalis* XFS7) were isolated for the production of lignolytic, cellulolytic and xylanolytic enzymes and value-added products.
5.	Documentation of valuable information regarding the use of traditional practices of healthcare system.	Ethno-botanical information was collected through several visits, questionnaires, open interviews and group discussions from herbal practitioners of Himachal Pradesh.
6.	Exploring traditional medicinal plants used by herbal practitioners of Himachal Pradesh.	Various traditionally important medicinal plants were explored by observing their effectiveness against various disease-causing pathogenic strains. The significant activity exhibited for extracts of selected medicinal plants tends to support their traditional use as anti-infective agents.
7.	Phytochemical analysis, purification of bioactive components from extract.	Extraction of bioactive components to further increase the effectiveness of plant extract.

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	NA
Exit Strategy:	• Promotion of local health practices via a participatory process has helped in enhancing the health security of rural communities. • Documentation and database collection will further be helpful in preparation of action and development plans for the sustainable development of IHR. • New ideas/strategies in combination with traditional resources/practices will help balance economic and ecological interests with due regards to socio-cultural principles. • Developing economically viable industry in view of the enormous resources available in IHR's will contribute to the nation's economic and social growth. • This will not only help in providing self-employment opportunities and but will also improve and uplift the life, economy and social status of the tribal and rural populations. • Therefore from the present work it could be analysed that herbal alternates can effectively substitute modern medicines, as modern medicines are costly and owes many side-effects. • Therefore, effective monitoring of herbal medicine for safety by regulatory authorities is very necessary and it requires collaboration between botanists, phyto-chemists, pharmacologists, and other major stake-holders. • The tribal community is unaware of the worth of traditional wealth, so they are exploiting these treasures ruthlessly. • So an initiative at government level such as deciding minimum support price for marketing of the medicinal herbs will not only help these communities economically but will also can be beneficial for the environment. • On the other hand switching to biofuels for transportation needs would reduce energy dependency on oil imports and could boost rural development, providing farmers with an additional source of income. • In this way, the new fuels could offer considerable potential to promote rural development and improve economic conditions in emerging and developing regions. • The present study has successfully demonstrated conversion of pre-treated wheat straw and corn stover hydrolyzate to fermentable sugars and ultimately to ethanol using the microbes. • Bioethanol production from such crop residues and wheat straw provides additional income to the farmers without compromising the food and nutritional security.

	• Thus, at the field level, promotion of crop cultivation will contribute to boost rural development and can considerably improve the framework for a second-generation biofuel industry. • Also smallholders stand to benefit directly from the additional income generated by selling crop residues. • Additionally, the farmers can take advantage of the new market opportunity and supply the crop residues in large quantities on regular basis to the industry that provides linkages between farmers, input dealers and processors. • Active stakeholder involvement including education and empowerment of farmers along with technical solutions and product manufacturing can also assist tremendously. • Even though the issue of crop residue burning touches many sectors, such as agriculture, environment, economy, education, social aspects, and energy. • In general, liquid biofuels produced from lignocellulosic biomass can significantly reduce our dependence on foreign oil, create new jobs, improve rural economies, reduce greenhouse gas emissions, and improve national security.

22/12/22

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Place: Shimla (HP)
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