MBUST Technical Report No. 2/2021

Reconnaissance for Natural Products Research in Nepal

Technical Report

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July, 2021

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Science and Technology Development Board
Lalitpur, Nepal

(This report should be cited as:
Shrestha, Sabina (2021): Reconnaissance for natural product research in Nepal.
Madan Bhandari University of Science and Technology Development Board, Lalitpur, Nepal)

Preface

Madan Bhandari University of Science and Technology Development Board (MBUSTDB) is undertaking preparations for the establishment of a research-oriented world-class university. In this context, MBUSTB is engaging experts for identification of potential areas for research and teaching, which has the potential of directly contributing to economic development of the country.

This publication presents the outcome of a study related to the identification of research areas related to exploration and utilization of indigenous natural resources, the outcomes of which have the potential of directly contributing to country's economic development. This study is a part of wider studies aimed at exploring the potential of natural products for biomedical, technological and agricultural applications.

MBUSTDB highly appreciates the remarkable hard work and dedication of the author – Dr. Sabina Shrestha – for preparing this publication. MBUSTB appreciates and thanks all other individuals and institutions who contributed to bringing out this publication.

Prof. Rajendra Dhoj Joshi
Chairperson
Madan Bhandari University of Science and Technology Development Board

Acknowledgements

I am grateful to Professor Rajendra Dhoj Joshi, Chairman of Madan Bhandari University of Science and Technology Development Board (MBUSTB) for giving me an opportunity of consultancy service for natural products research for exploration and utilization of indigenous natural resources of Nepal for biomedical, technological and agricultural application.

I would also like to express my gratitude to Prof. Sanjay Nath Khanal, Prof. Bhakta Bahadur Ale, Er. Bidya Ratna Bajrachharya and board members of MBUSTB for providing stimulating suggestions and their insights into need and opportunities in Nepal. I am also thankful to Mr. Shambhu Adhikari, Mr. Rabi Manandhar, Ms. Mira Maharjan, Mr. Rabi Manandhar Sayami and Mr. Laxman Khadka of MBUSTB for official administrative support.

I would also like to express my gratitude to Prof. Nam-In Baek, Kyung Hee University and Prof. Jaehong Han, Chung-Ang University and Prof. Somi Kim Cho, Jeju National University of South Korea their advice for issues identification and instrumental selection for natural products research. I am grateful to Prof. Rameshwor Adhikari, Research Centre for Applied Science and Technology (RECAST) for inspiring advice on fields needing crucial work in Nepal.

My thanks and appreciation to my colleagues Dr. Bhushan Shrestha, Dr. Subash Adhikari, Dr. Sudip Pandey, Dr. Sagar Regmi and Dr. Rinoj Gautam for their cordial support in lively discussions, field trips and helpful suggestions.

I am also thankful to Prof. Tej Kumar Shrestha and Mr. Atmaz Kumar Shrestha at Himalayan Biome Research Institute Pvt. L. for their supportive role for conducting this work.

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List of Abbreviations

CC	Column Chromatorgraphy
DPR	Department of Plant Resource
EIMS	Electron Impact Mass Spectrometry
ESIMS	Electrospray Ionization Mass Spectrometry
EtOAc	Ethyl acetate
FABMS	Fast atom bombardment mass spectrometry
FDA	Federal Drug Administration, USA
GC	Gas chromatography
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH
HACCP	Hazard Analysis Critical Control Point
HPLC	High Performance Liquid Chromatography
IR	Infrared spectroscopy
LC	Liquid chromatography
MeOH	Methanol
MRLs	Maximum Residue Levels
MS	Mass Spectroscopy
MSDS	Material Safety Data Sheet
n-butanol	*n*-BuOH
NMR	Nuclear Magnetic Resonance
NTFP	Non-Timber Forest Products
RP	Reverse Phase
TLC	Thin Layer Chromatography
UHPLC	Ultra-High-Performance Liquid Chromatography\

Executive Summary

The scientific and technological interventions are essential for national development. The herbal heritage of Nepal represents outstanding diversity and a rich source of bioactive components. The use of herbs for healing purposes had been in practice in Nepal since time immemorial. Plant's natural products are secondary metabolites which differ from primary metabolites (carbohydrates and proteins) and semantides (nucleic acids) and have distinctly different biological functions. The identification of species with benefit and production of standard products with quality assurance can give deserved reorganization to the products. The understanding and use of food with antioxidant potential as functional food can provide several health benefits and give healthier alternatives. The uses of phytochemicals in flavour and fragrance have a wide range of applications in industries related to medicine, food, beverage and cosmetics. Madan Bhandari University of Science and Technology (MBUST) development board has prioritized much anticipated research in this field. This reconnaissance for natural products research in Nepal was carried out for finding potential resources for medicine, nutraceutical and agricultural applications. The report embodies following outputs in chapters 1-8:

1. Plant resources of Nepal for medicinal, nutraceutical and agricultural application. Identification of potential plant samples from various parts of Nepal.
2. Natural product types, its utilization and local and global market.
3. Potential natural products for commercialization in national and international markets.
4. Techniques for isolation and extraction of bio-active compounds.
5. Potential research projects for development of natural products and natural product-based proto-type development, patenting, production and marketing.
6. National/international networking and collaboration.
7. Topics for PhD research and Master's degree research.
8. Cost estimation for lab establishment in MBUST for isolation and extraction of compounds from natural products.

The coordinated research effort encompassing growers/collectors, suppliers, manufacturers and consumers can give required outcomes in herbal trade. The formation of Ayurvedic and traditional medicine repositories, implementing intellectual property rights (IPR) polices and the compliance with global market trends are areas needing attention for successful marketing of Nepalese products. The adherence and addressing of legislative requirements related to

environmental protection, food safety and hygiene are necessary for product development. The major areas in Nepal for natural product research include**:**

- Authentic identification of medicinal plant material
- Documentation of surviving ethnobotanical knowledge
- Capacity building in cultivation and processing of products
- Search novel medicinal and functional food material
- Validation of herbal material
- Standardization of products
- Exploration of commercial prospects, value addition, industrial production,
- ISO and other regulatory certifications for lab establishment

The accredited procedure from the stage of production to processing and product delivery can ensure quality products. The principal component analysis, dietary supplements and biopesticides for organic agriculture and food grade preservatives are some of the sectors needing urgent intervention. The main constraint in use of natural products lies in the identification, isolation, and extraction of bioactive compounds which require specialized and diligent effort. There exist several well-established procedures for extraction and isolation of natural products from various sources using chromatographic techniques. The necessity of use of compounds in bioassay as well as to find out mechanisms it is necessary to isolate compounds. The spectroscopic techniques including Nuclear Magnetic Resonance (NMR), Mass Spectroscopy (MS) and Infra-Red Spectroscopy (IR) can be used for elucidation of compound structure. Further, High Performance Liquid Chromatography (HPLC), Gas chromatography (GC/MS) and Liquid chromatography Mass Spectroscopy (LC-MS/MS) techniques can be used for metabolites profiling. Therefore, there is a need to establish instrumentation facilities, national and international collaborative efforts of academia and industries to impart technical advancement and entrepreneurship to bring forth the products form himalayan herbal heritage for humankind.

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Introduction

Natural products are defined as organic compounds in the molecular weight range 100-2000. In a broader sense, the term natural products can also be applied to bulk substances from nature, such as crude plant material, foodstuffs, resins and exudates from plants or extract of plant material (water and alcoholic extracts). Research in natural products chemistry comprises isolation, purification and determination of structure of compounds, which are helpful for comprehensive study of naturally occurring organic compounds. Nature is a source of valuable organic compounds such as phenolics, alkaloids, terpenoids, sterols and several others. The integrated approach in natural products research provides key for drug discovery and also provides opportunity to reveal new industrial applications. The applications of natural products in medicine, functional food and cosmetics are ever growing (He et al., 2019; Yuan et al., 2016; Zhu et al., 2012). In addition, together with nanotechnology there are applications of natural products in several other fields also such as electronics, textiles, food science, energy sectors and agriculture (Caruthers et al., 2007; Kah, 2015; Sastry et al., 2011). The advancement in technology provides the use of natural products as the basis of new drug discovery that are more environmentally sound, economical, and effective (Mandal et al., 2018).

Methodology

The methodology comprised of literature review as well as individual and group discussions. Pertinent bibliometric information sources studied are the Web of Science, Scopus, Mendeley, ChemSpider, SciFinder and Google Scholar. Several keywords like plant's name, phytochemical, Ayurvedic herb, essential oil, biopesticide, nanomaterial, antioxidant, anti-inflammation, immunomodulatory, bioassay and traditional medicine were used to obtain a range of papers for analysis. It involved review papers, research papers, bulletins and official websites. The experimental procedure concept are based on conventional standard protocols given in literature (Bucar et al., 2013; Molyneux and Schieberle, 2007; Sarker and Nahar, 2012) and from own practical experiences (Shrestha et al., 2012; Shrestha et al., 2013 b,c; Shrestha et al., 2016 a,b).

CHAPTER 1: Plant resources of Nepal for medicinal, nutraceutical and agricultural application

1.1 Literature Review

There are 13,067 plant species of plants in Nepal (Chaudhary et al., 2020) which includes algae (1,001 species; (Prasad, 2013), fungi (2,467 species; (Adhikari, 2016), lichens (792 species; (Olley and Sharma, 2013), bryophytes (1,213 species; (Pradhan, 2016), pteridophytes (580 species; (Fraser-Jenkins et al., 2015), gymnosperms (41 species; (K.K. Shrestha et al., 2018), and angiosperms 6,973 species (Groombridge and Jenkins, 2002) have been described from Nepal.

Estimates for the number of medicinal plant species in Nepal range from 701 (DPR, 2007) to 1,700 species (Baral and Kurmi, 2006). It is estimated over 2,000 species of NTFPs plants in Nepal are considered to be potentially useful for food and medicine (Chaudhary et al., 2020). 143 species of commercially important medicinal plants have been reported (Bhattarai and Ghimire, 2006). The medicinal plant resource of Nepal have application in medical systems, including Ayurveda, Tibetan and Unani, and in folk medicine (Gewali and Awale, 2008) and enlisted 30 species of national priority herbs. There are 32 species including Shilajeet (rock exudate) of non-timber forest products and spices in Nepali markets with commercial value (ANSAB, 2020).

High-altitude mushrooms of Nepal such as Yarsagumba (*Ophiocordyceps sinensis*) is well known for its medicinal and functional food value (Shrestha et al., 2012; Shrestha et al., 2013a). Polysaccharides are the major active components of *O. sinensis* with a wide range of bioactivities including immunomodulation, antitumour, antioxidation, and hypoglycemic effects (Yan et al., 2014). The commercial possibilities of another similar mushroom *Cordyceps militaris* is immense (B. Shrestha et al., 2012). Cordycepin as important bioactive compound in *C. millitaris* and its therapeutic effects have been reported (Guo et al., 2016; Khan and Tania, 2020; Phan et al., 2018). Therefore, there is a need for research on Yarsagumba and other *Cordyceps* mushrooms.

Among species several biological activities from *O. sinensis* have been reported that include anti-inflammatory, antioxidant, anti-tumor, anti-metastatic, immunomodulatory, antimicrobial, insecticidal, hypolipidemic, hypoglycemic, anti-ageing, lipolytic, neuroprotective, renoprotective effects, etc. There are seven classes of chemical constituents in natural and

fermented mycelium of *O. sinensis* such as sterols, nucleoside compounds, saccharides and sugar derivatives, fatty acids and other organic acids, proteins, vitamins and inorganics (Zhu et al., 1998a,b).

It is found that *O. sinensis* is characterized by high levels of mannitol and seven other constituents including carbohydrates (glucose, mannitol, trehalose) and amino acids (aspartate, glutamate, lysine, threonine) and had better modulating effects on the gut microbiota compared with *C. militaris* (Ji et al., 2020). Therefore, research of *O. sinensis* fermented culture is still needed to address demand.

Cordyceps (*Cordyceps militaris*) has been extensively cultivated worldwide due to development of culture technique with possibility of growing on substrate like brown rice which exclude requirement of insect host and there is also presence of cordycepin content which is valued for its pharmacological effects (Xie et al., 2009).

Turmeric is a key spice widely used in Nepalese as well as many Asian countries. The curcuminoids compounds such as curcumin, demethoxycurcumin and bisdemethoxycurcumin present in turmeric are known for their therapeutic uses. There is a report of high bis-demethoxycurcumin content in turmeric (*Cucurma longa*) of Kathmandu and identified as suitable for large-scale production (Tønnesen et al., 1989). Curcumin is the principal curcuminoid present in turmeric. There is possibility of improved dissolution behavior in drug nanocrystal-loaded solid dosage forms and bioavailability of cucurmin (Ravichandran, 2013). The reports of recent advancement including antiviral components (Mounce et al., 2017), phytochemical constituents and pharmacology (Yadav and Tarun, 2017), Alzheimer's disease treatment (Chainoglou and Hadjipavlou-Litina, 2020) and antitumor effect (Pan et al., 2020) emphasizes on importance of research on turmeric components.

1.2 Medicinal, nutraceutical and agricultural application of Nepalese Plants

1.2.1 Medicinal Application

Among medicinal plants of Nepal those which are collected in substantial quantities and commercially available are 32 species (Appendix I). Himalayan medicinal plants possess great potential for discovery of novel molecules and new sources of active compounds, mainly

because of the environmental stress to which they are subjected. For example, it was found that the content of podophyllotoxin, which is isolated from podophyllin (a resin produced by species of the genus *Podophyllum*, commonly known as 'may-apple'), is much higher (4.3% of dry weight) in the Himalayan species *Podophyllum hexandrum* than in the American species *P. peltatum* (0.25%) (Jackson and Dewick, 1984). The evaluation of herbs used in Ayurvedic medicine production for its known efficacy and standardization of components used in formulation are still not enough. There is a need for further research for finding bioactive components, their quantification and application. There are 24 species of Medicinal plants prioritized for research and development by the Department of Plant Resource, Nepal Government (Appendix II). On the basis of marketing reports there are some species of plants which need urgent attention from natural products research point of view to get their benefit rather than their traditional export as raw materials are listed in Table 1.

Table 1. Potential plant resources of Nepal for medicinal, nutraceutical and agricultural application

S.N.	Species	Common Name	Application		
			Medicinal	Nutraceutical	Agricultural / Industrial
1.	*Acacia catechu* (L.f.) Willd	Khayer	+	-	+
2.	*Acacia rugata* (Lam.) Fawc. & Rendle	Sikakai	+	-	-
3.	*Aconitum heterophyllum* Wall. ex Royle	Atis	+	-	-
4.	*Aconitum spicatum* (Brühl) Stapf.	Bisjara	+	-	-
5.	*Acorus calamus* L.	Bojho	+	-	+
6.	*Aegle marmelos* (L.) Corrêa	Bel	+	+	-
7.	*Alnus nepalensis* D.Don	Utis	+	-	+
8.	*Amomum subulatum* Roxb.	Alaichi	+	+	+
9.	*Asparagus racemosus* Willd.	Satawari	+	+	-
10.	*Artemisia vulgaris* L.	Titaepati	+	+	+
11.	*Azadirachta indica* A.Juss.	Neem	+	-	+
12.	*Berberis aristata* Sims	Chutro	+	+	+
13.	*Bergenia ciliata* (Haw.) Sternb.	Pakhanbed	+	-	-
14.	*Cannabis sativa* L.	Bhaang	+	+	+
15.	*Chlorophytum borivillianum* (Baker) Engl.	Seto Musli	+	+	-
16.	*Choerospondias axillaries* (Roxb.) B.L.Burtt & A.W.Hill	Lapsi	+	+	+
17.	*Cinnamomum glaucescens* (Nees) Hand.-Mazz.	Sugandha kokila	+	-	+
18.	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Tejpat	+	-	+
19.	*Cinnamomum zeylanicum* Blume	Dalchini	+	+	+
20.	*Curcurma longa* L.	Beshar	+	+	-

21.	*Dactylorhiza hatagirea* (D.Don) Soó	Panchaunle	+	-	+
22.	*Delphinium denudatum* Wall. ex Hook.f. & Thomson	Nirmansi (Jadwar)	+	-	-
23.	*Dioscorea deltoidea Wall. ex Griseb.*	Bhyakur	+	-	-
24.	*Eclipta prostata* (L.) L.	Bhringraj	+	+	-
25.	*Elaeocarpus serratus* L.	Rudraaksya	+	-	-
26.	*Ephedra gerardiana* Wall. ex Stapf	Somlata	-	+	+
27.	*Ganoderma lucidum* Karst	Rato Chyau	+	+	+
28.	*Gaultheria fragrantissima* Wall.	Dhasingre	+	-	+
29.	*Hippophae salicifolia* D.Don	Dalechuk	+	+	+
30.	*Holarrhena pubescens* Wall. ex G.Don	Indrajau	+	-	-
31.	*Juglans regia* L.	Okhar	+	+	+
32.	*Lindera neesiana* (Wall. ex Nees) Kurz	Siltimur	+	+	+
33.	*Leucas cephalotes* (Roth) Spreng.	Dronpuspi	+	-	-
34.	*Mahonia napaulensis* DC.	Jamane mandro	+	+	+
35.	*Morchella conica* Pers.	Gucchi chyau	+	+	+
36.	*Moringa oleifea* Lam.	Sahijan	+	+	+
37.	*Nardostachys grandiflora* DC.	Jatamansi	+	-	+
38.	*Neopicrorhiza scrophularifolia* (Pennell) D.Y.Hong	Kutki	+	-	-
39.	*Ophiocordyceps sinensis* (Berk.) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora	Yarsagumba	+	+	+
40.	*Paris polyphylla* Sm.	Satuwa	+	-	-
41.	*Parmelia nepalensis* Taylor	Jhyau	+	-	-
42.	*Persea odoratissima* (Nees) Kosterm.	Kaulo	+	-	+
43.	*Phyllanthus emblica* L.	Amla	+	+	-
44.	*Piper longum* L.	Pipla	+	+	+
45.	*Podophyllum hexandrum* (Royle) T.S.Ying	Laghupatra	+	-	-
46.	*Prinsepia utilis* Royle	Dhatelo	+	+	+
47.	*Pterocarpus marsupium* Roxb.	Bijayasal	+	-	+
48.	*Rauwolfia serpentina (L.) Benth. ex Kurz*	Sarpagandha	+	-	-
49.	*Rheum austral* D. Don	Padamchal	+	-	-
50.	*Rhododendron anthopogon* D. Don	Sunpati	+	+	+
51.	*Rhododendron arboreum Sm.*	Laliguras	+	+	+
52.	*Rhus parviflora* Roxb.	Satibayer	+	+	+
53.	*Rubia manjith* Roxb. ex Fleming	Majitho	+	-	+
54.	*Sapindus mukorossi Gaertn.*	Ritha	+	-	+
55.	*Shorea robusta* Gaertn.	Saal	+	-	-
56.	*Swertia chirayita* (Roxb. ex Fleming) H. Karst.	Chiraito	+	-	+
57.	*Taraxacum officinale* Wigg.	Tuki phul	+	+	+
58.	*Taxus wallichiana* Zucc.	Lauth salla	+	-	+
59.	*Terminalia bellirica* (Gaertn.) Roxb.	Barro	+	+	+
60.	*Terminalia chebula* Retz.	Harro	+	+	+
61.	*Tinospora sinensis* (Lour.) Merr.	Guduchi, Gurju	+	+	-
62.	*Valeriana jatamansi* Jones	Sugandhawal	+	-	+

63.	*Vitex negundo* L.	Simali	+	+	+
64.	*Zanthoxylum armatum* DC.	Timur	+	+	+
65.	*Zingiber officinale* Roscoe	Aduwa	+	+	+

1.2.2 Nutraceutical Application

It has been reported that wild edible plants are comparable in terms of nutritive values with commercial fruits, and thus they can be promoted as alternative sources of nutrition (Bajracharya, 1980; Sundriyal and Sundriyal, 2001). Among wild edible fruits of Nepal those which can be found abundantly in wild and can be cultivated and are also rich in bioactive components are *Berberis asiatica, Hippophae salicifolia, Mahonia nepaulensis, Myrica esculenta, Choreospondias axillaris, Phyllanthus emblica, Prinsepia utilis, Rhus parviflora, Zizyphus incurva*. These plants are rich in antioxidants and can be used for development of functional food with health benefits and also prevention and cure of disease.

Among species the use of mushroom for nutraceutical application are gaining attention due to the presence of its high quality of proteins, polysaccharides, unsaturated fatty acids, mineral substances, triterpenes sterols and secondary metabolites, with various health benefits such as immunodeficiency, cancer, inflammation, hypertension, hyperlipidemia, hypercholesterolemia and obesity. There are 147 edible species of mushroom in Nepal (Adhikari, 2014) and development of mushroom cultivation can bring socio-economic transformation (Raut, 2019) in Nepal. Among cultivated species of mushrooms the cultivation of expensive mushroom like *Cordyceps militaris* has begun in Nepal. However, it is still beyond the purchasing capacity of the general Nepalese population as it can cost up to NRs. 3,50,000 (fresh weight). Further, the development of technology for mycelium culture of *Ophiocordyceps sinensis* and also research for its cultivation for fructification is essential to compete with the production process in which other nations are already moving ahead.

1.2.3 Agricultural Application

The growth in organic food market necessitates further need of biocides that can be usable in production, storage and transportation process of products. Biocides are usually less toxic and target pest and effective in small quantities. The increasing demand for residue free crop protection products is expected to boost the demand for biocides applicable in agriculture in future. There are some species of plants known for their use as pesticides (Baral and Kurmi, 2006). On the basis of availability of species some species of potential

plants for biocide production are listed in Table 2). Among species, leaves of *Zanthoxylum armatum* (timur) contain and *Juglans regia* (Okhar) are source of 2-undecanone and eugenol, respectively which are well known for their biocide potential. The standardization of formulation with Good Manufacturing Practices (GMPs) with quality assurance for Good Agricultural Practices (GAPs) will be instrumental in development of organic agriculture. Species such as *Sapindus mukorossi* (Ritha) and *Cinnamomum tamala* (Tejpat) are traded in substantial quantities of 447.8 tons and 202.8 tons, respectively from western development region of Nepal (Pyakurel et al., 2014). There can be the possibility of making formulations using species like *Zanthoxylum armatum, Juglans regia*, *Sapindus mukorossi* and *Cinnamomm tamala*.

Table 2. Potential plants with biocidal properties usable for agricultural application

S. N.	**Scientific Name**	**Common Name**	**Nepali Name**
1.	*Zanthoxylum armatum* DC.	Prickly Ash tree	Timur
2.	*Curcurma longa* L.	Turmeric	Beshar
3.	*Lindera neesiana* (Wall. ex Nees) Kurz	Spicebush	Siltimur
4.	*Vitex negundo* L.	Five-leaved chaste tree	Simali
5.	*Jatropa curcas* L.	Curcas nut	Sajiwan
6.	*Falconeria insignis* Royle Syn. *Sapium insigne* (Royle) Trimen	Twallo tree	Khirro
7.	*Diploknema butyracea* (Roxb.) H.J.Lam	Indian butter tree	Chiuri
8.	*Rhododendron lepidotum* Wall. ex G. Don	Rhododendron	Bhale Sunpaati

Commercial possibility of biocide and its scope for economy and/or industrialization

- Production of biocide for organic farming (production, storage, transportation)
- Reduce dependence of synthetic pesticides and utilization own natural resource
- Prevention of human health hazard
- Industrial production of effective formulation

1.3 Collection and identification of potential plant samples from various parts of Nepal

1.3.1 Yarsagumba (*Ophiocordyceps sinensis*) and other *Cordyceps* mushroom

Yarsagumba *Ophiocordyceps sinensis* (Berk.) Sung *et al*. is a well-known entomogenous fungus distributed in alpine nival zone of trans-Himalayas of Nepal, India Bhutan and Tibetan Plateau of China). It is popularly regarded as one of the most-prized herbs. In Ayurvedic literature, this herb was mentioned as Bhu-Sanjivani (Shrestha, 2010). It is regarded as highly effective herbal medicine for several diseases including cancer, hypoglycemia, asthma, hypercholesterolaemia, sexual dysfunction, immunodeficiency, etc. (Zhu et al., 1998a, 1998b). It is well known as tonic, aphrodisiac, cardiotonic and expectorant (Baral and Kurmi, 2006).

Other *Cordyceps* fungi reported from Nepal include *Metacordyceps liangshanensis, Cordyceps coccinea, Cordyceps ishikariensis, Cordyceps militaris, Cordyceps martialis, Cordyceps pruinosa, Ophiocordyceps formicarum, Ophiocordyceps gracilis, Ophiocordyceps nepalensis, Ophiocordyceps nutans, Ophiocordyceps sinensis*, and *Ophiocordyceps sphecocephala* (Shrestha, 2011). In Nepalese *Ophiocordyceps* the alcholic extract contained adenosine and cordycepin 0.288 mg/g and 0.346 mg/g in comparison to pure water extract 0.0918 mg/g and 0.102 mg/g (Kumar et al., 2013).

In Nepal, though species collected from various parts are available *Ophiocordyceps* from western Nepal (Mustang, Dolpa and Darchula districts) is preferred for the quality and size, whereas those with smaller size originating from Sankhuwasabha district are also popular for relatively cheaper price and easy availability. Yarshagumba (*Ophiocordycpes sinensis*) from Dolpa and similar forms from Sankhuwasabha (*Cordycpes* spp.) were obtained (Figure 1). The *Cordycpes* samples were extracted and analyzed on the basis of (BHMA, 1996) and (WHO, 1999). The combination of partition with solvents ethyl acetate, *n*-butanol and adsorption in planar chromatography or thin layer chromatography (TLC) on Silica gel $60F_{254}$ plate was used

to visualize metabolite profile. TLC solvent systems of different concentration and spray reagents were used and observed under ultraviolet lamp to obtain metabolite profile and their retention factor (R_f) on TLC. The thin layer chromatography revealed the presence of components in the sample of Dolpa more prominent and defined than that of Sankhuwasabha. The R_f value in Silica gel 60F_{254} plate in 20:3:1 chloroform: methanol: water revealed presence of some marker components. TLC visualization showed metabolite profile of six prominent compounds R_f 0.21, 0.40, 0.58, 0.65, 0.76 and 0.85. Among compounds sterols at R_f 0.21 and 0.40 can be marker compounds (Figure 2).

Figure 1. Yarsagumba (*Ophiocordyceps sinensis*), B. Other *Cordyceps* mushrooms

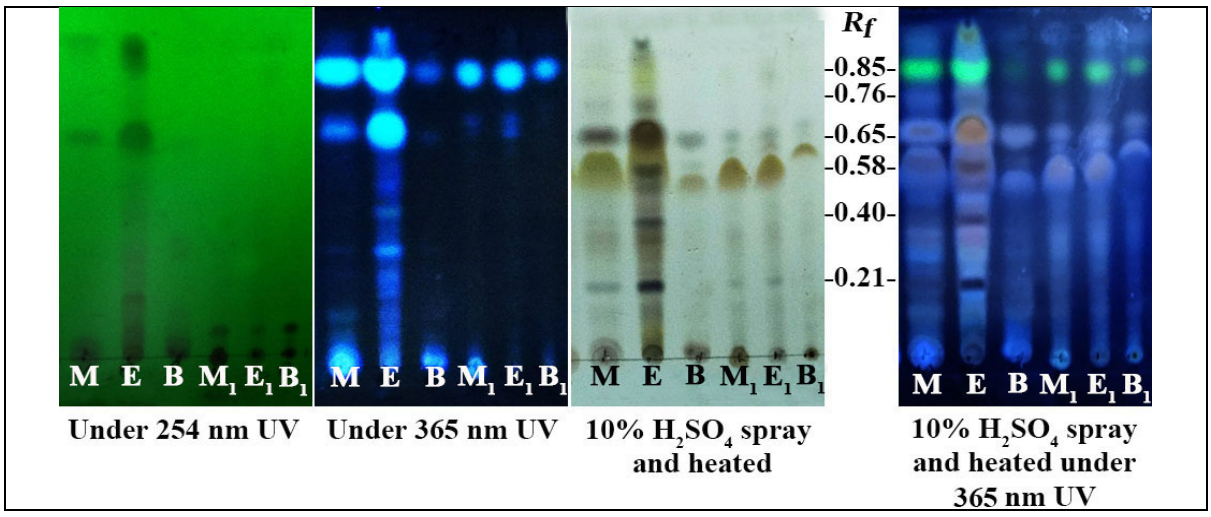
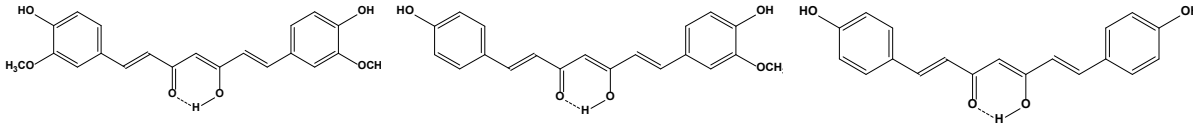


Figure 2. Visual comparison of metabolite profile of *O. sinensis* in methanol (M), ethyl acetate (E), butanol (B) fractions and other *Cordyceps* spp. in methanol (M_1), ethyl acetate (E_1), butanol (B_1) fractons on Silica gel 60F_{254} plate

1.3.2 Turmeric (*Curcuma longa* L.)

Turmeric *Curcuma longa* is used for medicinal properties and as condiment for food preparation. In Nepal *Curcuma longa* is cultivated in tropical and subtropical regions for domestic purposes and few sectors in the tropical sector have started commercial cultivation. However, still sufficient quantity is not produced and as there was import of 7,78,250 kg from India and Ethiopia of turmeric from mid-July to mid-September 2020 (https://customs.gov.np/page/fy-207677). The cost of turmeric is NRs. 60/- per kilogram for unprocessed fresh turmeric in whole sale market. The dried powder of turmeric costs on average NRs. 500. Turmeric cultivated in Bhaktapur and Nawalparasi had been procured. GON, 2020. Statistical Information on Nepalese Agriculture 2075/76 (2018/19) Ministry of Agriculture and Lifestock Development, Kathmandu (GON, 2020).

The dominant volatile components in *Curcurma longa* are *α*-turmerone, curlone, ar-turmerone, *β*-sesquiphellandrene, *α*-zingiberene, germacrone, terpinolene, ar-curcumene, and *α*-phellandrene (Dosoky et al., 2019). The yellow colored pigment in turmeric curcuminoids are used in natural food colorant. Curcuminoids (Figure 3) have been reported for wide range of pharmacological activities such as anti-inflammatory, anticancer, antioxidant, anti-angiogenic and immunomodulating effects (Fadus et al., 2017). Curcurmin is also known for prevention of Alzheimer's disease (Morales et al., 2017; Serafini et al., 2017; Yang et al., 2005).



curcumin demethoxycurcumin bis-demethoxycurcumin

Figure 3. Cucurminods in turmeric

Turmeric cultivated in warmer climates were found to have higher curcumionoid content than turmeric samples from cooler climates, the southern part of Nepal was found to have two times higher content of curcuminoids than turmeric from the northern parts of Nepal (Poudel et al., 2019). They also reported that there was variation in curcurmin content ranged from 79 mg/g (Kalikot) to 65 mg/g (Chitwan). In case of demethoxucurcumin 30.87 mg/g (Kalikot) to 86.96 mg/g (Sunsari). The bisdemethoxycurcumin content ranged from 32.07 (Kalikot) to 95.33 (Dhangadi). It is noteworthy that highest value for curcumin, demethoxucurcumin and bisdemethoxycurcumin for samples of South Korea were only 48.83, 14.01 and 5.53 mg/g which indicates cultivated species in Nepal are of high potential in cucurminods content. Product formulation with *Curcurma longa* content and specified curcumin quantification and

blending with its piperine (from *Piper longum* and *P. nigrum*) combination which can increase 20 fold bioavailability can have market for health benefit.

1.3.3 Rhododendron (*Rhododendron* spp.)

There are 33 species of rhododendron in Nepal (GON, 2018). *Rhododendron arboretum* is most abundant in Nepal. *R. arboreum* starts flowering from third week of March and highest bloom was recorded between the fourth week of April to the second week of May (Ranjitkar, 2013). The flowers of *R. arboretum* is rich source of ascorbic acid 33.12±0.03, total anthocyanins 29.67±0.85 and total phenols 34.14±0.43 mg/100g (Bhatt et al., 2017). Anthocyanidins delphinidin, cyanidin, petunidin, pelargonidin, peonidin, malvidin and flavonols quercetin, 5-*O*-methylquerecetin, myricetin, 5-*O*-methylmyricetin, kaempferol and 5-*O*-methlkaempferol are known to occur in species of rhododendrons (Arisumi et al., 1985). Rhododendrons are rich in natural dye like anthocyanins that can be used as potential photosensitizers in solar cells (Kim et al., 2013) and food industry (Liang-fang, 2013) which are important from the commercial utilization point of view. Therefore, the integration of traditional drug discovery methods together with modern biotechnological techniques has stimulated a renewed interest in this field of isolation, purification, characterization and development of natural product molecules (Agarwal et al., 2020). Rhododendron (*Rhododendron* spp.) samples will be obtained during flowering time during field trips to eastern Nepal.

Rhododendron flowers extracts possess antimicrobial property against *E.coli* maximum zone of inhibition (17 mm) at extract concentration 50mg/ml and *Bacillus cereus* and *Bacillus subtilis* showed minimum inhibition (8mm each) at extract concentration of 25mg/ml (Kashyap et al., 2017). Some species of rhododendron are used in Nepal for their medicinal properties. *Rhododendron anthopogon* (Sunpaate), *R. arboretum* (Laligurans), *R. barbatum* (Kalo Chimal), *R. campanulatum* (Nilo Chimal), *R. cinnabarinum* (Saano Chimal), *R. falconeri* (Gurans, Korlingaa), *R. lepidotum* (Bhaale sunpati), *R. setosum* (Jhuse Sunpati) are known for various medicinal uses (Baral and Kurmi, 2006)**.** *Rhododendron anthopogon* flower contains triolein,3-hydroxy-3-phenyl-propanamide, methyl oleanolate, ursolic acid, 2-hydroxy-4-methoxyl-phenol, 5-methoxyfurfural-hydroxy-coumarin, 23-hydroxy-ursolic acid, β-sitosterol, quercetin, myricetin, ancubin, myricetin -3′-*O*-β-*D*-xyloside, hyperin (Zhou et al., 2010).

1.4 Conclusion

Plants are an important economic resource of Nepal. They are an indispensable source of medicinal, nutraceutical and agricultural application. However, there is limited cultivation practice for these resources and most of the supplies come from natural habitat and few from cultivated sources. The forest user group and non-timber forest product group initiation have played significant roles in collection and marketing of plant based products. The identification of potential plants with market value and production of standard products with quality assurance can give Nepal much needed recognition for the products of Nepal.

The metabolite profiling studies are performed with state-of-the-art high-resolution LC-MS tools that apply the (i) high resolution of ultra-high-performance liquid chromatography (UHPLC) for the chromatographic resolution of isomers and (ii) high-resolution MS methods for molecular formula assignment. While chromatography is usually performed using reverse phase (RP) liquid chromatography (LC), valuable alternative methods rely on the use of hydrophilic interaction liquid chromatography (HILIC) or supercritical liquid chromatography (SFC) for the profiling of very apolar or lipophilic natural products. The establishment of structural links among natural products with molecular analysis and the determination of common features of extract composition can provide valuable information (Wolfender et al., 2019). The isolation, identification and quantification of marker components using spectroscopic analysis using Nuclear Magnetic Resonance (NMR), Infrared spectroscopy (IR) and mass spectroscopy from agro-forestry products can be useful tools in product's quality assurance.

The use of medicinal plants have various applications including anticancer, anti-inflammatory, anti-obesity, anti-diabetes, anti-melanogenesis and wound healing. Food with antioxidant potential and their application in functional food can provide several health benefits. Further, there can be commercial application in flavour and fragrance from essential oil for food, beverage and cosmetics can expand avenues of industrial application. The use of compounds from agriculture and forest waste for organic agriculture practice for pesticide free cultivation and storage can provide much needed alternatives for synthetic pesticides.

CHAPTER 2: Natural product types, their utilization and local and global market

2.1 Literature Review

There are several scaffolds of natural products which have been integral part of human civilization and there is growing need of utilization. The exploration of the chemical diversity of extracts from various biological sources has led to major drug discoveries. The collection and trade of medicinal plants remained as one of the sources of rural livelihood in Nepal (Ghimire et al., 2008; Sharma et al., 2017). The population of world is expected to grow by over a third until 2050, which suggests, in near future, mankind will face multiple challenges as: (1) more food (and fiber) have to be produced to feed the growing population; (2) more feed stocks for a potentially huge bioenergy market and 3) more medicines (*inter alia* of natural origin) to cure human diseases and disorders (Doering and Sorensen, 2018).

Natural products include a diverse group of substances from various natural sources such as plants, bacteria, fungi, insects, arachnids, marine organisms, and higher-order animals. The term "natural products" also refers to complex mixtures from these products and the isolated compounds derived from them. Further, it also includes vitamins, minerals, probiotics—i.e., live microorganisms, bacteria in most cases, that are intended to have health benefits—and special diets for medical conditions or health outcomes (NIH, 2020).

2.2 Natural Products Utilization and Local and Global Market

2.2.1 Utilization of Natural Products in Nepal

2.2.1.1 Medicine

Ayurvedic Medicine has been in practice in Nepal since ancient times and Susruta Samhita and Charaka Samhita are important repositories on herbal medicinal use. Further, Nepali Nighatnu (Devkota, 1968) and Chandranighantu (SVVS, 2012) are valuable compilations representing continued use of herbal resources in treatment in Nepal. Herbal export from Nepal accounts for a source of income in rural Nepal. Singhadurbar Baidhyakhana has inherited 350 years of legacy and prepares 110-145 types of Ayurveda medicines.

According to the Macroeconomic Report of Nepal Rastra Bank, the country exported herbal products worth Rs1.02 billion in 2017-18 which included turmeric, Sichuan pepper (timur),

asparagus (kurilo), cinnamon, spikenard (jatamasi) among others are the country's main exportable herbs. Most of the herbs from Nepal are exported to Indian medicine factories, departmental stores and resold in other countries (The Kathmandu Post, 2018). However, the majority of exports from Nepal are in the form of raw materials collected from the wild and some are cultivated.

Recently the Department of Plant Resource (DPR) has developed a Material Safety Data Sheet (MSDS) for 20 essential oils produced in Nepal for aromatic plants and the other 10 are in process. The domestication and Germplasm conservation of more than 172 species are done at nine botanical gardens of DPR at seven districts covering both tropical and temperate zones. DPR has conducted pharmacognostical research of 20 species of essential oils. Biochemical analysis of *Rhododendron arboreum* leaf extract was performed for anti-diabetes. This type analysis are the initial steps for safeguarding a sustainable market for farmers, collectors and processors and will give standard products to the international market (Dhungel, 2020).

There are 325 medicinal plant processing enterprises in Nepal. Among them Singhdurbar Vaidya Khana (Kathmandu), Bhaskar Herbaceuticals Pvt. Ltd. (Birgunj), Classical Herbal Products Pvt. Ltd. (Banasthali, Kathmandu), Dabur Nepal (Kathmandu), Gorkha Ayurveda Company (Gorkha), Herbs Production and Processing Co. Ltd (Kathmandu), Himalayan Bio Trade Pvt. Ltd. (Kathmandu), Lumbini Aayurved Pharmacy P. Ltd (Butwal), Natural Resource Industries Pvt. Ltd. (Kathmandu) and Siddha Formulation Pvt. Ltd. (Rupandehi) are ten main medicinal plant or plant product exporting enterprises of Nepal (Chapagain et al., 2020). In Nepal Jadibuti Association of Nepal (JABAN), the Ayurvedic Medicine Producers Association of Nepal (AMPAN), the Herbal Entrepreneur Association Nepal (HEAN), the Nepal Herbs and Herbal Products Association (NEHHPA) and the Federation of Community Forestry User's Nepal (FECOFUN) leading centers working in the field of medicinal plants concerned business.

2.2.1.2 Functional Food, Nutraceutical, Flavour and Fragrance and Cosmetic Application

The orthodox tea (*Camellia sinensis*) is famous for antioxidant and antibacterial composition and from Nepal 90% of the products is exported. The production of tea accounts for NRs. 1,766.38 million or USD 15.856 million (Kalauni et al., 2020) in Nepal. Other than *Camellia sinensis,* tea of *Moringa oleifera*, *Thymus linearis, Ocimum sanctum* and *Cinnamomum zeyllanicum* are some other plants used for preparation of tea in Nepal. Fruit candy of

Choerospondias axillaris (lapsi), *Phyllanthus emblica* (amala) and *Tamarindus indica* (imli) are some of production with possibility of market value for their functional food and nutraeutical value. Juice of *Rhododendron arboretum* (laligurans), *Hippophae salicifolia* (dalechuk) and *Aegle marmelos* (bael) are also produced in Nepal. Traditionally herbal decoctions such as Chyavanprash, Chitrak Haritaki, Mimiya, Wasableha and Shilajeet are some of the preparations with nutraceutical values are in use in Nepal. Nepalese honey collected from high altitude regions contained more antioxidants than honey of low altitude region (Neupane et al., 2015). Honey is one of the good sources of nutrients and its usage has continued due to several health benefits. The oil of *Zanthoxylum armatum* (timur), *Zingiber officinale* (adua), *Brassica campestris* (tori) and *Sesamum orientale* (till) are some of the flavor ingredients used in Nepal. In case of fragrance, essential oil is added in incense used in religious rituals and also used in massage and aromatherapy.

2.2.1.3 Agriculture Application as Biocide and Preservative

Plants such as *Artemesia indica, Azadirachta indica, Curcurma longa, Nicotiana tobacum, O. sanctum, Vitex negundo, Zingiber officianale* and *Zanthyoxylum armatum* are used to deter insects are some promising plants in usage (Neupane, 2004). There are serval plants products available in Nepalese market which can be used for various product development (Table 3). However, for operating industry and large scale production cultivation practice needed to be done for continuous and standard supply of raw materials. Plant species such as *Boerhaevia diffusa* is known for not only decreased disease symptom severity but also capable of protecting the plants against infection by viruses (Awasthi and Verma, 2006).

Table 3. List of plants with possibility for product development

S.N.	Botanical Name	English Name	Nepali Name	Type of Product
1.	*Aegle marmelos*	Golden apple, Bengal quince	Bael	Nutraceutical marmalade
2.	*Amomum subulatum*	Large cardamom	Alaichi	Essential oil
3.	*Asperagus racemosus*	Asparagus	Satawari	Tubers diuretic, aphrodisiac, tonic
4.	*Cannabis sativa*	Cannabis	Bhang	Seeds carminative, astringent, aphrodisiac, anthelmintic, anti-inflammatory
5.	*Choerospondias axillaries*	Nepalese hog plum	Lapsi	Nutraceutical marmalade
6.	*Cinnamomum tamala*	Indian cassia	Tejpat	Essential oil for flavor and biocide

7.	*Cinnamomum zeyllanicum*	Cinnamon	Dalchini	Essential oil for flavor and food additive
8.	*Curcurma longa*	Turmeric	Beshar	Nutraceutical, colour dye
9.	*Diploknema butyracea*	Butternut	Chiuri	Seed fat used in ointment
10.	*Gaulthera fragrantissima*	Wintergreen	Dhasingare	Essential oil
11.	*Matricaria chamomilla*	Chamomile	Chamomile	Essential oil for flavour, as herbal tea
12.	*Mentha arvensis*	Mentha	Mentha	Essential oil for flavour, as herbal tea
13.	*Nardostachys jatamansi*	Spikenard/Jatamansi	Jatamansi	Essential oil
14.	*Neopicrorhiza scrophulariflora*	Picrorhiza	Kutki	Rhizome improves appetite and stimulates gastric secretion.
15.	*Ocimum sanctum*	Basil	Tulashi	Essential oil
16.	*Ophiocordyceps sisnensis*	Cordyceps	Yarsagumba	Used as tea for toning lung kidney, chronic cough, weakness and impotency
17.	*Paris polyphylla*	Love apple	Satuwa	Rhizone used as anthelmintic and tonic
18.	*Piper longum*	Long piper	Pipla (long)	Essential oil for flavor and food additive
19.	*Piper pedicellatum*	Round piper	Pipla (round)	Essential oil for flavor and food additive
20.	*Rhododendron anthopogon*	Rhododendron	Sunpate	Essential oil for flavour, fragrance. Flower as herbal tea
21.	*Rhododendron arboreum*	Rhododendron	Laligurans	Nutraceutical, Flower as herbal tea, juice
22.	*Rhododendron lepidotum*	Rhododendron	Bhaale sunapaati	Essential oil for flavour, fragrance. Flower as herbal tea
23.	*Rubia manjith*	Madder	Majitho	Natural dye
24.	*Sapindus mukorossi*	Soap nut	Ritha	Organic surfactant, emulsifier
25.	*Swertia chirayita*	Chiretta	Chiraito	Used for intermittent fever and as tonic
26.	*Terminalia chebula*	Chebulic myrabalan	Harro	Fruit carminative and tonic
27.	*Terminalia bellirica*	Myrabalan	Barro	Fruit astringent, tonic
28.	*Thymus linearis*	Himalayan thyme	Ghodaa Marchha	Essential oil, tonic
29.	*Zanthoxylum armatum*	Prickly ash	Timur	Essential oil for flavor and biocide
30.	*Zingiber officinale*	Fresh ginger / Dried ginger	Aduwa / Sutho	Essential oil for flavour

2.2.2 Utilization of Natural Products in Global Context

2.2.2.1 Medicine

The natural products have a wide range of pharmacophores and a high degree of stereochemistry (Drewry and Macarron, 2010), which give special structural versatility, sometimes beyond human imagination, made them all-time important for new drug development. They have the attributes of metabolite-likeness which also made them special. The compounds may act as a substrate for one or more of the many transporter systems and are helpful in transporting to their intracellular site of action (Harvey et al., 2015). The recent developments in genomic research made advancement in understanding of biosynthetic processes. It is also revealed that there are many areas with untouched biosynthetic pathways needing exploration. The pharmaceutical sector is facing the challenges of new effective and safe medications in most of the therapeutic use due to the limitations and ineffectiveness of available drug, natural products are being appreciated as the foundation of novel leads which can pave path further development and are expedited by emerging technologies.

Among medicinal materials derived from natural sources taxol (paclitaxel) which is a potent anti-neoplastic and anti-mitotic taxane drug deserves special mention. It is known to bind to the N-terminus of β-tubulin and stabilizes microtubules arresting the cell cycle at the G2/M phase. The microtubule damage induces apoptosis through a JNK-dependent pathway followed by a JNK-independent pathway, perhaps related to the activation of protein kinase A (PKA) or of Raf-1 kinase, resulting in phosphorylation of Bcl-2. Taxol is extracted with difficulty and in generally low yields, of the order of 100 mg/kg in the case of taxol, from the trunk barks of different *Taxus* species, 10-deacetylbaccatine III is precursor of Taxol is extracted much more readily and in better yields (300 mg/kg of leaves) from *Taxus* spp. leaves. semi-synthetic processing of 10-deacetylbaccatine III can convert it into Taxol (Liu et al., 2016)**.** Taxol is used in treatment of ovarian, breast, lung, bladder, prostate, melanoma, esophageal, and other types of solid tumor cancers as well as Kaposi's sarcoma. This also signifies the importance of search for natural products based compounds for treatment of diseases.

The uses of antibiotics are still considered one of the most significant interventions in saving human life. There is still an alarming rise in the failure to treat bacterial infections due to the generation of antibiotic-resistant “superbugs” (Ali et al., 2018; Blair et al., 2015). The

conventional antibiotic targets are cell wall synthesis (beta lactams), cell membrane (polymyxubs), RNA polymerase (rifamycin), nucleic acid synthesis (quinoiones), protein synthesis and foliate synthesis. The reinvigoration of natural-product-based research for antibiotic discovery and utilization of the classic tools of synthetic chemistry, microbiology, and chemical biology have been applied in novel ways to combat bacterial pathogenesis (Abouelhassan et al., 2019). The actinomycetes found in the rhizosphere of the alder tree (*Alnus nepalensis*) had the potential of killing multi drug resistant bacteria (Baniya et al., 2019), Further, search of microorganisms from different habitat of Nepal can be source of antibiotic.

The most recent calamity the world faced due to COVID-19 pandemic which has led to search for natural remedies for boosting immunity for protection of health and regaining vigor and vitality after COVID-19 infection. The phytoconstituents obtained from *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Giloy), *Ocimum sanctum* (Tulsi) as regarded as remedial option against COVID-19 (Shree et al., 2020). The facility for conducting test of natural products is necessary for research in this field to find out potential of Himalayan herbal materials.

2.2.2.2 Functional Food, Nutraceutical, Flavour and Fragrance and Cosmetic Application

German institutes, Gesellschaft für Internationale Zusammenarbeit (GIZ) and Deutsche Import Promotion Desk (IPD) are supporting a model project promoting the export of Medicinal and Aromatic Plants (MAPs) have done considerable work for promoting MAPs of Nepal. It is reported that the global sales of flavour and fragrance manufacturers increased by 5.5% annually to € 22 billion (2012-2016) and there is growing demand for natural cosmetics and natural flavorings. In case of trade with Germany (Europe) for example in Nepali supplies of essential oils are still very little, only 0.1% of the total German import value in 2016 (Blom and Trujillo, 2018). The key features of most of the products of Nepal include collections from the wild which are all-natural, adulteration free, organic and there is 3000 years of history back-up the herbs, spices and essential oils from Nepal. Nepalese products include food (ginger, turmeric, Sichuan pepper (timur), black cardamom, long peeper, cinnamon, cosmetic ingredients (essential oil, fatty oils, dyes, henna, floral water, aromatherapy (essential oil), natural dyes and possibility of development of pharmaceuticals. Curcumin, the yellow pigment of *Curcuma longa*, in EU it bears a status of food ingredient (E100) and is present in many food additives (Georgiev, 2014). Exports of essential oils from Nepal are relatively small, but are on the rise.

Between 2010 and 2015, exports of essential oils rose by a compound annual growth rate of 11% from US$ 974 to 1,626 thousand. By volume, exports rose from 21 to 37 tons. The USA has become the largest destination (by value), followed by France, Belgium, Germany, UK and Canada, while exports to India is dropped by 30%. Most Nepal oils are limited supply oils. The best opportunities for MAPs and oils from Nepal are in three following key segments: premium flavour (natural health food and organic food), premium beauty and personal care products and Pharmaceutical (herbal traditional medicines) (Boeckel, 2017) .

2.2.2.3 Agriculture Application as Biocide and Preservative

Biocides are applied in order to deter, control or kill harmful and unwanted organisms. Biocides can not only adversely affect harmful organisms but also humans, the environment and endangered species. There are four class and 23 different product types (PAN Europe, 2020)

I. Disinfectants and general biocidal products

1. Human hygiene biocidal products
2. Private area and public health area disinfectants and other biocidal products
3. Veterinary hygiene biocidal products
4. Food and feed area disinfectants
5. Drinking water disinfectants

II: Preservatives

6. In-can preservatives
7. Film preservatives
8. Wood preservatives
9. Fibre, leather, rubber and polymerised materials preservatives
10. Masonry preservatives
11. Preservatives for liquid-cooling and processing systems
12. Slimicides
13. Metalworking-fluid preservatives

III: Pest control

14. Rodenticides
15. Avicides
16. Molluscicides
17. Piscicides
18. Insecticides, acaricides and products to control other arthropods
19. Repellents and attractants

IV: Other biocidal products

20. Preservatives for food or feedstocks
21. Antifouling products
22. Embalming and taxidermist fluids
23. Control of other vertebrates

2.3 Conclusion

There is still a need for identification of Nepalese medicinal materials and new edible botanicals with functional food and nutraceutical value and prevention of human health hazards. The exploration of diversity from extreme environmental niches and endemic diversity can be an important source. Further, sstandardization of herbal products and value addition and marketing is needed.

The organic cultivation solutions include nano fertilizer and biocides (production, storage, transportation). Reduce dependence on synthetic pesticides and utilization of our own natural resources. The commercial production of effective formulations based on essential oils produced in Nepal for organic farming and biocidal use do have both industrialization possibility and economic gain.

The challenges and opportunities in Nepal in natural product research include**:**

- Authentic identification of medicinal plant material
- Documentation of surviving ethnobotanical knowledge
- Capacity building in cultivation and processing of products
- Search novel medicinal and functional food material
- Validation of herbal material
- Standardization of products
- Exploration of commercial prospects, value addition, industrial production,
- ISO and other regulatory certifications for lab establishment

2.3.1 Market Access Requirements in Global Context

The understanding and fulfilling of global market trends is required for successful marketing of Nepalese products. The obligations of fulfilling legislative requirements are

mandatory and subject to penalties if they are ignored, especially in Europe and USA. In the case of natural products, fields related to environmental protection, food safety for consumers and hygiene are applicable. The regulatory barriers may obstruct entry to USA and Europe. In Food legislation, Hazard Analysis Critical Control Point (HACCP rules) must be respected and a Certificate of origin must be submitted. In Europe, there are regulations on requirement of Maximum Residue Levels (MRLs), traceability and a phytosanitary certificate.

In case of cosmetic regulation in the USA, the Federal Drug Administration (FDA) requires that every ingredient must be substantiated for safety before going to market. In Europe, a cosmetic safety assessment must be carried out and regulations regarding herbal traditional medicines are extremely strict. The best option is to export the MAP or oil as herbal food supplement and care should be taken regarding claims.

In the case of the European Union, the General Food Law (Regulation (EC) 178/2006) prohibits the introduction of unsafe food to the EU market and defines the traceability of its ingredients through all stages of production, processing and distribution. The importer/ trade partner must be able to give clarification. Hygiene of foodstuffs (Regulations (EC) 852/2004; 853/2004; 854/2004) is based on the HACCP principles. EU legislation restrict pesticides and contaminants in EU food specifying MRLs of contaminants.

To ensure and improve the quality use of best quality and timely harvest, storage and extraction of material with proper distillation and storage in hygienic handling and organic certification can contribute to enhance the quality of the product.

CHAPTER 3: Potential natural products for commercialization in national and international markets

3.1 Introduction

There are considerable interests in herbal drugs, medicines, and natural product-based herbal products and it is increasing. The bioactive chemical compounds both in pure form and as homogenous extracts have been derived from medicinal plants. The research in this field has made it possible for the development of new drug leads. They also represent an excellent source of molecules for the production of food additives, functional foods, nutritional products, and nutraceuticals for the growing number of natural food companies. There is growing interest in herbal products and bioactive components for immunity boosting mechanisms in wake COVID-19 pandemic (Galanakis et al., 2020; Nilashi et al., 2020). The important associations in this are Nepal Forest Industry Association, JadiButi Entrepreneurs Association of Nepal (JABAN), and Nepal Herbs and Herbal Products Association (NEHHPA). This report embodies the natural products of Nepal with potential for commercialization in national and international markets in order of priority in terms of economic significance.

3.2. Literature Review

There are approximately 20,000 different medicinal plants in 91 different countries in the world (Sasidharan et al., 2011). The plantation and production of crops and using their bioactive natural compounds employing extensive extraction processes require significant research and market-related considerations (Belwal et al., 2018). The global trade in medicinal plants has increased 3% annually since 2010, with an estimated total import of 673,564 tons in 2014 valued at 2.7 billion USD and the global medicinal plant market is dominated by China and India in both export and import (GIZ, 2017). There are 701 species of medicinal plants in Nepal (DPR, 2007). Some compilation of Nepalese herbal diversity and phytochemical constituents exist in literature (Gewali and Awale, 2008; Watanabe et al., 2005). It is estimated over 2,000 species of NTFPs plants in Nepal are considered to be potentially useful for food and medicine (Chaudhary et al., 2020). In Nepal there exist 32 species including Shilajeet (rock exudate) are in trade (ANSAB, 2020). Other than herbal non timber forest products (NTFP), the export of rosin and resin acid export value in F.Y. 2017/18 amount was NRs. 1.59 billion and export volume in F.Y. 2017/18 140 tones (MOC, 2019). The Nepalese herbal material supplied to India and China includes 300 species (Pyakurel et al., 2019) and the annual export has been

estimated to be between 7,000 and 27,000 tones at a value of USD 11 to 48 million [in 2020 value, inflation-adjusted figures derived from (Olsen, 2005) by (Caporale et al., 2020)].

There had been some effort in herb cultivation and processing in Nepal but significant herbal material collected from Nepal are either sent to India and China or other countries and whenever those countries stop importing herbal material remains in Nepal. There is a need for identification, prioritization, conservation, propagation, cultivation, processing and marketing in coordination with government and non-government organization(s), communities and private sector involvement in the public private partnership model (Dhungel, 2020). There is a need of attaining international accreditation and international recognition of test results that require sustained commitment and investment over many years (World Bank, 2018).

3.3 Natural Products for Commercialization in National and International Markets in Order of Priority in Terms of Economic Significance

This is a substantial share of the export of Ayurvedic products from Nepal and it is valued at around USD 7.5 million in 2015, though it is a fraction of Indian medicinal plant export (USD 290 million in 2017) or essential oil export from France which includes extracts from imported Nepalese plants (USD 399 million in 2017) (World Bank, 2018). There are 32 species of MAPs traded in and from Nepal (Appendix I) and have significant economic contribution in terms of value and volume. Eleven species have been prioritized for agro-technology development by the Department of Plant Resource (listed in Appendix II) (Kalauni and Joshi, 2018). There are several species of Nepal that are source of important natural products important from nutraceutical (Shrestha et al., 2017) and organic agriculture point of view. The potential sectors of commercialization in national and international markets are as follows:

3.3.1 Standardized Ayurvedic Herbal Material

New botanical drug for which extensive prior human use is not available, a more extensive nonclinical pharmacological/toxicological assessment is warranted. This assessment should be similar to that for non-botanical drugs (e.g., synthetic drugs), and recommendations should follow appropriate regulating bodies in Nepal like Department of Drug Administration (DDA), and also International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and U. S. Federal Drug Administration's (FDA) guidelines. Batch-to-batch variations (e.g., a variation in chemical composition) are known to exist in different batches of the botanical drug substances. It is of both scientific and regulatory

interest to learn the impact of such variations on the therapeutic effect of botanical drug products. Sufficient quantities of the botanical raw material and drug substance from the different batches should be retained for future chemical characterization and/or pharmacological/toxicological testing (FDA, 2016).

In context of Nepal the drug development of botanicals particularly in case of Ayurvedic drug is still challenging as botanical drug product usually consists of more than one chemical constituent, it may be technically challenging to use standard pharmacokinetic measurements to substantiate the systemic exposure of a botanical drug in animals. Some effort in Standards of quality for raw and processed materials have been documented in Nepal recently (Srivastava, 2019). Monitoring representative chemical constituent(s) in a botanical drug product using a sensitive analytical method can provide information regarding systemic exposure. The chemical constituents of a drug product that contribute to toxicity or pharmacology should be assessed in the pharmacokinetic/toxicokinetic studies.

Plant species included in export data of Nepal (Appendix I) and Caporale et al., (2020) are the possible candidate plants needed standardization for value addition. *Delphinium himalayai, Swertia chirayita, Paris polyphylla, Neopicrorhiza scrophulariiflora, Valeriana jatamansi* and *Zanthoxylum armatum* are most potential species commercialization in national and international markets due to their bioactive components and they are produced in substantial quantity in Nepal. Plant species prioritized for standardized medicinal material development are listed in Table 4.

Table 4. Medicinal plants needing prioritization for standardized medicinal material development

S.N.	Species	Remark
1.	*Cinnamomum glaucescens*	Contains sabinene, 1,8 cineol, α-terpenol and methyl cinnamate . Used in perfumery and traditional medicine for demulcent (soothing inflammation), stimulant, analgesic, antiseptic, astringent, and carminative properties (DPR, 2007).
2.	*Crocus sativa*	Source of crocins and volatile components safranal, crocetin, picrotoxin. Fragrant, stimulant, tonic, aphrodisiac, anti-spasmodic, diuretic (Husain et al., 1992).
3.	*Delphinium himalayai*	Juice of the root is given in cases of cough and colds. An infusion of the root is put in wounds in the hooves of cattle to expel worms or kill germs (Gurung and Pyakurel, 2017).

4.	*Gaultheria fragrantissima*	Major constituent is methyl salicylate. Aromatic stimulant, carminative and antiseptic (DPR, 2007)
5.	*Morchella conica*	Used as delicious food and tonic (Gurung and Pyakurel, 2017).
6.	*Nardostachys grandiflora*	Source of valeranone, spirojatamol, oroseol, jatamansin. Substitute for valerian. Oil air tonic, rhizome considered tonic, stimulant, antispasmodic, diuretic, stomachic and laxative (DPR, 2007).
7.	*Neopicrorhiza scrophulariiflora*	Contains kutkin, kurrin, kutkiol, kutkisterol (Watanabe et al., 2013). Rhizome useful in dropsy, antiperiodic fever, anaemia an jaundice (DPR, 2007).
8.	*Paris polyphylla*	Source of pariphyllin A, pariphyllin B, parsterone, polyphylin D and trillin. Rhizome is anthelmintic and tonic (Buckingham, 1994).
9.	*Phyllanthus emblica*	Source of chebulic acid, vitamin C, root contains ellagic acid. Fruits are refrigerant, diuretic, laxative and one of the ingredient of Triphala (DPR, 2007).
10.	*Piper longum*	Piperine, kadsurin A, cepharadione A. Roots ae carminative, useful in bronchitis, abdominal pain, fruite spike used as spices ad used in cold cough, asthma, hiccup (Watanabe et al., 2005).
11.	*Rhododendron anthopogon*	Triterpenoids, flavonoids and sterols are the major constituents of *R. anthopogon*. Relaxing uterus and alleviating dysmenorrhea and for uterine relaxing menstruation pain. Its essential oil β-cadinene (11.4%) is antimicrobial (Gewali and Awale, 2008)
12.	*Taxus wallichiana*	Taxanes, including paclitaxel, 10-deacetylbaccatin III, baccatin III, isotaxiresinol, secoisolariciresinol. Anticancer use in breast and uterus cancer, also used in asthma and bronchitis (Watanabe et al., 2005).
13.	*Valeriana jatamansi*	Major components were isovaleric acid (52.95%), patchouli alcohol (18.20%), 3-methyl pentanoic acid (6.89%). Its oil is antifungal and antibacterial. The aqueous extract of together with pentobarbital sodium enhanced sedative and hypnotic effect (Gewali and Awale, 2008)
14.	*Zanthoxylum armatum*	Compounds linalool (58.3%), limonene (24.46%) and methyl cinnamate (8.92%) present in fruit. *Z. armatum* is a part of patented medicinal formulation to treat toothache, bleeding gums, swollen gums. It is larvicidal and leach repellent (Gewali and Awale, 2008).

3.3.2 Essential Oil and their Processing for Aromatic Components for Food Additives, and Fragrances / Cosmetic and Biocidal Products

Plant species such as *Ocimum sanctum* (Tulshi), *Curcurma longa* (Besar), *Zanthoxylum armatum* (Timur), *Lindera neesiana* (Siltimur), *Thymus linearis* (Ghodae maarchaa), *Mentha arvensis* (Babari), *Cyperus rotundus* (Mothe) are in use in Nepal as condiments and various Ayurvedic preparation based on them, so can be of use in food grade biocide preparation. Plant species commercially cultivated and processed essential oils are listed in Table 5.

Table 5. Commercially cultivated and processed essential oils in Nepal

S.N.	Scientific Name	Nepali Name	2019/2020 estimated Production
1.	*Cinnamomum glaucescens*	Sugandhakokila	2,400 kg
2.	*Cinnamomum tamala*	Indian bay leaf, Tejpat	1,000 kg
3.	*Cymbopogon flexuosus*	Lemongrass oil	3,840 kg
4.	*Cymbopogon martinii*	Palmarosa oil	2,129 kg
5.	*Cymbopogon winterianus*	Citronella oil	5,716 kg
6.	*Matricaria chamomilla*	Chamomile oil	1,006 kg
7.	*Mentha arvensis*	Mentha	11,016 kg
8.	*Nardostachys jatamansi*	Sugandhawall	720 kg
9.	*Ocimum basilicum*	Basil oil	3,840 kg
10.	*Zanthoxylum armatum*	Winged prickly ash	2,400 kg

3.3.3 Functional Food and Nutraceuticals

The phytochemicals present in functional foods have a role in preventing disease and promoting health. Functional food and nutraceuticals have an important role in enhancing the body's immune system, circumventing various diseases including heart disease, hypercholesterolemia, digestion and ageing (Rao, 2003). Fruits and fruit juice are known for cognition and brain health (Keservani et al., 2016) and their phenolic compounds are antioxidant phytochemicals plays role in functional and health-promoting properties (Swallah et al., 2020). Plants with functional food and nutraceuticals value are listed in Table 6.

Table 6. Plants prioritized for standardized functional food and nutraceuticals development

S.N.	Species	Remark
1.	*Aegle marmelos*	Marminal, xanthotoxol, anthraquinone. Ripe fruit aromatic used in diarrhoea and dysentery whereas run ripe fruit is laxative, and dyspepsis, stomachache (Watanabe et al., 2005).
2.	*Berberis aristata*	Berberine, berbamine, caffeic acid, quercetin, rutin, chlorogenic acid, and meratin (Gewali and Awale, 2008)
3.	*Choerospondias axillaris*	Epigallo-catechin, catechin, epicatechin and their galloylated derivatives (Li et al., 2015)

4.	*Cordyceps militaris*	Adenosine, iso-sinensetin, dimethylguanosine immunity, Cordycepin, xylitol (Jiang et al., 2011; Jin et al., 2018; Wada et al., 2017). It is known for rreduction of blood sugar, blood lipids, resist tumour delay aging (Wei et al., 2020). It is also known for several properties such as pro-sexual, anti-inflammatory, immunomodulatory, anti-oxidant/anti-aging, anti-tumour / anti-cancer / anti-leukemic, anti-proliferative, anti-angiogenetic, anti-metastatic, anti-microbial, anti-bacterial, anti-viral, anti-fungal, anti-protozoal, insecticidal, larvicidal, anti-fibrotic, steroidogenic, hypoglacaemic, hypolipidaemic, anti-diabetic, anti-HIV, anti-malarial, anti-fatigue, neuroprotective, liver-protective and other synergistic activities.
5.	*Curcuma longa*	curcumin (curcumin I), demethoxy curcumin (curcumin II) and bis demethoxy curcumin (curcumin III), ar-turmerone (51.8%) ar-turmerol (11.9%) (Singh et al., 2002; Thomas et al., 2011).
6.	*Eclipta prostrata*	Echinocystic acid, wedelolactone. It provides relief from headache, fever, glandular swelling elephantiasis, cuts and wounds (Watanabe et al., 2005).
7.	*Ephedra gerardiana*	Norephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrine. It is sympathomimetic agent and finds its application as stimulant, decongestant and appetite suppressant (Gewali and Awale, 2008)
8.	*Ganoderma lucidum*	Triterpenoids and polysaccharides. Its triterpenoids have been reported to posses hepatoprotective, anti-hypertensive, hypocholesterolemic and anti-histaminic effects, anti-tumour and anti-engiogenic activity, effects on platelet aggregation and complement inhibition. Polysaccharides, especially *β-D*-glucans, have been known to possess anti-tumor effects through immunomodulation and anti-angiogenesis (Boh et al., 2007)
9.	*Hippophae salicifolia*	Major oil constituent, oleic (31.9%), linoleic (27.9%), linolenic (17.8%) and palmitic (16.3%) acids. Its oil is potent antioxidant and anti-cancer cure, cardiovascular risk reducer, skin diseases and gastrointestinal ulcer and liver protectant, also used in cosmetics, health products and nutraceuticals (Gewali and Awale, 2008)
10.	*Mahonia napaulensis*	Neprotine, umbellatine. Antidysenteric and anti-diarrhoeic, diuretic (Watanabe et al., 2005).
11.	*Ophiocordyceps sinensis*	Amino acids, cyclic dipeptides, saccharides and sugar derivatives, steroids, nucleotides and nucleosides, polyamines, saturated and unsaturated fatty acids, mannitol, oligosaccharides, polysaccharides and steroids including ergosterol, delta-3 ergosterol, ergosterol peroxide, sitosterol, daucosterol and campesterol are present in *O. sinensis*. It is known to improve physical endurance, reproductive functions, cardiovascular, circulatory and respiratory system, renal hepatic system, prevent cancer (Gewali and Awale, 2008)
12.	*Phyllanthus emblica*	Phyllanemblinin B, phyllaemblicin A. Common cold, constipation, anaemic, jaundice, dyspepsia and rich source of Vitamin C (Watanabe et al., 2005).
13.	*Pyrus communis*	Arbutin, chlorogenic acid, epicatechin, epigallo-catechin, epigallocatechin 3-gallate (Cui et al., 2005; James-Martin et al., 2015). Treat allergic inflammatory diseases, positive effects on the hepatic enzymes by either enhancing the activity of alcohol dehydrogenase (ADH) and aldehyde dehyrogenase (ALDH) and

		thereby assisting in aldehyde and alcohol clearance from the system (James-Martin et al., 2015).
14.	*Taraxacum officinale*	Butyrolactones and butanoates namely taraxiroside A–F, acylated *c*-butyrolactone glycosides. Effective in hepatic disease, diabetes mellitus, rheumatoid arthritis, cancer and jaundice. It has anti-hyperglycemic, anti-oxidants, anti-inflammatory, anti-allergic and anti-coagulant properties (Choi et al., 2018)

3.4 Natural Dye

Natural dyes are more popular due to their nonhazardous nature and wide applicability such as pharmaceuticals, food, cosmetics and leather. Natural dyes are used in a wide range of applications such as textile/fabric dyeing, drug dyeing, food dyeing and solar cells. Among dyes curcumin is the best known component of curcuminoids used in various food industries for coloring. It is used in dairy products, beverages, cereal, pickles, sausages and also used in hair care and skin care cosmetics (Bhandari et al., 2020) among them plants with potential of use as natural dyes are tabulated in Table 7.

Table 7. Plants with natural dye development possibility

S.N.	Species	Parts Used	Colour
1.	*Acacia catechu*	Bark	Brown, black
2.	*Adhatoda vasica*	Leaf	Yellow
3.	*Butea monosperma*	Flower	Yellow, orange
4.	*Crocus sativus*	Flower	Yellow, orange
5.	*Curcuma longa*	Rhizome	Yellow
6.	*Embellia ribes*	Fruit	Red
7.	*Hypericum cordifolium*	Leaves	Black
8.	*Indigofera tinctoria*	Leaf	Blue
9.	*Mallotus philippiensis*	Fruit	Red
10.	*Punica granatum*	Bark	Yellow
11.	*Rubia cordifolia*	Root	Red
12.	*Zingiber officinale*	Rhizome	Brown

3.5 Agriculture Application as Biopesticide

The saponin rich plant such as *Sapindus mukorossi* (rittha) and *Senegalia rugata* (shikakai) can be used as emulsifiers and surfactants. *Diploknema butyracea* commonly known as Chiuri is a rich source of tritepenic saponins which exhibit insect control, antifungal, moluscicidal and

plant growth stimulant activity (Walia et al., 2017; Waller, 1999). The phorbol esters are extracted from the seeds of *Jatropha curcas* (Sajiyon) as bio-insecticides to protect crops from pre- and post-harvest pests. Their insecticidal mechanism was found to be due to a stimulatory effect on protein kinase C (PKC) enzymes. These esters are categorized as green because they are not toxic to living organisms, and readily decompose in UV light and through the microbial activity in soil; moreover, they were found to be friendly towards beneficial insects (Ashraf et al., 2014). Further, *Adhatoda vasica* and *Zanthoxylum armatum* are well known for their pesticide effect. There are several plant species that can be utilized for their biopesticide properties (Table 8).

Table 8. Potential plant species with biopesticide properties

S. N.	Scientific Name	Common Name	Nepali Name	Reference
1.	*Acorus calamus*	Sweet flag	Bojho	Ansari et al., 2013
2.	*Adhatoda vasica*	Malabar nut	Ashuro	Ansari et al., 2013
3.	*Allium sativum*	Garlic	Lasun	Nuruzzaman et al., 2019
4.	*Artemisia vulgaris*	Mugwort	Titaepati	Neupane, 2004
5.	*Annona squamosa*	Custard apple	Sitaphal	Neupane, 2004
6.	*Amomum subulatum*	Cardamom	Alaichi	Satyal et al., 2012
7.	*Azadirachta indica*	Neem	Neem	Ansari et al., 2013 Neupane, 2004
8.	*Calotropis gigantea*	Crown flower	Ank	Habib and Karim, 2016
9.	*Curcurma longa*	Turmeric	Beshar	Walia et al., 2017
10.	*Datura stramonium*	Thorn apple	Datura	Neupane, 2004
11.	*Diploknema butyracea*	Indian butter tree	Chiuri	Walia et al., 2017
12.	*Gaultheria fragrantissima*	Wintergreen	Dhasingre	Walia et al., 2017
13.	*Jatropa curcas*	Curcas nut	Sajiwan	Lengai et al., 2020
14.	*Juglans regia*	Walnut	Okhar	Mominul Islam and Widhalm, 2020
15.	*Lindera neesiana*	Spicebush	Siltimur	Ansari et al., 2013
16.	*Melia azedarach*	Persian lilac	Bakaino	Neupane, 2004
17.	*Nyctanthes arbortristis*	night-flowering jasmine	Parijat	Mishra et al., 2019
18.	*Nicotiana tabacum*	Tobacco	Surti	Neupane, 2004, Lengai et al., 2020
19.	*Origanum majorana*	Marjoram	Muswan	Sedlaříková et al., 2017

20.	*Persicaria hydropiper*	Water pepper	Pirre	(Hossain and Khalequzzaman, 2018)
21.	*Ricinus communis*	Castor	Andir	Neupane, 2004
22.	*Sapindus mukorossi*	Soap nut	Rittha	Walia et al., 2017
23.	*Sapium insigne*	Twallo tree	Khirro	Khanam et al., 2008
24.	*Piper longum*	Piper	Pipala	Lengai et al., 2020
25.	*Vitex negundo*	Five-leaved chaste tree	Simali	Neupane, 2004
26.	*Zanthoxylum armatum*	Prickly Ash tree	Timur	Hieu et al., 2012
27	*Zingiber officinale*	Ginger	Aduwa	Neupane, 2004, Lengai et al., 2020

Plants species which are cultivated or those exported from Nepal are available in substantial quantities which have economic significance in immediate commercial value. Natural products such as raw herbs were exported from Nepal in past as there were few industries that can utilize such materials in Nepal in the past. There are some herbal based industries are uprising in Nepal nowadays but still other than essential oils other plant extracts are very little in econo*mic* chain. The extract of *Taxus wallichiana* was previously extracted in Hetauda by Machhapuchhre herbal (not working at present), further Gajurmukhi herbal in Kakarbhitta process green tea extract (polyphenol and caffeine) and Berberine stem extract (Berberic hydrochloride), and in Nepalgunj processing Ginger for (Gingerol) and Turmeric (Curcuminoids) exist (Dhungel, 2020).

Large cardamom *Amomum subulatum* is produced in substantial quantities in Nepal but very little amount is being used in Nepal as most of the product (≥6,500) tons are exported (J. Shrestha et al., 2018). Its seed and rind from Nepal contains 1,8-cineole is a major component 60.8 and 39 %. These oils are antibacterial (MIC ≥ 313 μg/mL) and appreciably active against the fungus *Aspergillus niger* (MIC = 19.5 μg/mL) (Satyal et al., 2012) which can be utilized for production of pesticides. The top ten species of commercial interest in Nepal and their estimated volume of production (Caporale et al., 2020):

1. *Acorus calamus* L. (99,676 kg)
2. *Artemisia* spp. (168,369 kg)
3. *Cinnamomum glaucescens* (Buch.-Ham. x Nees) Hand.-Mazz. (100,896 kg)
4. *Gaultheria fragrantissima* Wall. (993,414 kg)
5. *Juniperus indica* Bertol. (654,280 kg)
6. *Nardostachys jatamansi* (D. Don) DC. (353,803 kg)
7. *Phyllanthus emblica* L. (118,100 kg)
8. *Rhododendron anthopogon* D. Don (339,282 kg)

9. *Valeriana jatamansi* Jones (135,532 kg)
10. *Zanthoxylum armatum* DC. (301,209 kg)

3.6 Future Perspective

Plant species such as *Paris polyphylla*, *Nardostachys jatamansi*, *Neopicrorhiza scrophulariiflora*, and *Zanthoxylum armatum* are at high risk (Pyakurel et al., 2019). There are several issues such as land rental and aggregating land, investment in physical infrastructure; requirement of R & D; inputs (fertilizer and seeds); extension services; adequate support for building firm capabilities; quality infrastructure (for testing and certification) sustainability and access to foreign markets (World Bank, 2018). The foreign direct investment (FDI), and research support to identify the intensity of specific MAPs in their final products.

The Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) of India has enlisted nearly 600 medicinal plant products, 52 minerals, and 50 animal products are commonly used in traditional Ayurvedic preparations. Medicinal plants are easily contaminated during growth, collection, and processing and there is a problem of adulteration of raw materials, which affects quality of the product. Several markers, such as taxonomic, chemical, genomic, and proteomic markers, help identify herbal drug components. In developed countries chemical fingerprinting and marker-based assessment of raw materials and active ingredients are used for assuring its quality. However, though the concept was recently introduced the Indian GMP regulation does not provide any guidelines for marker-based identification and only 44% perform chemical marker–based studies for their formulations, at government testing laboratories or private laboratories. Marker-based studies are further limited because reference standards are not available for all the herbs/plants used in medicinal preparations.

There is a need of bridging growers/collectors and suppliers providing raw materials to domestic manufacturers and ultimately consumers. Nepalese manufacturers can also grasp the field of personal products segment and there is a need for e-commerce and skills development. The formation of Ayurvedic and traditional medicine repositories can be beneficial for protecting Nepal's cultural heritage and traditional medicine systems, implementing intellectual property rights (IPR) polices.

Human health and immunomodulation has been of great concern in present world scenario due to COVID-19 pandemic. The immunomodulatory compounds in Nepalese botanicals can be great resource. The research on edible botanicals such as *Ophiocordyceps sinensis*, *Curcurma longa* and fruits such as *Mahonia napaulensis* and *Eclipta prostate* are needed for immunomodulatory nutraceuticals development.

3.7 Conclusion

There is an increasing trend in demand for plant based natural products and bioactives worldwide and a number of pharmaceutical, food, and cosmetics industries are essentially doing research for product development. The main constraint in use of natural products lies in the identification, isolation, and extraction of bioactive compounds which is a specialized and tedious task. The steady increase of the herbal product market and growing concern and adverse effect of synthetic chemicals will continue. There had been several species of plants identified and tested to date for their pharmaceutical importance, but limited species have been utilized and achieved commercial success. In Nepal there exists ISO/IEC 17025:2017 accredited laboratory of the Department of Plant Resource for certification of medicinal and aromatic plants.

Though the demand for medicinal plant products is growing outside Nepal, exports to China and India are hampered by transportation costs, Chinese and Indian regulations, and inability to compete on prices with similar local goods. The requirement of costly certifications —such as in Good Management Practices (GMP) or organic methods—impedes export to the European Union, North America, and Japan; these markets have, however, been accessed by larger companies exporting certified essential oils. Nepalese enterprises lagged behind Indian companies in terms of product marketing (Caporale et al., 2020). There is a need for rigorous research utilization of available advanced technology to achieve ideal food and drug development requirement including drug delivery, biocompatibility and without adverse effects. The establishment of supply chain including production, processing, manufacture, quality control and consumer reach is necessary to get benefit of herbal resources.

CHAPTER 4: Techniques for isolation and extraction of bio-active compounds

4.1 Introduction

The search and findings of novel chemical structures within natural resources continues to be a crucial strategy for the discovery of biologically active substances. Several sources such as plants, bacteria, fungi, insects, arachnids, marine organisms, and higher-order animals are mines of natural products. The term “natural products” refers to complex mixtures from these products and the isolated compounds derived from such sources. There have been more than 300,000 secondary metabolites identified so far from the biodiversity and some have been structurally elucidated but biological functions have not been evaluated yet.

Importance of natural products

1. Structural diversity
2. Mostly relatively small (<2000 Da)
3. Possess ‘‘drug-like’’ properties (i.e., possibility of being absorbed and metabolized)

The research on natural products begins with selection of species based on ethnopharmacological information, folkloric reputations, or traditional uses, and also random selection of species not previously worked by other researchers. There are basically two strategies of extraction of compounds from natural products:

I. Isolation of compound focused

a. Isolation and identification of compounds from natural sources followed by biological activity testing (mainly *in vivo*).
b. Chemotaxonomic investigation.

II. Bioactivity focused

a. Bioassay-guided (mainly *in vitro*) isolation and identification of lead compounds from natural sources.
b. Production of natural products libraries.
c. Production of active compounds in cell or tissue culture, genetic manipulation, natural combinatorial chemistry

4.2 Methods

The extraction and isolation of compounds involves several steps including extraction using suitable solvent, concentration of extract, solvent partition, chromatographic separation and spectroscopic analysis as well as bioassays (Figures 3 and 4). The conventional methods are adopted from Bucar et al., (2013) and Sarker and Nahar, (2012).

4.2.1. Extraction Process

1. Drying and grinding of plant material preferable in ambient temperature in room or shade dry to prevent microbial fermentation and subsequent degradation of metabolites is required. Homogenizing fresh plant parts (either of leaves, flowers, wood, bark or fruit etc.) evenly to facilitate homogenous drying and facilitate extraction. Suitable solvent system is needed to be used depending of type of compounds present or needed to be extracted. Plant extracts contains many compounds and on the basis of their solubility they can be largely divide into non-polar and polar components. The commonly used solvents are:

a. Nonpolar: *n*-hexane, chloroform ($CHCl_3$)

b. Medium polarity extraction: ethyl acetate (EtOAc), dichloromethane (DCM).

c. Polar extraction: water, ethanol, methanol (MeOH).

4.2.2 Extraction Method

Extraction processes can employ non-polar (water-immiscible) or polar (water-miscible) solvents (Table 9). The selected solvent should have a low potential for artifact formation, a low toxicity, a low flammability, and a low risk of explosion. Further, it should be economical and easily recycled. Evaporation of the organic solvents or freeze-drying (of aqueous solutions) yields dried concentrated crude extracts. Some conventional extraction methods are:

a. Maceration at room temperature by soaking the material with the solvent with stirring

b. Steam distillation and micro wave steam distillation

c. Soxhlet

d. Supercritical fluid extraction using supercritical CO_2

e. Sublimation

f. Ultrasound-assisted extraction by placing the material in solvent in an ultrasonic bath

g. Microwave-assisted extraction

Table 9. Physicochemical properties of some common solvents used in natural products extraction

S.N.	Solvent	Polarity index	Boiling point (°C)
1.	*n*-hexane	0.0	69
2.	Dichloromethane	3.1	41
3.	*n*-butanol	3.9	118
4.	iso-propanol	3.9	82
5.	*n*-propanol	4.0	92
6.	Chloroform	4.1	61
7.	Ethyl acetate	4.4	77
8.	Acetone	5.1	56
9.	Methanol	5.1	65
10.	Ethanol	5.2	78
11.	Water	9.0	100

4.2.3 Fractionation

There are different types of column chromatography (CC), size-exclusion chromatography (SEC) and solid-phase extraction (SPE) can be employed to get the fraction containing required compounds.

4.2.4 Isolation

The chromatographic techniques utilizing different types of columns compounds can be isolated. Some of chromatographic techniques include:

1. Thin-layer chromatography (TLC)
2. Preparative thin-layer chromatography (PTLC)
3. Open-column chromatography (CC)
4. Flash chromatography (FC)

Some modern chromatographic techniques are:

1. Vacuum liquid chromatography (VLC)
2. Flash chromatography (e.g., Biotage)
3. Solid-phase extraction (e.g., Sep-Pak)

4. Countercurrent chromatography (CCC)
5. High-performance thin-layer chromatography (HPTLC)
6. High-performance liquid chromatography (HPLC)
7. Hyphenated techniques (e.g., LC-MS, LC-NMR, LC-MS-NMR)

4.2.5 Structure Elucidation

In most cases of extraction and isolation of natural products, the end point is the identification of the compound or the conclusive structure elucidation of the isolated compound. There are many useful spectroscopic methods of getting information about chemical structures, but the interpretation of these spectra requires specialists with detailed spectroscopic knowledge and wide experience on compounds derived from natural products. The advancement in the area of artificial intelligence and computing some programs are available for automated structure elucidation.

The spectroscopic techniques that are utilized for the structure determination of natural products are as follows:

1. Ultraviolet-visible spectroscopy (UV-vis): Provides information on chromophores present in the molecule. Some natural products, such as setrols, flavonoids, alkaloids, and coumarins, to name a few, can be primarily characterized (chemical class) from characteristic absorption peaks.

2. Infrared spectroscopy (IR): Different functional groups such as—OH,—NH_2, aromaticity, and so on, present in a molecule can be determined by infrared spectra.

3. Mass spectrometry (MS): The information about the molecular mass, molecular formula, and fragmentation pattern can be obtained using mass spectrometry techniques such as electron impact mass spectrometry (EIMS), electrospray ionization mass spectrometry (ESIMS), and fast atom bombardment mass spectrometry (FABMS).

4. Nuclear Magnetic Resonance (NMR): Reveals information on the number and types of protons and carbons (and other elements like nitrogen, fluorine, etc.) present in the molecule, and the relationships. Still NMR spectroscopy remains as technique which efficiently and

unambiguously elucidates complex structures of individual small molecules. The different NMR experiments that can be used for structure elucidation are classified into two major categories:

a. One-dimensional techniques: ^{1}H NMR, ^{13}C-NMR, ^{13}C-DEPT (Distortionless Enhancement by Polarization Transfer)

b. Two-dimensional techniques: ^{1}H-^{1}H COSY (Correlation Spectroscopy), ^{1}H,^{13}C-HMBC (Heteronuclear Multiple Bond Correlation),^{1}H,^{13}C, HSQC (Heteronuclear Single Quantum Correlation).

There are limitations in information that can be obtained from spectroscopic techniques (Table 10). The compound's structure elucidated is with NMR Spectroscopy confirmed with its mass determination using High Resolution Fast Atom Bombardment Mass Spectrometry (HR-FABMS). In addition to the above-mentioned spectroscopic techniques, X-ray crystallographic techniques provide information on the crystal structure of the molecule, and polarimetry offers information on the optical activity of chiral compounds.

Table 10. Comparative uses and limitation of spectroscopic techniques

Spectroscopic techniques →	**Nuclear Magnetic Resonance (NMR)**	**Mass spectrometry (MS)**	**Infrared spectroscopy (IR)**
Sensibility	Low	High	High
Informative	High	Low	Low
Interpretation	**Full structure**	Molecular formula	Functional group

4.2.6 Quantification

The amount of bioactive compounds in extract determines the effectiveness of formulation in which extracts were used. High-performance liquid chromatography HPLC, Liquid chromatography/ Mass Spectroscopy (LC/MS) and Gas chromatography/ Mass Spectroscopy GC/MS techniques are utilized for assessment of the quantity of compounds in extracts. Further, HPLC, LC/MS and GC/MS can be used for determining purity of isolated compounds. The degree of purity of the test compound is necessary as the most reliable assay result can be obtained with a compound of 100% purity which excludes any possibilities of having activities resulting from minor impurities. Most of the modern bioassays are microplate-based and can

be conducted using a small amount of extract, fraction, or compound for the assessment of activity.

MS-based methods are also capable of rapid detection of trace-level compounds in extract. LC coupled to high-resolution mass spectrometers such as Time-of-Flight (TOF) and Fourier Transform (FT) is also useful for high-throughput screening and identification of metabolites in natural resources. The GC/MS (Gas chromatography/Mass spectroscopy) suitable in identification hydrophobic or volatile small molecules such as flavor and aroma constituents (Molyneux and Schieberle 2007).

Nowadays computerized treatment of mass spectral data and software for pre-processing of LC–MS data are available and very useful for peak picking, ion extraction, organization and classification of data. The Bruker Data Analysis and Bruker Profile Analysis for Bruker, MassHunter for Agilent, MarkerLynxTM for Waters or publicly available software such as XCMS, MZmine, and MET–IDEA can handle data from different instruments. As structurally similar metabolites share similar fragmentation patterns, molecular families tend to cluster together within a network (Nguyen et al., 2013; Yang et al., 2013).

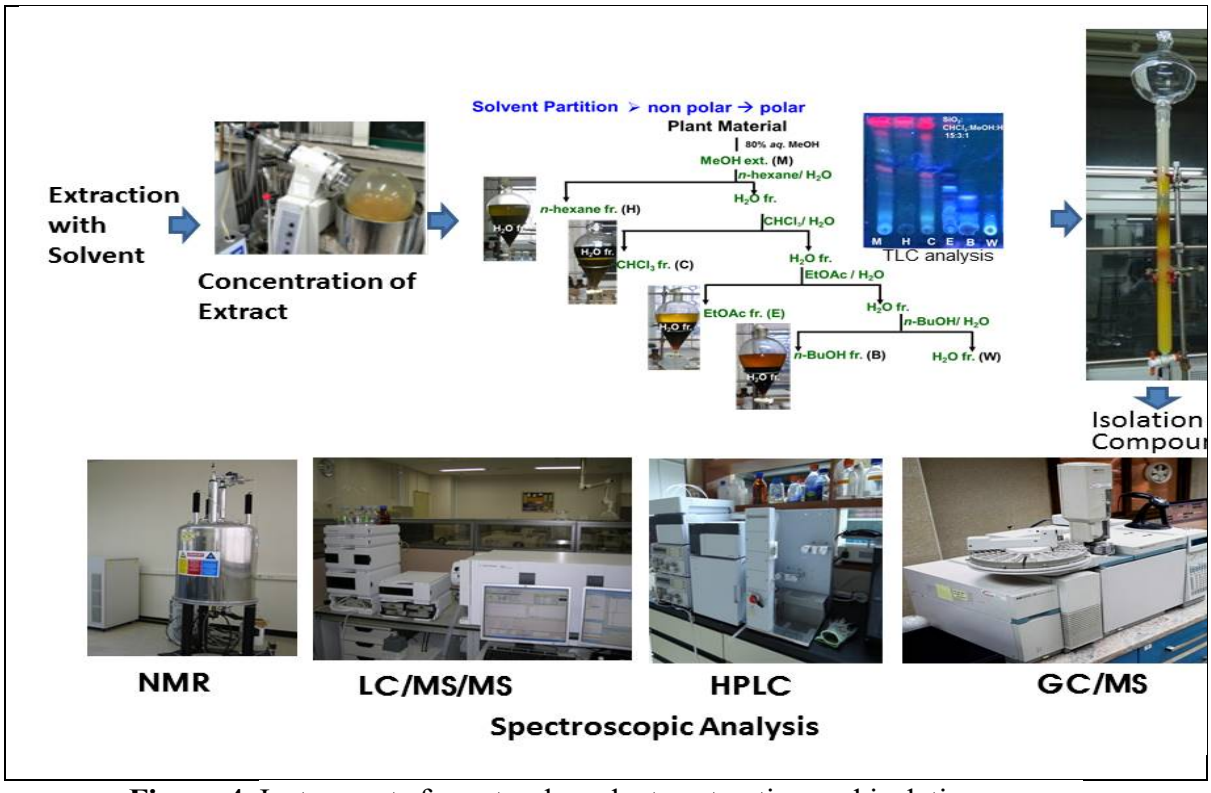


Figure 4. Instruments for natural products extraction and isolation process

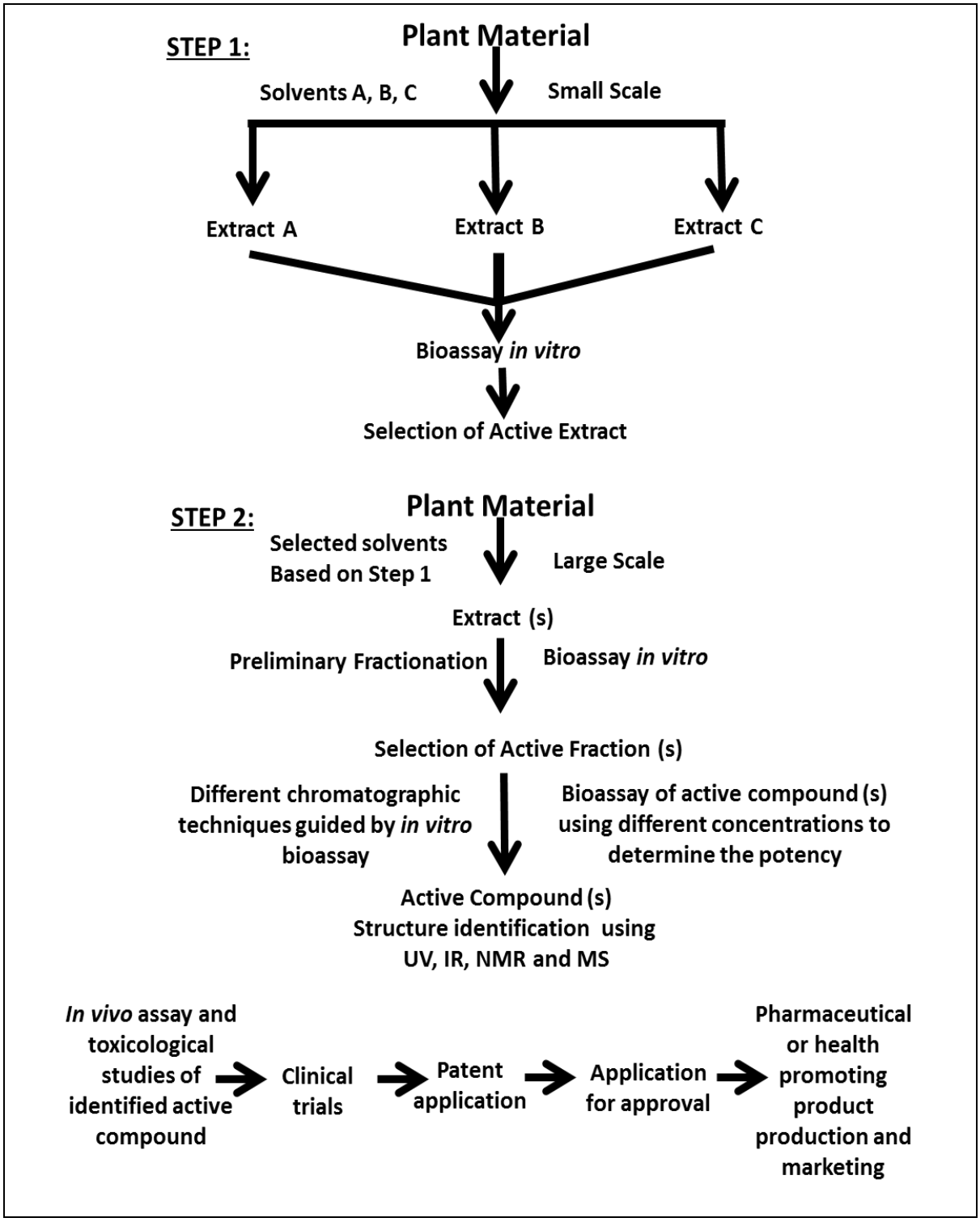


Figure 5. Schematic outline of bioassay-guided natural products research

4.2.7 Assays

Several assays including chemical, biological, or physical assays are necessary to exactly pinpoint the target compound(s) from a complex natural product extract. To find out bioactive

compounds, natural product research these days is more focused on isolating target compounds (assay-guided isolation) rather than trying to isolate all compounds present in any extract. The target compounds can be of certain chemical classes, have certain physical properties, or possess certain biological activities. Therefore, appropriate assays should be incorporated simultaneously in the extraction and isolation protocols are useful for finding active components. Various bioassays for example, antibacterial, antifungal, anticancer, antidiabetic, etc. can be performed for crude extract, chromatographic fraction, mixture, or a pure compound. Such assay can involve the use of in vivo systems (clinical trials, whole animal experiments), ex vivo systems (isolated tissues and organs), or in vitro systems (e.g., cultured cells).

4.3 Extraction and Isolation Techniques for Compounds from Some Target Species

4.3.1 Extraction and Isolation of Compounds from *Ophiocordyceps sinensis* and other Fungal Species

O. sinensis is a well-known and high value medicinal fungus of Nepal known for its ability to treat a broad spectrum of human diseases concerned with the functions of the lung, kidney, immune system, and also for its ability to enhance the quality of life and physical performance. Polysaccharides have been identified as the major active components of with a wide range of bioactivities including immunomodulation, antitumour, antioxidation, and hypoglycemic effects. The fermentation products from different strains of *O. sinensis* are in extensive use in recent years, mainly in China, Japan, and Korea. However, the relationship between structural features, solution behavior, space conformation, and their bioactivity is unclear due to the structural diversity and complexity of polysaccharide molecules.

The analysis by GC-MS provides quick and reliable information on type and quantity of components in the sample. Therefore, the initial step in extraction and isolation of *O. sinensis* of Nepalese origin can be an important step in its research. The natural *O. sinensis* or cultured mycelia dried and ground into fine powder will needed to be derivatized (as volatility is necessary for using GC or GC/MS as analytical method) and proceed with GC-MS analysis using following methods (Zhang et al., 2020):

Extract fifty milligrams powder of each sample
(naturally occurring *O. sinensis* or mycelium culture sample) with 2 mL of 50% (v/v) methanol–water mixture containing 100 µL ribitol (400 µg/mL in water)
↓
After ultrasonic-assisted extraction for 60 min, centrifuged at 12000 rpm for 10 min at room temperature and filter through 0.22 um filter
↓
Transfer two hundred microliters of the supernatant to a 2.5 mL tube
↓
Add eighty microliters of methoxyamine hydrochloride (20 mg/mL in pyridine) to the dried residue and incubated at 37 °C for 120 min
↓
Silylate sample for 60 min at 37 °C by adding 50 µL *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA)
↓
Transfer the derivatized samples to glass vials for GC–MS analysis

Process for extraction and identification plan for compounds from *O. sinensis* and other *Cordyceps* species for sample in small amount

The analysis of gas chromatography together with mass spectral analysis can provide the metabolic profiling of *O. sinensis* which can be used for understanding quality as well as difference in natural and cultured mycelia. There is a possibility of isolation of compounds when samples can be available in bulk such as 1 kilogram or above dry material. Such isolated compounds can be utilized for bioactivity analysis.

The dried and powdered fungal sample
↓
Extract using 80 % aqueous methanol (MeOH) at room temperature for 24 h, followed by filtration
↓
Evaporate filtrates in a vacuum
↓
Suspend in H_2O (3 L) and then successively extract with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH)
↓
Concentrate organic and aqueous layers to get fractions of EtOAc, *n*-BuOH, and H_2O
↓
Column chromatography for isolation of required compounds

Process for extraction and identification plan for compounds from *O. sinensis* and other *Cordyceps* species for sample in bulk amount

4.3.2 Extraction and Isolation Plan for Compounds from *Curcurma longa*

Turmeric contains 3-6% polyphenolic compounds, collectively known as curcuminoids, which is a mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcumin is the most important constituent of curcuminoids recognized for their biological activity, food safety and the broad range of pharmaceuticals. Curcumin is safe, non-toxic and strong natural antioxidant. It is known for anti-inflammatory, anti-HIV, anti-bacterial, antioxidant, nematocidal, antiparasitic, antispasmodic and anticarcinogenic activities (Niranjan and Prakash, 2008). Other than curcurmin there are several compounds such as sesquiterpenes named as curcumenone; dehydrocurdione; (4 S, 5 S)-germacrone 4, 5-epoxide; bisabola 3, 10-diene 2-one; arturmerone (Roth et al., 1998); bisacumol; bisacurone; curcumenol; isoprocurcumenol; zedoaronediol; procurcumenol; epiprocurcumenol; germacrone-13-al. 4- hydroxybisabola-2, 10-diene-9-one; 4,5-dihydrobisabola-2, 10-diene; 4-methoxy5-hydroxybisabola-2, 10-diene-9-one; 2, 5-dihydroxybisabola-3, 10-diene and procurcumadiol (Ohshiro et al., 1990). Some other compounds named as curlone (Kiso et al., 1983); α-turmerone; βturmerone; terpinolene; α-phellandrene; curcumadiol; labda-8 (17)-diene-15, 16-dial and acidic polysaccharides are also present in turmeric. The extraction and isolation of curcuminoids other components can be done with following method.

The dried and powdered rhizome samples from C. *longa*

↓

Extract using 80 % aqueous ethanol (EtOH) at room temperature for 24 h, followed by filtration

↓

Evaporate filtrates in a vacuum

↓

Suspend in H_2O (3 L) and then successively extract with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH)

↓

Concentrate organic and aqueous layers to get fraction of EtOAc, *n*-BuOH, and H_2O

↓

Column chromatography for isolation of required compounds from EtOAc, *n*-BuOH and H_2O fractions

Process for extraction and isolation plan for compounds from *Curcurma longa* rhizome

Bioassay of isolated compounds to find uses and also use for profiling of compounds for quality of turmeric from different parts of Nepal as there is significant difference in components of

turmeric from different parts of Nepal (Poudel et al., 2019; Tønnesen et al., 1989). The isolated curcurmin can be used for its nanoparticle preparation. Curcumin content in turmeric rhizome represents approximately 3.5% w/w of the dried rhizome, and the residue generated during its extraction contains other compounds such as cellulose and starch which are useful in the food industry. There is possibility that nanotechnology could provide a new approach to support the full use of the rhizome and generate new ingredients for the food industry (Serpa Guerra et al., 2020). The isolation of major components from EtOAc, *n*-BuOH and H_2O fractions can be useful for identification of compounds but also will be useful for nanoparticle based research.

4.3.3 Extraction and Identification for Compounds from *Rhododendron* Species

Rhododendron is one of the naturally occurring plants which possess various health benefits, such as prevention and treatment of diseases associated with heart, dysentery, diarrhea, detoxification, inflammation, fever, constipation, bronchitis, asthma and cancer (Tiwari et al., 2020). The flower colours can be categorized into four groups – red, purplish pink, purple and white. The anthocyanins present in petals are delphinidin, cyanidin and malvidin 3-*O*-arabinoside-5-*O*-glucosides, cyanidin 3,5-di-*O*-glucoside, 3-*O*-galactoside and 3-*O*-arabinoside, and delphinidin 3-*O*-glucoside. The red-flowered species mainly contained cyanidin monoglycosides and are with much higher total anthocyanin content than purplish pink- and purple-flowered species (H. Du et al., 2018).

Extract dried petals (30–50 mg) with 3 ml methanol containing 0.2% formic acid
Sonicate extract at 20 °C for 20 min and then kept at 4 °C for 24 h
↓
Filter the supernatants through syringe filter
↓
Perform chromatographic analysis LC/MS of identification of compounds
↓
Identification of required compound from nanoparticle based assay
↓
Extraction of material in bulk amount using aqueous methanol MeOH (80%) and obtaining EtOAc, *n*-BuOH and H_2O fractions
↓
Isolation of bioactive / photosensitizer compound

Process for extraction and identification plan for compounds from *Rhododendron* spp.

As natural dye extracted from rhododendron flowers is expected to use as a photosensitizer in dye sensitized solar cell the compounds present in it can give an idea on the quality of

performance of solar cells compared to the other dye solution. Further, for isolation of major components in *Rhododendron* can be done with extraction with aqueous methanol MeOH (80%) from EtOAc, *n*-BuOH and H_2O fractions.

4.4 Conclusion

There exist several well-established procedures for extraction and isolation of natural products from various sources. A precise protocol for extraction and isolation can be designed depending on target compound(s) needed to serve research purpose. In the case of unknown natural products, it will be necessary to try out pilot extraction and isolation methods to find out the best possible method. The selection of methods needed to be done weighing the advantages and disadvantages of available methods focusing on their efficiency as well as total cost involved. The availability of various hyphenated techniques (e.g., LC-MS, LC-NMR, LC-MS-NMR), it is now possible to determine the compounds present in extract or fraction while separation is being carried out. The necessity of use of compounds in bioassay as well as other systems of experimentation and finding out mechanisms, it is necessary to isolate compounds. The progressive progress in the area of separation technology there are a variety of extraction and isolation methods as well as application of newer approaches. The GC-MS and HPLC analysis equipment exist in some of government centers like Department of Plant Resource (Thapathali, Katmandu), Nepal Academy of Science and Technology (Khumaltar), and Nepal Bureau of Standards & Metrology (Machhaphokhari, Kathmandu) and Department of Food Technology and Quality Control (Babarmahal, Kathmandu). However, equipment like Nuclear Magnetic Resonance (NMR) is still lacking in Nepal which is required for complete structure elucidation. Therefore, establishment of instrumentation facility with NMR and other such as GC-MS, LC-MS and FAB-MS can greatly contribute to research and utilization of the herbal heritage of Nepal.

CHAPTER 5: Potential research projects for development of natural products based proto-type, patenting, production and marketing

5.1 Research Areas for Natural Products Based Research Projects

There are limited natural products based on research and documentation of Nepalese herbal materials (Gewali and Awale, 2008; Watanabe et al., 2013, 2005). The technological advances and the methodological development have revolutionized the screening of natural products as well as provided an opportunity to re-establish natural products as a source of drug leads with several possibilities. The application of technologies that have impact on the discovery and validation of novel drugs includes: (a) improvement in natural product sourcing, (b) development of a streamlined screening process, (c) validated synthetic organic technology and biosynthesis, (d) application of *in vitro, in vivo and in silico* approaches for efficacy evaluation. The extensive research, production of processed products and their standardization are of utmost importance for getting actual benefit for Nepal from worldwide herbal trade. The natural products based research projects for implementation are as follows:

i. Collection and herbal resource mapping

The information on herbal materials, their growing region and ethnobotanical knowledge inventory are necessary for sustainable utilization of resources. Such information will be useful for improvement of cultivation techniques to obtain high value organic herbal products. The proper identification of herbal material, understanding of region of occurrence, and parts and method of traditional use can provide important information for their utilization in medicine, cultivation and marketing strategy.

ii Evaluation of Nepalese herbal resource

The evaluation of herbal products for principal active components for claimed efficacy and determination of toxicity can provide imputes for use in medicinal and other applications. Evaluation of phytochemistry and pharmacological properties of commercially important as well as those of other underutilized Nepalese plant materials can provide information on possible field of application of their bioactive components. Research on neuroprotective, anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, anthelmintic, anti-hyperglycemic and antihypertensive properties of plant materials for efficacy of herbal

materials can open several avenues of medicinal use. Further, standardization of herbal product based on active components are necessary for quality control of products.

iii. Evaluation of functional food materials

The evaluations of the risks and benefits of herbal dietary supplements are necessary for making marketable products. There are several kinds of wild edible fruits available naturally in Nepal which can be used in community forestry for production that will benefit the local community as well as be base for the functional food product industry.

iv. Evaluation of antimicrobial and bio pesticide from herbal materials

Phytochemistry and antimicrobial evaluation of herbs are useful for use as food and fruit preservatives and health care products. The formulation for oral and skin care products do have market potential as well as such practice can support the healthier way of life. The essential oils produced in Nepal can be utilized for such purposes. Preparation of biopesticides from herbal materials for organic cultivation is another sector with huge benefit marketwise as well as for better perspective of humankind.

v. Principal component analysis herbal essential oil

Flavor and fragrance components of essentials are much sought after due to their aesthetic appeal in the human mind. The flavor enhancing compounds are used in preparation of culinary delight as well as used in bakery and confectionary products. The uses of essential oil in massage and aromatherapy have several health benefits. The essential oil components are also an indispensable source of biopesticide which are ecofriendly as they are biodegradable and alternative for synthetic pesticide. Therefore, quantification and qualitative analysis of essential oil composition in herbal materials are necessary.

vi. Microorganisms resource identification for pharmaceuticals

Microbial natural products are well known for their potential therapeutic activities with desirable pharmacokinetic properties for clinical development. There are several natural products which have reached market for example antibiotics (erythromycin A, vancomycin, penicillin G, streptomycin and tetracycline), antifungal agents (amphotericin B and griseofulvin), cholesterol-lowering (lovastatin, anticancer agents daunorubicin, mitomycin C and bleomycin), immunosuppressant (rapamycin, mycophenolic acid and cyclosporine A) (Lam, 2007). Yarsagumba (*Ophiocordyceps sinensis*) is acclaimed fungal resource with several pharmacological potential known for beneficial effects (S. Shrestha et al., 2012; S Shrestha et

al., 2013) which grow naturally in the alpine region of Nepal. The component analysis of natural harvest as well as culture of suitable strain of *Ophiocordyceps sinensis* and other fungal species can be a source of yet untapped resources of Nepal.

vii. Nano practical synthesis from herbal extract

The extracts of different plant parts such as leaves, flowers, and fruits, as well as several plant-derived compounds, have capacity to reduce metals, forming nanostructures. Such green-synthesized nanoparticles have similar properties as their chemically synthesized counterparts. Plant compounds such as phenolic, amino, carboxyl and hydroxyl groups, proteins, and amino acids do possess reducing capacity. There is a need for more research in pure solutions of active components found in plant extracts to gain more insights into the efficacy of such compounds.

viii. Computer-aided drug discovery and *in silico* chemicobiological analysis

In silico analysis of herbal compounds can facilitate hit identification, hit-to-lead selection, optimize the absorption, distribution, metabolism and analyze toxicity safety issues. These processes efficient drug discovery, design, development and optimization. The computational *in silico* design includes ligand-based drug design (pharmacophore, a 3D spatial arrangement of chemical features essential for biological activity), structure-based drug design (drug-target docking), and quantitative structure–activity and quantitative structure–property relationships which can reduce use of animal model and time frame for understanding drugs and their targets.

Some potential research projects in the fields of natural products chemistry for application in Nepal are as follows:

1. Development of organic herbal cultivation techniques high value herbal products: Plant species such as *Neopicrorhiza scrophulariiflora* (Kutki) and *Paris polyphylla* (Satuwa) are critically endangered due to increase in their trade. *N. scrophulariiflora* contains important compounds such as caffeoyl glycoside, cucurbitacin glycoside, cyclopentanoid monoterpenes, hydroquinone glycoside, iridoid glucoside, non-glycosidic iridoid, phenol, phenyl glycoside, phenylpropanoid, phenylethyl glycoside and secoiridoid glycoside with therapeutic properties for liver disease, immunity, cardiovascular, nervous, dermatological, musculoskeletal, digestive, respiratory and genito-urinary systems (Rokaya et al., 2020). Some important compounds such as diosgenin, Paris saponin I-III, diosgenin and methoxy-

protopolyphyllin I, C22-hydroxyprotopolyphyllin I, C22-methoxy-protopolyphyllin II, polyphyllin VI, and polyphyllin VII from *Paris polyphylla* are known for anticancer, anti-leishmania, haemostatic, anthelmintic, Alzheimer, immuno-stimulating, anti-tyrosinase, antibacterial, spermicidal, antifungal and antiviral properties (Bhat et al., 2017). The development and organic herbal cultivation techniques and sustainable harvest of such resources are needed to lessen the pressure of harvest in nature. *N. scrophularia* costs RMB 120-140/kg (approx. NRs. 2,200-2,600) and *P. polyphylla* costs RMB 150-300/kg (approx. NRs. 4,500-5,500) in the whole sale market of China (He et al., 2018). This can proceed with trial farming and organic cultivation technology development in the MBUST facility.

2. Standardization of commercial herbal materials with phytochemical and pharmacological properties: Such practice can bring uniformity and quality product in herbal products. For example, the quantification of diosgenin in *Paris polyphylla* can add value to its herbal material which is in high demand in the international market (He et al., 2018).
3. Development of dietary supplements from wild edible fruits. Dietary supplement with quantification of bioactive components can enhance the value of herbal materials: Phenolic and flavonoid content and antioxidant potential in *Myrica esculenta* fruit can be evaluated for making its dietary supplement possibilities. Further, analysis of its anthocyanin content can provide insight into its beneficial use.
4. Biopesticides development from herbal materials for organic cultivation: Pest control in organic cultivation is challenging and is sought after for management of organic cultivation. As biopesticides are biodegradable and will not have toxic residue which make such organic products are productive to farmers and consumers. Application of biopesticide of edible source origin such as *Curcuma longa* (Turmeric, Beshar), *Amomum subulatum* (Cardamom, Alaichi) and *Zanthoxylum armatum* (Prickly Ash tree, Timur) can be useful for control of many pests.
5. Utilization of herbal extracts for health care products: Herbal extracts such as essential oil rich in menthol are useful for making oral rinse, soap material, nasal inhaler, deodorant etc. The research work on essential oil can take place with Gas Chromatography - Mass Spectroscopy (GC-MS) instrumentation MBUST facility installed at MBUST.
6. Mushroom and fungal mycelium based nutraceutical products development: Herbal tea prepared from mushroom such as *Ganoderma lucidum* and *Cordyceps millitaris* are in good demand in the market. The ethanol and water extract of such mushrooms can make available more components and can make them instantly soluble in herbal infusion.

7. Nano particle synthesis using herbal extracts for dye-sensitized solar cells and biomedical use: Nepalese herbal materials such as *Curcuma longa* (Turmeric, Beshar), *Pterocarpus marsupium* (Bijaysal) and *Rhododendron arboreum* (Laligurans) are rich source of compounds with dyeing properties.
8. *In silico* analysis of herbal components of commercially important herbs of Nepal: The computational methods include structure-based and ligand-based chemoinformatics processes which can be used for virtual screening of compounds. In structure-based approach molecular dynamics, docking and binding cavity analysis are used. The ligand-based techniques include Quantitative Structure Activity Relationships (QSAR), estimation of drug likeness, prediction of adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, pharmacophore identification and similarity searching are conducted (Pereira and Aires-de-Sousa, 2018). Further, computational methods can be used to optimize the delivery process of the compound molecule loaded on a nanomaterial destined to the specific target site and overcome multiple barriers and on the drug release mechanism. This kind of facility not only compliment bioassay but will also help in finding ways of drug use and drug applications.

5.2 Technology Development

There can be several technological interventions from production of raw herbal material to processed products. The herbal production site and farming procedure can make a difference in herbal constituents. The modified farming practice such as hydrophonic cultivation can further enhance some of the components in herbal materials. Collection and sample processing methods including drying and irradiation can further determine the active component content.

There are some standard technologies that can be applied for natural products extraction from herbal materials and their isolation and standardization. The application of supercritical CO_2 extraction, microwave assisted extraction and ultrasonication can make difference in component quantity in extraction. Solvent extraction is another step that greatly influences the outcome of natural products. Depending on type of purpose there can be variation in technology that can be applicable. For example, aqueous methanol is preferred for extraction purpose but in case of extraction of food materials aqueous ethanol extraction is applicable. The applications of different chromatographic techniques are need to separate active components in extract. The use of spectroscopic techniques including Nuclear Magnetic Resonance (NMR),

Mass Spectroscopy (MS), and Infra-Red Spectroscopy (IR) needed to be used for elucidation of compound structure. Further High Performance Liquid Chromatography (HPLC), Gas chromatography (GC/MS) and Ultrahigh-pressure (or performance) liquid Mass Spectroscopy (UGPLC-MS/MS) techniques can be used for metabolites profiling.

In case of research on fungal and microbial metabolites there is requirement of growth chambers and fermenters for growing research materials. Moreover, for bioassay of extract and isolated compounds for finding their bioactivity suitable *in vitro*, *in vivo* and *in silico* laboratory facilities with cell culture and animal models as well as technical collaboration with laboratories with advanced facilities for neuroprotective, anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, anthelmintic, anti-hyperglycemic and antihypertensive assays are needed. The underlying cause of disease progression in humans can be free radicals and inflammation which leads to failure of immune response that in turn disturb the cellular microenvironment and phytochemicals are known for their effect in antioxidant and anti-inflammatory properties to counteract such conditions. Therefore, research in this field can give answers to the search for potential bioactive compounds. The advancement in testing in cell based assay systems can give a cutting edge technological need. Free radical scavenging activity to overcome reactive oxygen species (ROS) can be done with di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) and 2,20-azino-bis(3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) assays, the antibacterial screenings can be done for extracts as well as compounds of plants using disk diffusion assay of ATCC cultures of microorganism. The establishment of a cell culture lab can give added advantage of assay in different cell cultures including cancer and neuronal cells. There can be some very useful assays using UV-VIS spectroscopy can be performed such as angiotensin I-converting enzyme for antihypertensive (Balasuriya and Rupasinghe, 2011), α-glucosidase and α-amylase enzymes for anti-hyperglycemic (Lankatillake et al., 2021), inhibition of acetylcholinesterase and butyrylcholinesterase and tyrosinase for neuronal disease (Orhan et al., 2017) to find potential compounds .

5.3 Proto-type Development

The development of proto-type is necessary to streamline the process of product development which will function and will have flawless performance. There is a need of a careful research for production of consumer goods with market value and quality assurance. Some of products based on natural products of Nepal with national and international market are as follows:

i. Curcumin supplement tablet

Turmeric is used in some Ayurvedic preparation for its anti-inflammatory, anticancer, antioxidant, anti-angiogenic and immunomodulation function (Fadus et al., 2017). The turmeric cultivated in different geographical areas differ in curcuminoids content (Poudel et al., 2019). Product formulation with *Curcurma longa* content with specified curcumin and blended with piperine (from *Piper longum*) which increase 20 fold bioavailability can have a market for health benefit. Further, herbal materials such as turmeric, ginger and cardamom are cultivated in substantial quantities in Nepal. Their pulverized pure materials have application as condiments as well as can be used for products such as lozenges and candy.

The information of *Curcurma longa* and *Piper longum* with better quality production sites of Nepal is needed. Such information will be useful for getting substantial supply. Sample from such sites can be extracted and water extract, oleoresin, essential oil are needed to be evaluated for curcumin content using GC-MS facility to be established in MBUST. The selected sample with appropriate curcurmin content can be evaluated for antioxidant and anti-inflammatory properties. Such products can be marketed as food supplements and can be used in production of chewables such as lozenges and candy. A chewable candy can be made with sugar base, suitable lubricants, acidifying agents, preservatives and flavouring agents (up to 80% w/w of total composition) and effective concentration curcuminoids up to 20% w/w of the total composition can be applicable. Fruit extract and stevia can be used as alternatives for sugar substitutes. The natural flavours such as honey, molasses, maple sugar, or artificial flavours: menthol, xylitol, methyl anthranilate and ethyl caproate, at concentrations of 0.1-10% w/w can be applied for variation of taste.

ii. Wild edible fruits concentrate

Wild edible fruits are a rich source of polyphenol. Traditionally some fruits are used in Ayurvedic preparation and household use to impart colour, add flavor and deliver health claims. Therefore, products with assurance of beneficial compound content are in demand for such herbal preparations. Fruit extract such as *Myrica esculenta* (Kaphal) rich in flavonoid and anthocyanin can be used in smoothie, salad dressing, bakery products, ready meals, sauces and soups. *Myrica esculenta* is an underutilized fruit available during April-June in Nepal rich in anthocyanin compounds and are harvested in limited quantities and little amount reaches the market. The de-stoning of fruit and freeze drying of pulp for better preservation of beneficial compounds and research on total phenolic content, flavonoid content, antioxidant potential

using UV-Visible spectrophotometer can bring assurance to consumers the goodness of this fruit and also development of products such as concentrated extract cubes or powder form for ready to use can make it within the reach of consumers.

iii. Processed essential oil components

The aromatic compounds give each essential oil its characteristic essence. Essential oils are obtained through distillation utilizing steam or water or mechanical methods (e.g., cold pressing). There are several applications of essential oils due to their antiparasitic, bactericidal, fungicidal, virucidal, and insecticidal properties. Essential oils can be utilized to make oral rinse, hair and skin care products. There also exists the possibility of blending of fragrance components from essential oils. The essential oil component such as menthol can be purified and can be used in lozenges, nasal sprays, vapo-rubs, inhalers, and cough syrups.

iv. Preparation of biopesticides

Essential oil and other herbal materials for pest control can give beneficial impact in organic cultivation. The essential oil components can be used as pest deterrent and bio pesticide for crop protection. The botanicals including *Adhatoda vasica* (Asuro), *Zanthoxylum armatum* (Timur), *Sapindus mukorossi* (Ritha), *Diploknema butyracea* (Chiuri), *Jatropa curcas* (Sajiyon) and *Curcuma longa* (turmeric) extract can be used for preparation of bio pesticide.

v. Food grade natural dye and photosensitizer

There are 33 species of rhododendron in Nepal (GON, 2018). Rhododendron flowers are a rich source of natural dye like anthocyanins that can be used as potential photosensitizers in solar cells (Kim et al., 2013) and food industry (Liang-fang, 2013). Anthocyanidins such as delphinidin, cyanidin, petunidin, pelargonidin, peonidin, malvidin and flavonols quercetin, 5-*O*-methylquerecetin, myricetin, 5-*O*-methylmyricetin, kaempferol and 5-*O*-methlkaempferol are known from rhododendrons (Arisumi et al., 1985). The food grade product with qualitative and quantitative analysis of anthocyanins can be produced, and it can also be used as a photosensitizer in solar cells.

5.4 Patenting, Production and Marketing

The Nepal opening of industries started after the formation of Patent, Design and Trademark Law, 1993 (GON, 1935) it was amended in 2063 (2006) to incorporate some changes. The Patent, Design and Trademark Act 2022 (GON, 1965) which was amended in 2044 and 2063 is still in effect in Nepal. It defined patent, design and trademark as well as established procedure of registration of rights and remedy and compensation in case of violation. Further, The Copyright Act, 2059 (GON, 2002) made it possible to register exclusive rights to the creators of the works on their original and intellectual creations in Nepal. Colston, 1999 gave a comprehensive study of Intellectual Property Law of the United Kingdom within the international framework of conventions, treaties and agreements. In Nepalese context (Spakota and Kunwar, 2011) provided concepts of Intellectual Property Rights and different aspects of intellectual property, international instruments, development of the intellectual Property Rights and existing situation of Intellectual Property Rights in Nepal and existing problems and challenges. The absence of appropriate inventory of biodiversity resources and appropriate legislative infrastructure Nepal is the reason for lagging behind in implementation of IPR (Yonzon, 1999). Chaudhary (1998) highlighted some aspects of intellectual property rights and ethnomedicinal knowledge for biodiversity conservation in Nepal. The Copyright Act 2059 (GON, 2002) and Copyright Regulations 2061 (GON, 2004) of Nepal have addressed for originally produced works of literature, art, knowledge and science for the benefit of its creator. World Intellectual Property Organization Copyright Treaty, 1967 elucidated on the need of a worldwide protection of intellectual property. There is urgent need of well-defined mechanism for local people to provide their information in repositories, further research for record of indigenous knowledge for bioprospecting of Nepalese plant resources for providing deserved recognition (Shrestha et al., 2017). There is need of regulations in the form of 'Petty Patents' and a 'Sui generis' system for protection and promotion of traditional knowledge to prevent an unauthorized use by third parties and protection of traditional knowledge that may erode or negatively affect the life or culture of the communities who are in possession of such knowledge (Dua, 2014). Therefore, there is a need for protection of ethnomedicinal knowledge as intellectual property and concerned people should get their rightful justice in benefit sharing.

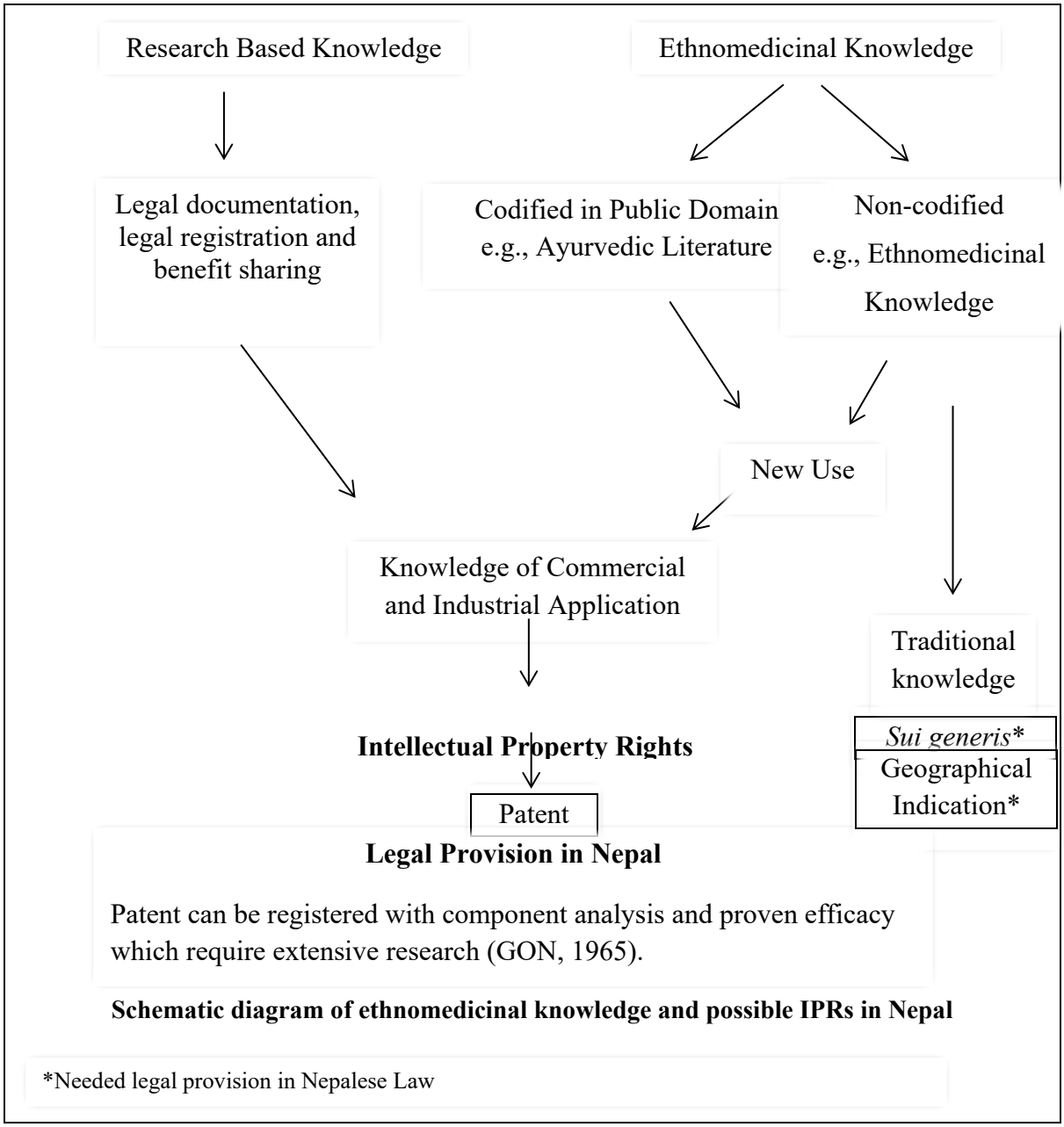
5.4.1. Acts, Regulations and National Strategy Concerning IPRs in Nepal

Acts, Regulations and National Strategy Concerning IPRs in Nepal are as follows:

1. Patent Design and Trademark Act 1965

2. Copyright Act, 2002
3. Copy Right Regulation, 2004
4. National Biodiversity Strategy and Action Plan 2014-2020

Medicine has played an important role in curing ailing human civilization. Nature is storehouse of medicine as plants have synthesized molecules which can treat various diseases. Herbal medicine is plant-derived material or preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants. In some traditions, material of inorganic or animal origin may also be present (WHO, 2005). Plants are the source of compounds that have been used as drugs, either in their original or semi-synthetic form (Salim et al., 2008). The knowledge which part of the plant cures what disease, how it is administered and what combination provides important clues in drug discovery. Therefore, the knowledge of practitioners of ethnomedicine is valuable in terms of IPRs. Understanding these facts, several countries like India, China, Korea, Thailand, Portugal, Peru, Costa Rica and Australia have taken an active role in securing IPRs of traditional knowledge.



Schematic diagram of ethnomedicinal knowledge and possible IPRs in Nepal

India has taken a comprehensive process for incorporating traditional medicine and benefit sharing (Dua, 2014). The Ayurved and traditional ethnomedicinal systems of Nepal and India are closely related due to geographical proximity and traditional ties. Therefore, in Nepal issues identified for genetic diversity and traditional knowledge are needed to be implemented (GON, 2018). There is an urgent need for laws concerning Intellectual Property right of traditional medicine be formulated and applied in Nepal. Nepal should abide by international treaty and make sure the ethnomedicinal intellectual property rights of indigenous communities protected in Nepal and elsewhere in the world and community get their rightful benefit.

5.4.2. Constraints of Patent Right Utilization in Nepal

Nepal is clearly lacking behind in incorporating intellectual property rights in some aspects in comparison to those done by the international community.

1. There is a need for regional centers for legitimate registration of indigenous knowledge that guarantee trade secrets. There should be provisions on mandatory disclosure of source and geographical origin of the biological material and associated ethnomedicinal knowledge used in the invention in patent applications.
2. The collective effort of the government body and indigenous people organization is required. Effective mobilization of local communities, organizations for collection of ethnomedicinal knowledge
3. Community, concerned organizations and university partnership should be developed to authenticate the ethnobotanical knowledge with scientific basis.
4. A national database of ethnomedicinal is needed and those with trade secret value should be protected.
5. The Patent, Design and Trademark Act, 2022 and Copyright Act 2059 needed to amend in light of present development.
6. A system for royalty collecting and sale of IPRs of ethnomedicine needed to be made.
7. Provision for buying such right of national interest from concerned persons and nationalize use, share benefits and also do further research for improvement including universities and industry should be made.
8. Effective Laws that apply the provision of accountability to those who breach laws can give benefit to actual owners of IPRs.
9. Proper remedy in case of violation of acts and compensation should be defined.
10. New acts for Intellectual property rights incorporating Petty Patents', 'Sui generis' and Geographical Indications (GI) without delay can give added advantage in this sector.

MBUST can contribute in resource identification from different parts of Nepal and develop a repository of information and bridge university and citizen scientists. This can bring into light the traditional knowledge and their innovative use. This can bring consciousness for registration of IPR and issues of sharing benefit to indigenous knowledge holders when such ideas are used. This can possibily make more people share their hidden knowledge and which can be of mutual benefit.

5.5. Production and Marketing

There is a need for systematic effort in proper collection or production of herbs, linking of processed or semi processed products with scientific basis and their industrial application for product development and marketing of the herbal products in Nepal. However, such practice is happening for limited quantities and for limited herbal materials. The nationwide herbal material collection, rating and grading of collection from nature as well as some of cultivated herbs are still not available. Most herbal materials collected from nature or cultivated are collected by middle men from villages and which ultimately reach Kathmandu or Nepalgunj market from where they are purchased by Nepalese consumers including herbal products manufacturer and rest are exported to India. The Herbs Production and Processing Co. Ltd. of Nepal government extracts essential oil from its own farming and also processes for some of essential oil based products. The Department of Plant Resource of Nepal Government does physicochemical analysis and estimation of essential oil to facilitate export of such oils. Department of Food Technology and Quality Control (DFTQC), Ministry of Agriculture and Livestock Development of Government of Nepal is the apex organization responsible for the enforcement of Food Act and Regulations. The department ensures the quality and safety of food and feed products in the country.

5.6 Conclusion

Herb contains several compounds, it is necessary to identify major components responsible for their bioactivity. The standardization of herbal products with identified active compounds and following the standard manufacturing processes to obtain a consistent amount of chemical compounds are necessary to get actual benefit of the product. Therefore, research consideration for i. single herb (complete or specified parts of plant) with principal component content, ii. Herbal or essential oil extract with principal component analysis, iii. Dietary supplements based on natural wild fruits and iv. Biopesticides for organic agriculture and food grade preservatives are major areas for production and marketing of Nepalese products. The proper accreditation procedure in every stage, from production in the farm, through processing, to distribution to the consumer can ensure quality products.

The standard guidelines such as GAP (Good Agrotechnological Practices), GLP (Good Laboratory Practices), GCP (Good Clinical Practices), GMP (Good Manufacturing Practices) ete. are needed to be followed during cultivation of medicinal plants and development of herbal products. There is need of adherence to international and domestic regulatory for agricultural and collection practices as well as responsible and sustainable harvest of agroforest product and also research, planning and advocacy for bringing Nepalese herbal product in forefront of human society.

CHAPTER 6: National and international networking and collaboration

6.1 Introduction

The remedy from use of natural products had been and it still is one of the much sought after products. The research and development for medicinal and health promoting use of natural products are increasing in the world due to technical advancement and growing understanding in the subject as well as emerging new application possibilities. Several national and international institutions are working for in depth understanding and finding utilization of natural products. The growing need and commercial possibilities in this field have also led to formation of several private enterprises worldwide. The relevant centers concerned with the natural products sector in Nepal and elsewhere in the world are listed together with their background information.

6.2 Important Natural Products Related Centers of Nepal

The leading centers concerned for development of natural products study and development in Nepal are as follows:

6.2.1 Department of Plant Resource, Thapathali, Kathmandu

https://dpr.gov.np

This department has been providing service especially in four sectors. i. Publish the Nepal Flora by keeping the record of flowering as well as non-flowering plants of Nepal, ii. In-situ and ex-situ conservation of rare, endangered and threatened species of the entire Flora of Nepal, iii. Development and handover of good agricultural and cultivation practices and technology of medicinal plants to the farmers and iv. Standardization and certification of essence of Medicinal plants collected in Nepal. The natural products research laboratory of the department was established in 1964 AD as the Royal Drug Research Laboratory. The name of the laboratory was changed to Natural Products Development Division again in 1999 AD.

6.2.2 Department of Drug Administration, New Baneshwor, Kathmandu

https://www.dda.gov.np/content/role-of-dda

This department regulates all functions relating to drugs like misuse and abuse of drugs and its raw materials, to stop false and misleading advertisements and make available safe, efficacious and quality drugs to the general public by controlling the production, marketing, distribution, sale, export, import, storage and use of drugs in Nepal. The important responsibilities of the department includes: i. Evaluation, Registration and licensing of medicines and health technology products, ii. Issuance of permit to conduct clinical trial, licenses to open pharmacy and recommendation letter to establish manufacturing unit, iii. Post marketing surveillance on medicines and health technology products, iv. Inspection and monitoring of facilities engaged in manufacture, sales, store and distribution of medicines and health technology products, and v. Testing of medicines before and after registration

6.2.3 Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur

https://nast.gov.np/natural-products-chemistry

Nepal Academy of Science and Technology (NAST) is an autonomous apex body established in 1982 to promote science and technology in the country. The Academy is entrusted with four major objectives: advancement of science and technology for all-round development of the nation, preservation and further modernization of indigenous technologies, promotion of research in science and technology and identification and facilitation of appropriate technology transfer. The Natural Products Chemistry Division of this academy has facilities for isolation and characterization as well as quantitative and qualitative evaluation.

6.2.4 Department of Food Technology and Quality Control (DFTQC), Babar Mahal, Kathmandu

http://www.dftqc.gov.np/

Department of Food Technology and Quality Control (DFTQC), Ministry of Agriculture and Livestock Development of Government of Nepal. DFTQC has played a pioneer role in laying down the foundation stone for the food quality control system, research and development in the field of food science and technology and nutrition support programs. This organization is responsible for the enforcement of Food Act and Regulations. It has been working for the implementation of Feed Act and Regulations as well. The department has a key role in

augmenting appropriate food processing and post-harvest techniques to promote agribusinesses. Any agro or forest-based edible products should be tested and verified by DFTQC for commercial production that does not pose harm to the human body. The herbal products are also required to be tested and certified by DFTQC.

6.2.5 Herbs Production & Processing Co. Ltd. (HPPCL), Kathmandu

https://hppcl.com.np/

HPCCL was established in 1981 as an undertaking of the Government of Nepal. HPPCL is the first Nepali company to harvest and promote the country's medicinal and aromatic plants (MAPs) to produce medicinal extracts and essential oils for pharmaceutical, food, and beauty and wellness industries, both within Nepal itself and abroad. The processing of rosin and essential oils are done by this company.

6.2.6 Singha Durbar Vaidyakhana Vikas Samiti (SDVKVS), Kathmandu

https://vaidyakhana.gov.np/

Singha Durbar Vaidyakhana Vikas Samiti (SDVKVS) is the manufacturer unit with four centuries of experience in the science of Ayurveda and herbal formulation. The medical formulas at SDVKVS are an inherited legacy which dates back reign of king Pratap Malla. (1624–74 A.D). This center pioneers in Ayurvedic knowledge and practices and is known to produce more than 150 products.

6.2.7 Patent, Design and Trademark, Department of Industries, Ministry of Industries, Commerce & Supplies Government of Nepal, Kathmandu

http://www.iponepal.gov.np/

The Patent, Design and Trademark Act has authorized the Department to protect the industrial property like patent, design and trademark. The Department acts as a semi-judiciary unit in case of protection of industrial property as well as in the settlement of disputes and other administrative procedures. The Department also acts as a focal point of all the international organizations/institutions including World Intellectual Property Organization (WIPO). Besides the membership of WIPO, Nepal has received a membership for Paris Convention for Industrial Property.

6.2.8 National Ayurveda Research and Training Center (NARTC), Ministry of Health and Population, Kirtipur, Kathmandu

https://nartc.org.np/

This is a national level research and training center under the ministry of Health, Government of Nepal is a premium research center established in 2009 AD with the aim of facilitating Ayurveda Health Researches and provide training to Ayurveda human resources for promoting and enriching the Ayurveda ecosystem in the country.

6.2.9 The International Centre for Integrated Mountain Development (ICIMOD), Kathmandu

https://www.icimod.org/

The International Centre for Integrated Mountain Development (ICIMOD) is an intergovernmental knowledge and learning centre working on behalf of the people of the Hindu Kush Himalaya (HKH) based in Kathmandu, Nepal and work in and for our eight regional member countries – Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal, and Pakistan. ICIMOD works to make people of the HKH more resilient, make the most of new opportunities, and prepare for change and also strengthens regional cooperation for conservation and sustainable mountain development.

6.2.10 Tribhuvan University

https://tribhuvan-university.edu.np/

Tribhuvan University (TU) is the foremost university in Nepal with various departments which provides courses in diverse disciplines at various levels. The Central Departments of the university including Chemistry, Botany and Biotechnology under the Institute of Science and Technology and Ayurveda Campus (http://www.iom.edu.np/?page_id=170) under Institute of Medicine to provide advanced level training in fields related to natural products chemistry. Research Centre for Applied Science and Technology (RECAST) https://recast.edu.np/ is the research center of Tribhuvan is involved in appropriate technologies designs and developments in the field of natural products, biotechnology, biodiversity conservation, food and agriculture, materials science and nanotechnology.

6.2.11 Pokhara University

https://pushas.edu.np/

Pokhara University has Bachelor and Master of Pharmaceutical sciences. The M.Sc. Pharmaceutical sciences has program for for Natural Products Chemistry and Clinical Pharmacy. The course facilitates students for a career in the pharmacy profession and strengthens existing pharmacy practices in the public and private sector organization. Also provide knowledge and practical skill for a career in natural medicine research and pharmaceutical care practice.

6.2.12 Kathmandu University

http://biot.ku.edu.np

The Department of Biotechnology is concerned for providing practical-based education and enhancing professional practices among our students. The department houses various research centers and laboratories to meet these objectives including KOICA Laboratory for Organic Farming, Molecular Biology Lab, Bio-informatics Lab, Cell Biology Lab, Biochemistry Lab, Environmental Biotechnology Lab, Industrial Biotechnology Lab, Chemistry Lab conducting B.Sc, MSc. and Ph.D. level research work. The department of pharmacy (http://pharm.ku.edu.np/) provides new knowledge and research to prepare students for wide variety of currently existing and potential future roles in pharmaceutical industries, hospitals, community pharmacy and their role to play in achieving desirable health outcomes in the patients or in the success of drug therapy.

6.2.13 Asia Network for Sustainable Agriculture and Bioresources (ANSAB), Kathmandu, Nepal

https://ansab.org.np

ANSAB works for building rich, healthy and productive ecosystems actively managed and sustainably used by formerly poor local communities. It also features adaptive people and resilient ecosystems able to cope with global climate change. The network aims in generating and implementing community-based, enterprise-oriented solutions that conserve biodiversity and improve the livelihoods of the poorest of the poor while bolstering national economic development and addressing climate change.

6.2.14 Chaudhary Group (C.G.) Biotech and Aayurveda Pvt. Ltd.

https://www.chaudharygroup.com/index.php/our-company/biotech-a-ayurveda.html

Chaudhary Group is setting up a GMP compatible high tech Ayurveda medicine manufacturing plant and an international standard R&D facility in its industrial complex Chaudhary Udyog Gram, Nawalparasi, Nepal. The current activities of the company include:

- Trading - Natural herbs are collected from cultivators, suppliers etc and after value addition are exported to India and also sold in the domestic market.
- Grinding and Oil Extraction - The unit also operates a Grinding and Oil Extraction unit in Nepalgunj, which is in the plains of Western Nepal where most of the raw herbs are available, for processing of the raw herbs collected in order to add value to the exported material.

The company aims to be a state-of-the-art research and development facility for personal care and life style product markets. In order to have a sustainable raw material base, CG also intends to get into research focused crop cultivation, farming and replication as part of its backward integration activity. Chaudhary Group is starting the cultivation of organic vegetables in a research driven and focused in - house farm in Kathmandu and three in-house farms in Nawalparasi. CG is simultaneously starting operations in the development of fruit orchards, industrial horticulture, animal husbandry and dairy etc.

6.2.15 Gorkha Ayurved Company Pvt. Ltd.

www.gorkhaayurved.com

The Gorkha Ayurved Company is a joint venture between Nepalese entrepreneurs and French NGO CIDR (French acronym for Center of International Development and Research), and German entrepreneurs. Production unit based at Gorkha, It was established in 1984 AD, with the aim of developing Nepal's tremendous potential. The company has been working in Gorkha District of Nepal to strengthen the economic condition of the remote villages involving local communities in collection, cultivation and processing of Ayurveda Health products. Gorkha Ayurved is registered as Private Limited, company in accordance to Nepal Company Act under the department of industry with the limited liabilities. The company is maintaining the quality of its products and focusing on the GMP criteria of the World Health Organization.

6.2.16 Dabur Nepal Private Limited

https://www.dnb.com/business-directory/company-profiles.dabur_nepal_pvt_ltd.40b111983698e9dd5a97a5a2019e7425.html#industry-info

Dabur was founded in 1884 in India and entered Nepali market in the year 1989 and started commercial production in year 1992. Dabur Nepal Private Limited provides pharmaceutical and personal care products. The Company offers a range of herbal products such as health supplements, digestive system and cough and cold medicine, tooth paste, energizers, shampoo, hair oil, skin care, food, and home care products.

6.2.17 Gajurmukhi Herbal Pvt. Ltd., Jhapa, Nepal

http://www.gajurmukhiherbal.com

Gajurmukhi one of the leading company with processing capacity of 4.5 metric tonnes per day and has been involved in organic farming in 150 Hectares of private lands and 350 hectares of community forest lands. The company products are organic certified by European Standard (EU) and American Standard (NOP). The every batch of essential oils is done GCMS (Gas Chromatography Mass Spectrometer) to analyze quality.

6.3 International Natural Products Related Centers of Excellence

6.3.1 State Key Laboratory of Bioactive Substance and Function of Natural Medicines, China

https://www.imm.ac.cn/en/organization.asp

State Key Laboratory of Bioactive Substance and Function of Natural Medicines (SKLBSFNM), Ministry of Science and Technology in 2011, was established on the foundation of the Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicines (The Ministry of Education), the Key Laboratory of Biosynthesis of Natural Products (The Ministry of Health), and State Key Subject of Pharmacy for research fields:

1. The discovery of bioactive substances of natural medicines.
2. The drug ability of bioactive substances of natural medicines.
3. The development of new technologies and methods.

The operation policy of the laboratory is “Open, Exchange, Cooperation and Competition.” SKLBSFNM is an integration of advanced resources, knowledge support and improved research platforms and systems. The laboratory’s tasks are to discover bioactive substances with novel structures and unique functions, to optimize their structures and clarify their molecular mechanisms. The goal of SKLBSFNM is to create a favorable environment, bring in high level pharmaceutical professionals and play a key role in the field of drug discovery.

6.3.2 The International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan.

https://www.iccs.edu/

ICCBS is one of premier research establishments in the developing world. Its reputation for scientific research and training extends far beyond the country's borders. ICCBS emerged from generous support of a private sector, and sustained by the government’s grants. In the last 51 years has earned major recognition from Pakistan, apart from training thousands of young researchers from home and abroad in frontier areas of science and technology. Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) is a center for molecular medicine, which deals with the understanding of the molecular basis of diseases and then developing appropriate strategies for their early diagnosis, management and elimination.

6.3.3 Natural Products Research Center, Korea Institute of Science and Technology, S. Korea

https://gn.kist.re.kr:8443/eng/main/main.do

Natural product research center was established to lead and facilitate the advancement of natural product industrialization in domestic and global markets by performing fast screening and uncovering precise mode of action (MOA) of bioactive natural products. The center mainly focuses on the discovery of the first target in the world applicable for functional foods and medicines based on multi-omics techniques. The center also does research on construction of high throughput screening systems and *in vivo* animal models by validating and optimizing the candidate target as well as development of a transformation technique to maximize the content of bioactive compounds in natural products.

6.3.4 Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK

https://www.ljmu.ac.uk/research/centres-and-institutes/centre-for-natural-products-discovery
The center undertakes high-impact natural products research, provides scientific research training and has taken an active role in research dissemination, advocacy, public engagement and social and economic contributions, as well as research capacity building.

6.3.5 All India Institute of Ayurveda, India

https://aiia.gov.in/

All India Institute of Ayurveda is an Apex Institute for Ayurveda in India. It aims at bringing a synergy between traditional wisdom of Ayurveda and modern tools and technology. The institute offers postgraduate and doctoral courses in various disciplines of Ayurveda and focuses on fundamental research of Ayurveda, drug development, standardization, quality control, safety evaluation and scientific validation of Ayurvedic medicine. The hospital is equipped with state of the art modern diagnostic tools and techniques which are used in teaching, training and research. The patient care is done primarily through Ayurveda of tertiary level. This institute also has an international collaborative centre for global promotion and research in Ayurveda.

6.3.6 National Center for Natural Products Research (NCNPR), University of Mississippi, USA

https://pharmacy.olemiss.edu/ncnpr/

The center was founded in 1995 to research, develop and commercialize potentially useful natural products. Based at the University of Mississippi, School of Pharmacy, NCNPR collaborates with academia, government and the pharmaceutical and agrochemical industries to create natural products that can be used to improve human health and agriculture as crops, pharmaceuticals, dietary supplements and agrochemicals. The National Center for Natural Products Research has been funded by the U.S. Food and Drug Administration (FDA) since 2001 to develop analytical methods and reference standards for botanical formulations sold as dietary supplements in the U.S. This fundamental research is an essential part of the FDA's strategy for increasing the quality and safety of products sold in this multi-billion dollar market.

6.3.7. Department of Oriental Medicinal Materials & Processing, Kyung Hee University, S. Korea

https://gskh.khu.ac.kr/index_eng/s2_class/class_2_detail.php?&ca_id=200204

The function of this department of Kyung Hee University is to investigate new potentials of oriental medicinal materials, developing new medicines and cosmetic materials using oriental medicinal plants. The center does research on pharmaceutically active compounds in ginseng and secondary metabolites from oriental medicinal plants. The research in the department concentrates on molecular level work on oriental medicinal plants, trying to extract pharmaceutically active compounds and develop potential medicines, functional foods, and cosmetics.

6.3.8. Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll- Institute (HKI), Germany.

https://www.leibniz-hki.de/en/mission-and-vision.html

HKI is a world-leading academic research center in the field of natural product research and infection biology of human-pathogenic fungi. Employing a multi-disciplinary approach the HKI develops new technologies that are used to identify natural products, to characterize their function and exploit their mechanisms of action in order to elucidate cellular processes. In addition, the HKI investigates the pathobiology of infections caused by human-pathogenic fungi. These investigations will find application in the use of natural product-based drugs for treatment and diagnosis of infectious diseases mainly caused by fungi. Specifically, the HKI is an internationally leading center in the following areas:

Genome Analysis (transcriptome, proteome, metabolome) of fungi and host cells

Molecular Biology of fungal secondary metabolite producers and pathogenic fungi

Natural Product Chemistry including structure analysis

Fermentation/Physiology

Systems Biology including establishment of data bases

Host/Fungal pathogen interaction

6.3.9 Laboratory of Medicinal Chemistry, N.D. Zelinsky Institute of Organic Chemistry Russian Academy of Sciences, Russia

https://zioc.ru/institute/laboratories/laboratory-of-medicinal-chemistry-n175

The laboratory is well known for qualitative and quantitative measurement techniques in a complex of physical and chemical determinations of structural and functional characteristics developed in ZIOC RAS form a basis of analytic measurements. The laboratory has more than 200 analogues of natural antimitotics - cell division inhibitors: combretastatin, podophyllotoxin, steganocin, glazovianin A and others have been synthesized from natural allylbenzenes, isolated from industrial extracts of parsley and dill seeds. A constantly updated collection of chemical compounds (about 200,000) has been created for screening for various types of biological activity. The laboratory has developed program for recording these compounds in the warehouse and on-line searching for both molecules and their NMR and mass spectra by structural fragment.

6.3.10 Laboratory of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan

http://www.f.u-tokyo.ac.jp/~tennen/index-e.html

Research on mechanism of formation of a wide variety of naturally occurring substances and multidisciplinary approach involving synthesis, enzymology, molecular biology, and structural biology is employed to address fundamental problems at the interface of chemistry and biology are key features of the laboratory. The researchers conducted in the laboratory includes the process of biosynthesis of natural organic compounds produced by plants and microorganisms, using not only the foundation discipline of organic chemistry, but also incorporating the methods of biochemistry and molecular biology in an effort to understand the enzymes that catalyze each biosynthesis reaction and the functions and control mechanisms of the genes that govern their expression at the molecular level.

6.3.11 Alexander von Humboldt Foundation, Germany

https://www.research-in-germany.org/en/research-funding/funding-programmes/avh-humboldt-research-fellowship-for-experienced-researchers.html

Alexander von Humboldt Foundation is an intermediary organization of German foreign cultural and education policy, the foundation aims to promote international cultural dialogue

and academic exchange. The foundation offers flexible sponsorship programmes for researchers at all stages of their careers. Such fellowships and awards enable outstanding scientists and scholars from abroad to complete long-term research stays in Germany. There are no specific quotas for specific countries or subjects; only personal academic achievement counts. The foundation also maintains close links with its alumni, the Humboldtians, after their stay in Germany and offers numerous alumni support programmes.

6.3.12 Tokyo Institute of Technology, Japan

http://www.apc.titech.ac.jp/~htanaka/

The laboratory aims to develop chemistry (molecules) and engineering to understand or control biological functions. As a goal of research, it is aimed to implement social implementation technology. This is a leading institute for synthesis of biological active natural products and application of chemistry to medicine and industry.

6.3.13 The American Chemical Society (ACS), USA

https://www.acs.org/content/acs/en/about.html?sc=180808_GlobalFooter_od

As a non-profit scientific organization with more than 140 years' experience, we are a champion for chemistry, its practitioners and our global community of members. The American Chemical Society (ACS) is focusing collaborative partnerships with sister chemical societies around the United Nations Sustainable Development Goals (SDGs) through Chemistry Enterprise Partnerships (CEPs). CEPs outline how the ACS will collaborate with a partner organization/sister chemical society.

6.3.14 Royal Society of Chemistry, UK

https://www.rsc.org/

Chemical Society of London was formed in 1841 when 77 scientists – including doctors, academics, manufacturers and entrepreneurs –with dialysis inventor Thomas Graham as their first President. Seven years later Queen Victoria granted a Royal Charter to the Society, confirming its purpose of "the general advancement of Chemical Science". The society has over 54,000 members across the world, an internationally-renowned not-for-profit publishing and knowledge business, and a reputation as an influential champion for the chemical sciences.

6.4 Necessity of National and International Network and Collaboration

It is an irony that though we have a very long history of use of natural products as herbal heritage and there had been gradual establishment of centers for development of this sector Nepal is still lagging behind. There is still need to establish network in Nepal to bring together grass root level people involved in herb collection and cultivation and source of ethnobotanical knowledge in Nepal, academia which can impart technical advancement and industrial entrepreneurs who can apply the research outcome from academia to make marketable products that can compete international need. The proper network within stakeholders of medicinal plant and essential oil fields is needed to strengthen business in this sector. The channelization of the network with international business and scientific research centers can help to get recent technology and market niches. It is very encouraging step that Madan Bhandari University of Science and Technology Development Board has taken initiative by signing a memorandum of understanding with National Business Initiative (https://nbinepal.org.np/), a private enterprise, to do research with business perspective integrating agroforestry products and human needs.

The human resource for research as well as laboratory facilities with equipment for structure determination using spectroscopic methods for structure elucidation and appropriate bioassay facility for determination of efficacy had been major limiting factors in the research of natural products research in Nepal. The development of herb and agroforestry based industry and export of processed products instead of raw material to international markets as well as use in national consumption can make lots of difference in trade of Nepalese products. Further, to assimilate technical advancement and new knowledge the collaborative research work with centers of excellence in an international arena are required.

6.5 Conclusion

The national and international collaboration for utilization of natural products and solving trade related issues such as quality testing and certification can ultimately help to reach international markets segments related to natural products. There are several aspiring micro, small and medium enterprises in Nepal involving personal care products, Ayurvedic and traditional medicine products who can benefit from research and innovation.

CHAPTER 7: Topics for PhD research and Master's degree research

7.1 Introduction

The academic programs of MBUST include the forest bio-material science and engineering, organic agriculture and artificial intelligence under consideration. The programs proposed for forest bio-material science and engineering (Yan, 2020) and organic agriculture (Amstel, 2020) include some courses related to natural products (Appendix III). Therefore, the topics and design of course are provided for natural products related fields.

7.2 Topics for PhD Research and Master's Degree Research

The applicable topics for PhD research and course for Master's degree research are as follows:

7.2.1. Herbal Resource Principal Component Analysis and Standardization

Species: *Neopicrorhiza scrophulariiflora, Paris polyphylla, Piper longum, Delphinium himalayai* and *Cannabis sativa* from known localities of production in Nepal.

1. Principal phytochemical constituents analysis of species with commercial trade value

Among plant species of Nepal *Piper longum, Delphinium himalayai, Neopicrorhiza scrophulariiflora, Paris polyphylla,* and *Cannabis sativa* are species with trade value due to their medicinal and other uses. The phytochemical analysis leading to standardization will be useful for the standardization of the raw as well as processed products. Further, identification of phytoconstituents will open avenues for their utilization in herbal medicine and other uses. The sustainable supply is also issue in Nepal as there is very little organized collection practice through community forestry user groups. For the research of phytochemical constituents sizable raw material (5-10 kg) for each plant will be needed to be collected so that there will be sufficient amount can be isolated and use for processing in Nuclear Magnetic Resonance (NMR) and other spectroscopic methods as well as bioassay. Herbal materials can be extracted with aqueous ethanol or methanol (80%), the concentrated extract will be required to partitioned using solvents like ethyl acetate, *n*-butanol and distilled water to get non polar and polar constituents. After partition and concentrated fractions and on application in column chromatography with bioassay guided fractionation the isolation of compounds can be done which can be identified using Nuclear Magnetic Resonance (NMR), Infra-Red, Ultra Violet

Visual and mass spectroscopic methods. The isolated major compounds can be used as marker compounds for High Performance Liquid Chromatography or Liquid Chromatography-Mass Spectrometry based quantification and standardization of herbal materials.

2. Comprehensive profiling and quantification of major component of herbal materials with LC-MS

The isolated compounds profiling and their quantification using LC-MS can help in building spectrum with major components of each plant which can be used for simultaneous quantification of marker compounds for qualitative analysis of herbal materials.

3. Evaluation pharmacological properties of herbal materials for antioxidative, anti-inflammatory, neuroprotective, anticancer, antibacterial, antifungal, antiviral, anthelmintic, anti-hyperglycemic and antihypertensive effects.

Establishment of laboratory for different assay will build up laboratory for herbal analysis system The facility of anti-oxidative assay such as di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) and 2,20-azino-bis(3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) the antibacterial screenings with disk diffusion assay of ATCC cultures of microorganism and some enzyme based assay using UV-VIS spectroscopy can be used such as angiotensin I-converting enzyme for antihypertensive (Balasuriya and Rupasinghe, 2011), α-glucosidase and α-amylase enzymes for anti-hyperglycemic (Lankatillake et al., 2021), inhibition of acetylcholinesterase and butyrylcholinesterase and tyrosinase for neuronal disease (Orhan et al., 2017) to find potential compounds which can provide platform for preliminary screening of useful compounds.

4. Study on phytochemicals from herbal material for skin and hair care products

Natural herbal based cosmetic preparations are in demand in market for their healing properties and soothing effect. The essential oil based herbal extracts can be targeted for skin and health care products.

5. Organic herbal cultivation and development of high value organic herbal products

The improvement in cultivation including new technologies such as hydroponic and aerophonic technology can produce herbal material rich in desired marker compounds. Evaluation of those methods can give technology for future cultivation.

7.2.2 Essential Oil Characterization and Product Formulation

1. Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and cultivated herbs

There can occur variation in components content in essential oil depending on habitat of occurrence and make them different in composition. Characterization of content can be useful for making essential oil based products.

2. Quantification of essential oil composition in herbal materials of Nepal and their antimicrobial property for use as food and fruit preservatives

Microbial contamination in food and fruits limit their shelf life. The research on essential oil-loaded encapsulation of natural oils in nanofibers can provide options for food grade preservatives (Ataei et al., 2020).

3. Essential oil based biopesticides development from herbal materials of Nepal

The use of pesticide and their residue is of serious concern, there is possibility of epigenetic harm due to synthetic pesticides (Yang et al., 2018), therefore alternative effective herbal materials are needed to be searched.

4. Formulation of flavor and fragrance components from essential oils produced in Nepal

Several essential oil are produced in Nepal such as *Cinnamomum glaucescens, Cinnamomum tamala, Cymbopogon flexuosus, Cymbopogon martini, Cymbopogon winterianus, Matricaria chamomilla, Mentha arvensis, Nardostachys jatamansi, Ocimum basilicum* and *Zanthoxylum armatum* for export purpose. The product formulation for use within Nepal can be more beneficial than export of essential oils.

5. Formulation for oral, skin and hair care products from essential oils produced in Nepal

Use of essential oil for oral, skin and hair care products are and their demand is growing in the market. The product formulation for these purposes can make possible utilization of essential oil produced within Nepal.

7.2.3 Functional Food and Medicinal Materials Analysis

1. Principal constituents analysis and evaluation of the beneficial herbal tea

Camellia sinensis (tea) is a rich source of epigallocatechin (Kalauni et al., 2020). Further, evaluation of other herbal products used as tea infusion such as leaves of *Taxus baccata* and *Hippophae goniocarpa* can value add such products. Dietary supplements rich in flavonoid and polyphenol content are known for several health benefits.

2. Analysis of bioactive components and functional food value of edible mushroom from organic cultivation

Edible cultivated mushroom such as *Agaricus bisporus, Pleurotus ostreatus, Ganoderma lucidum,*and *Cordyceps militaris* are source of components with function value which can be evaluated for promotion as health food (Du et al., 2018). Evaluation of bioactive components evaluation can be used for their functional food development.

3. Analysis of bioactive components from *in vitro* culture of Yarsagumba (*Ophiocordyceps sinensis*) and other *Cordyceps* species

Ophiocordyceps sinensis is the most revered mushroom of Himalayas. The wild harvests as well as mycelium culture do have a market in the world. The bioactive components of in vitro culture can be compared with those of wild harvest to provide trust in the beneficial effect of cultured products (Kharkwal, 2016).

4. Microbial secondary metabolite analysis for antibiotic and antifungal properties

Antibiotic resistance is one of the major setbacks in treatment of diseases of microbial origin. Several antibiotic substances capable of treatment of such conditions have been isolated and are in pharmaceutical use (Pala et al., 2019). Therefore, culture of microbes from different sources for harvest of their secondary metabolites can provide an opportunity to find effective antibiotics and antifungal components.

7.2.4 Computer-aided Drug Discovery and *in silico* Chemicobiological Analysis

1. Computational methods for structure-based and ligand-based chemoinformatics processes of bioactive components from Nepalese herbs

The virtual screening of compounds for their possible bioactivity for principal components of herbal material can direct the utility of isolated compounds from herbal materials.

2. Application of computational methods for optimization of herbal components for target site in medicinal and pesticide use

In structure-based approach molecular dynamics, docking and binding cavity analysis are useful for such purposes. The ligand-based techniques including Quantitative Structure Activity Relationships (QSAR), estimation of drug likeness, prediction of adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, pharmacophore identification and similarity searching are (Pereira and Aires-de-Sousa, 2018) can facilitate understanding compounds possible function delivery process. Therefore, research in computer-aided drug discovery can provide new avenues of discovery and innovation.

7.3 Design of Master's Course

The understanding of plant taxonomy, botanical nomenclature, species identification, morphological features and microscopy are needed for species identification. This will provide understanding of authentic botanical and other sources, alternative substitutes and adulterants. The sample preparation, method development, molecular structure determination, chemical profiling, data processing and interpretation, value chain improvements are needed to be incorporated in the course. The use of nuclear magnetic resonance (NMR), infra-red spectrometry, UV-vis spectroscopy, mass spectrometry and sensitive hyphenated techniques such as GC-MS and LC-MS are useful for qualitative and quantitative analysis. Following are some course topics applicable for Master's and PhD course:

7.3.1 Core Subjects

Course Code	Course Name	Course Modules
NP-1	Taxonomy of medicinal and aromatic plants and phytochemistry	Plant collection and herbarium technique; taxonomic libraries, plant identification, checklists and keys; retrieving taxonomic data, web-based systematics.

NP-2	Bio-organic chemistry and natural products based drug discovery	Secondary metabolites and their biosynthetic pathways; alkaloids, steroids, flavonoids / polyphenols / tannins; terpenoids, polyketides, proteins / peptides, saponins
NP-3	Separation and chromatographic techniques	Planar chromatography – basic principles, sample application, development of plates, visualization of plates; Column Chromatography – column packing, sample loading, detection; principles of chromatographic techniques, normal and reversed phase, bonded phase chromatography; selection of mobile phases; automated flash chromatography; Hyphenated techniques: Introduction to GC-MS and LC-MS techniques
NP-4	Principle and applications of Nuclear Magnetic Resonance and spectral analysis	^{1}H NMR and ^{13}C NMR Spectroscopy: Chemical shifts, coupling constant, advanced 1D NMR experiments such as NOE, DEPT experiment. Quantitative NMR methods. Homonuclear 2D NMR: H- H COSY, NOESY, DQF-COSY, TOCSY, C-C correlations INADEQUATE. Heteronuclear 2D NMR: HSQC/HMQC, HMBC structure elucidation: examples from alkaloids, flavonoids, sterols, coumarins, and triterpenes.
NP-5	Principle and applications mass spectroscopy, infra-red spectroscopy and UV-VIS spectroscopy	Applied spectroscopic methods for structure elucidation: High-Resolution Fast Atom Bombardment Mass Spectrometry (HR-FAB/MS), electrospray ionization (ESI), Matrix-Assisted Laser Desorption/Ionization High-Resolution Time-of-Flight Mass Spectrometry (MALDI HR TOF/MS), nitrogen rule, mass defect, diagnostic fragment ions; - Ultraviolet bands for carbonyl compounds, unsaturated carbonyl compounds, dienes and conjugated polyenes; Fieser Woodward rules for conjugated dienes and carbonyl compounds - Ultraviolet spectra of aromatic and heterocyclic compounds - Steric effect in biphenyls; Characteristic vibrational frequencies of alkanes, alkenes, alkynes, aromatic compounds, alcohols, ethers, phenols and amines - detailed study of vibrational frequencies of carbonyl compounds (ketones, aldehydes, esters, amides, acids, anhydrides, lactones, lactams and conjugated carbonyl compounds).

NP-6	Quality control and marketing strategy	Medicinal plant based industry; export and import of plant materials, threatened / endangered medicinal plants.

7.3.2 Elective Subjects

Course Code	Course Name	Course modules
EL-1	Ayurveda and herbal formulations	Plant-derived molecules for medicine, perfumery, cosmetic, agrochemicals, dyes and pigments
EL-2	Computational chemistry	Introduction and numerical methods; classical simulations; quantum mechanical calculations; biomolecular simulations; role of *in silico* approaches for suitable targets in drug discovery
EL-3	Herbal cultivation	Technology for commercial production of some selected medicinal and aromatic plants.
EL-4	Microbial cultivation for bioactive compounds	Biotechnology of propagation and production of antibiotic and non-antibiotic drugs using microbes
EL-5	Bioactivity analysis	Bioassay-directed fractionation, rapid screening methods, toxicity, enzyme inhibition, pharmacological activity for anti-inflammatory, neuroprotectors, immunomodulators, biopesticides
EL-6	Essential-oil	Supercritical fluid extraction; microwave assisted, ultrasonication, extraction of volatile oils; flavor and fragrance products

7.3.3 Mandatory Non-credit Subjects

Course Code	Course Name	Course modules
NC-1	Literature and research methodology	Literature review, research hypothesis and proposal formulation, statistical analysis
NC-2	Ethnobotany	Colleting, documenting and validating traditional knowledge, bioactive component discovery; case studies of development of drug from folk medicine: e.g. curcumin, artemisinin, taxol
NC-3	Intellectual property right, trademark and patent right and research ethics	Importance of intellectual property right and trademark registration, patent right protection, ethical research and obligation of country's law in product development
NC-4	Trial cultivation	Selected medicinal and aromatic plants cultivation in controlled condition.
NC-5	Entrepreneurship and communications	Market need analysis, product development, technical writing and marketing skills

NC-6	Seminar	Gain knowledge of the latest findings in natural products research and biotechnological advancement, journal publication; presentation and discussion skills.

7.4 Conclusion

The utilization of plant resources from nature and agriculture sectors are much anticipated fields that can bring ultimate progress of Nepal as the land is bestowed with species diversity and climate. The setback which is depriving us from this possible gain is lack of trained human resource not only for processing but also growing and collecting. The research in organic farming of herbal and food resources can give quality production as well as help in penetration of the organic market niche. The exploration and sustainable utilization of non-timber forest products as well as timber products with medicinal value can be beneficial not only to Nepal but all natural products utilization sectors. The research and human resource outputs from natural products study is therefore directly connected to the rural economy and export quality products and innovations in this area can bring herbal based entrepreneurship and industrial development.

CHAPTER 8: Cost estimation for lab establishment for the isolation and extraction of compounds from natural products

8.1 Introduction

Natural products chemistry research involves extraction of plant materials, concentration of extract, isolation of compounds and identification of compounds using different spectroscopic techniques (Figure 5). The natural products laboratory requires a section with wet lab and instrumentation facility. The extractions of plant material and column chromatography leading to isolation of compounds are carried out in wet laboratory (Figure 6). The equipment concerned with sophisticated spectroscopic analysis such as Fourier transform infrared spectroscopy, UV-Visible spectroscopy, Nuclear Magnetic Resonance Spectroscopy (NMR) are required to be placed in instrumentation facility.

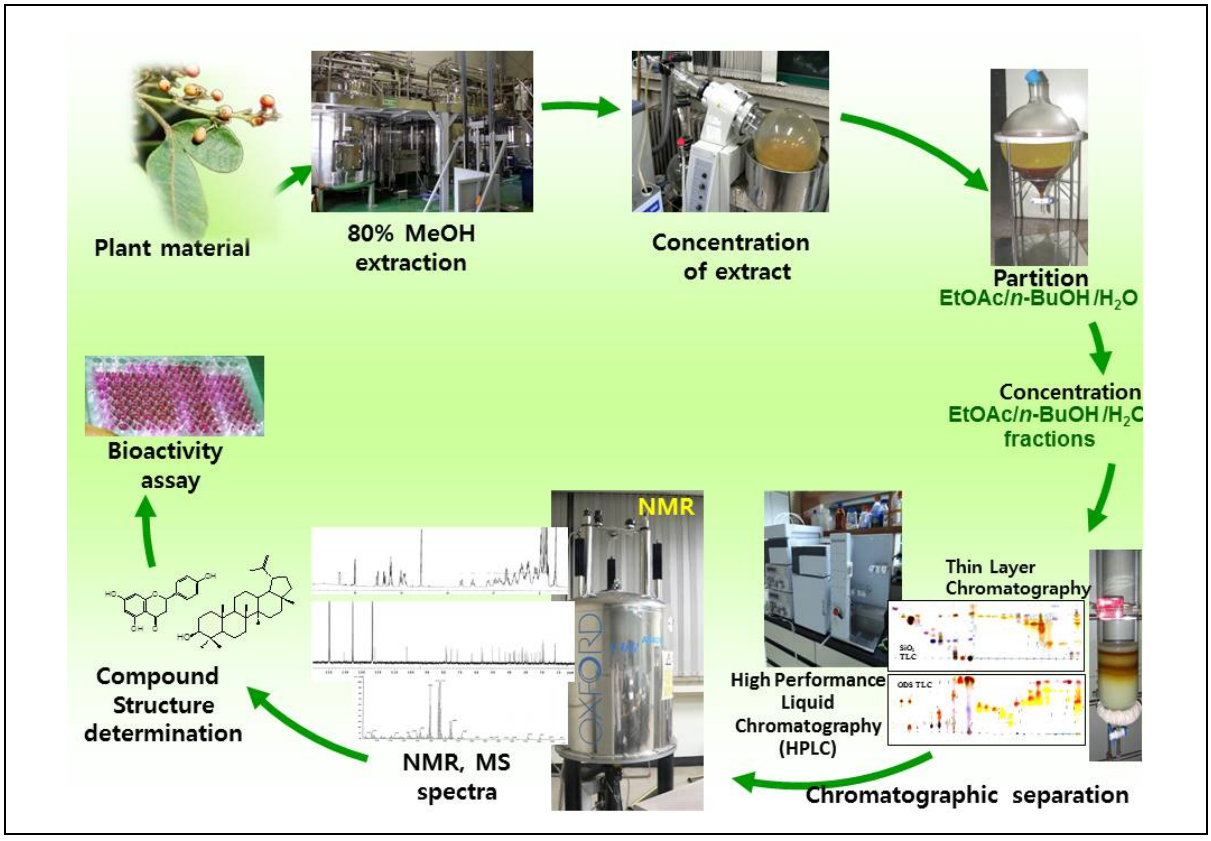


Figure 6. General stages of natural products research

The specialized spectroscopic analysis equipment such as High-performance liquid chromatography (HPLC), Gas chromatography mass spectrometry (GC-MS), Liquid Chromatography with tandem mass spectrometry (LCMS/MS) can be placed within instrumentation facility or within wet chemistry laboratory.

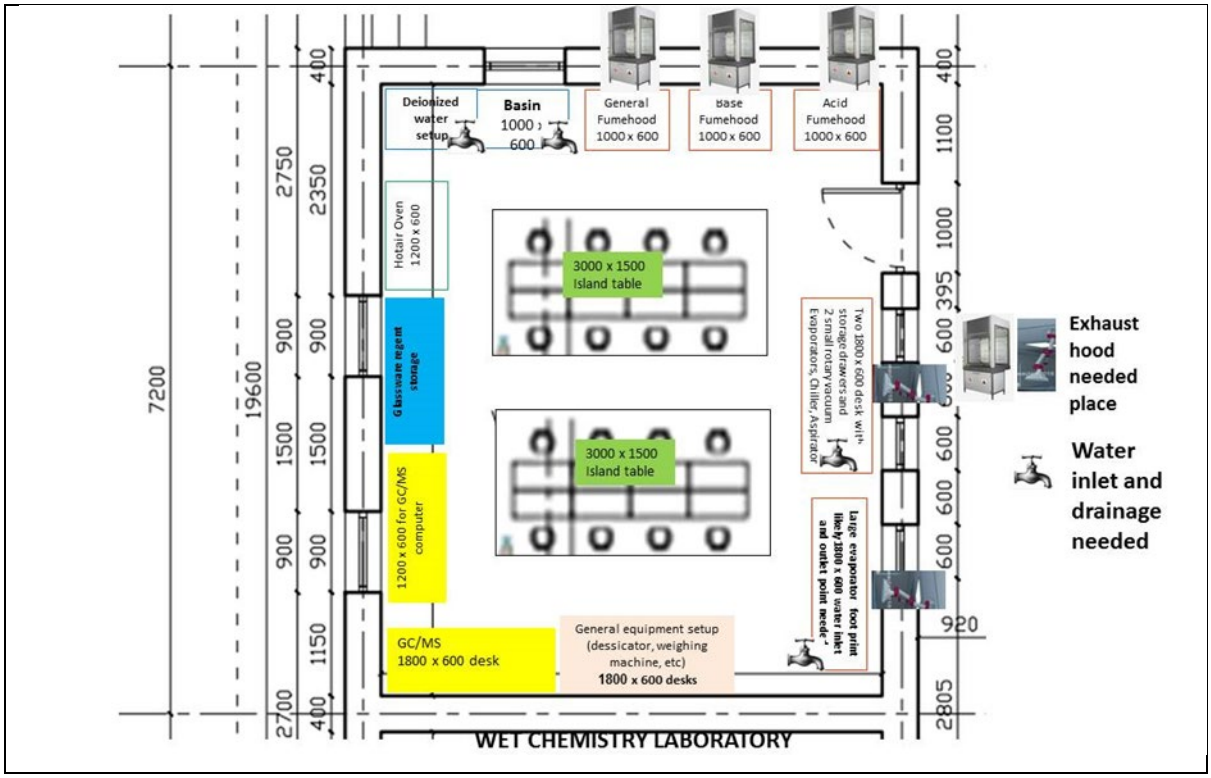


Figure 7. A typical wet chemistry lab setup

Among equipments the Nuclear magnetic resonance (NMR) spectrometer with needs specialized facility. The higher the magnetic field gives the more dispersed or separated the NMR spectrum so equipment with a 500 MHz or more capacity superconducting magnet system can provide better research possibilities. The floor of the laboratory with NMR equipment must be sufficiently rigid to reduce the vibration from adjacent dynamic loads to a negligible level (no single peak vibration greater than 20 µg acceleration from 5 to 100 Hz). Ground floor or basement sites are preferred as the natural resonant frequencies of most building structures are typically at low frequencies and horizontal in direction. The cement slabs on grade are preferred to suspended floors because they are stiffer by nature and generally have less low frequency vibrations. Use of firestone tire antivibration systems is not recommended at sites with vibrations below 20 Hz or at any site with large vibrations in the horizontal direction. The ground or basement sites are ideal to minimize building vibrations. High rise buildings may oscillate at frequencies below 1 Hz. Such oscillations may be noticeable in upper floors and are impossible to control. The room with size 6.1 m x 6.1 m (20ft x 20 ft) can be appropriate (Figure 7). There is a need of a minimum 1.5 m (5 ft) space from any wall. [Source:

http://www.emory.edu/NMR/docs/Varian%20NMR%20Installation%20Planning%20Guide%20020406.pdf and http://www2.chem.uic.edu/nmr/downloads/BASHCD10/pdf/z31276.pdf]

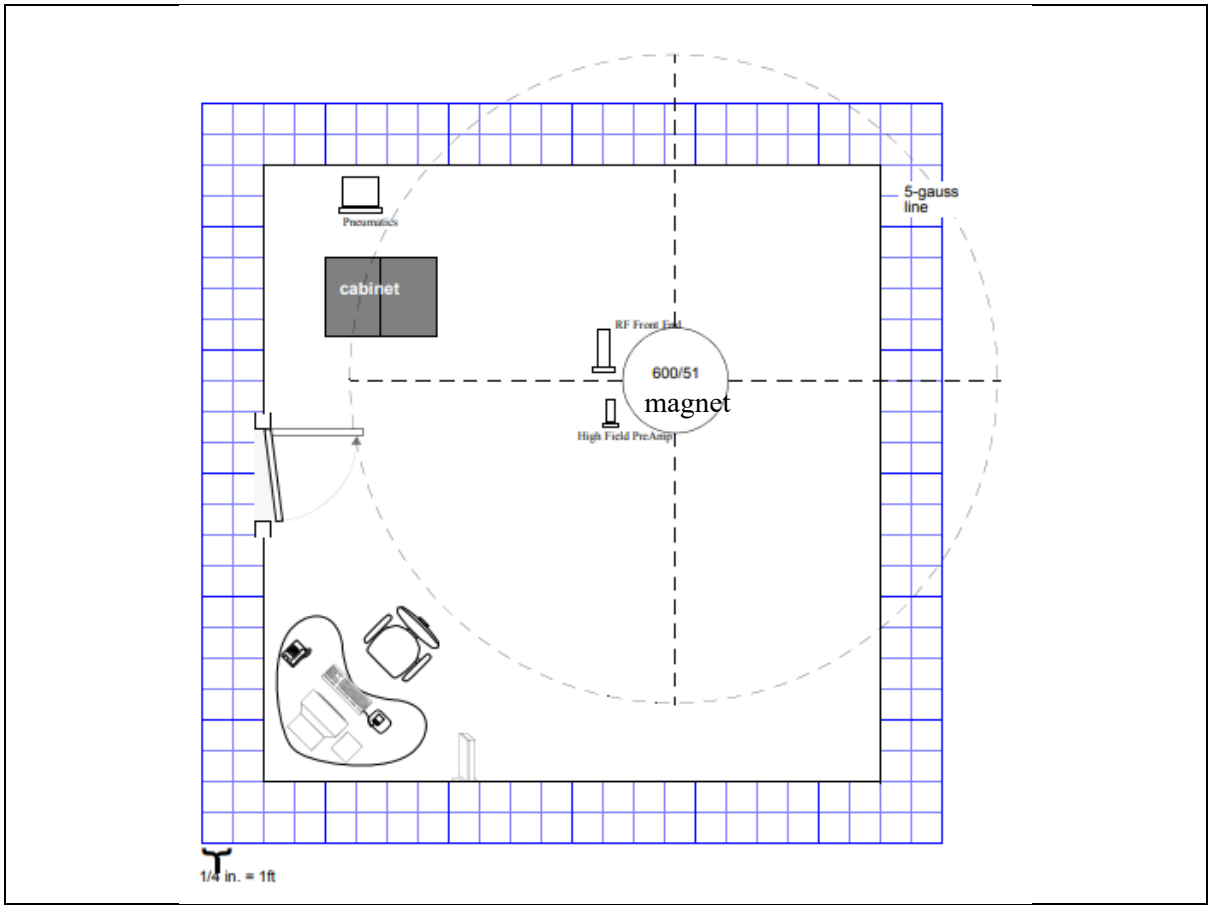


Figure 8. NMR room layout

http://www.emory.edu/NMR/docs/Varian%20NMR%20Installation%20Planning%20Guide%20020406.pdf

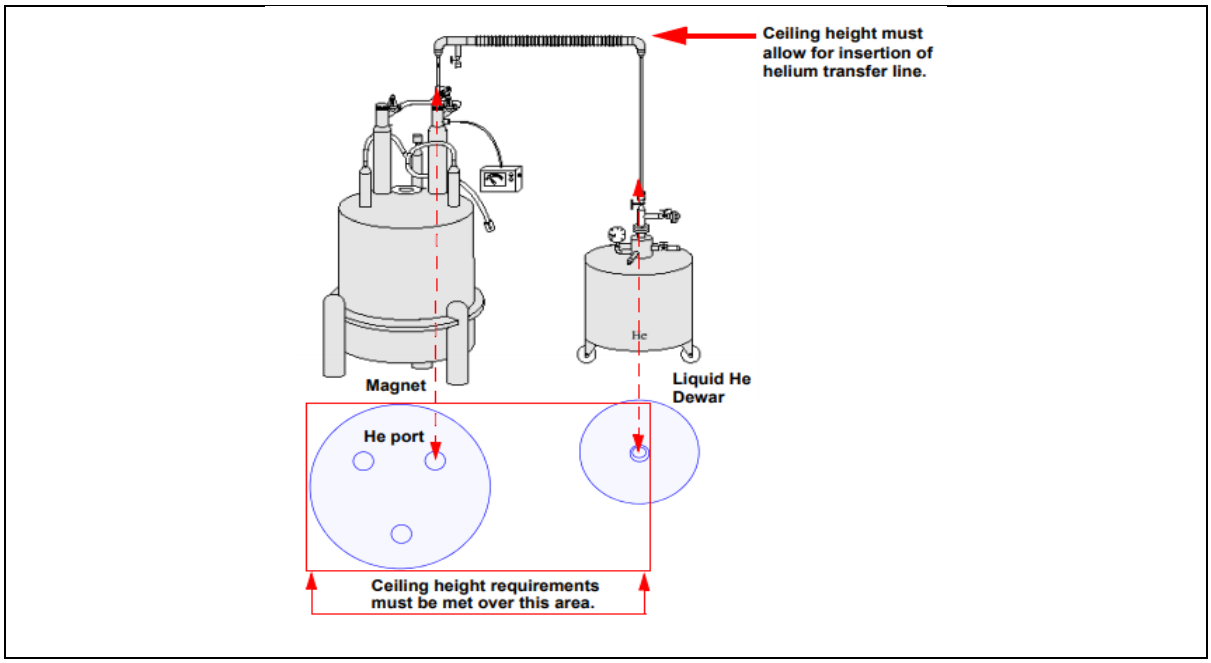


Figure 9. Ceiling height requirement

Source: http://www2.chem.uic.edu/nmr/downloads/BASHCD10/pdf/z31276.pdf

The celiing height requirement can be 3.5-4 m depending on equipment structure and appropirate space for helium transfer line (Figure 8).

NMR magnet system is three dimensional, consideration must be given to floors above and below the magnet, as well as to the surrounding space on the floor the magnet resides (Figure 9). The magnetic field exerts attractive forces on equipment and objects in its vicinity. These forces, which increase drastically approaching the magnet, may become strong enough to move large equipment and to cause small objects or equipment to become projectiles. [Source: http://www2.chem.uic.edu/nmr/downloads/BASHCD10/pdf/z31276.pdf]

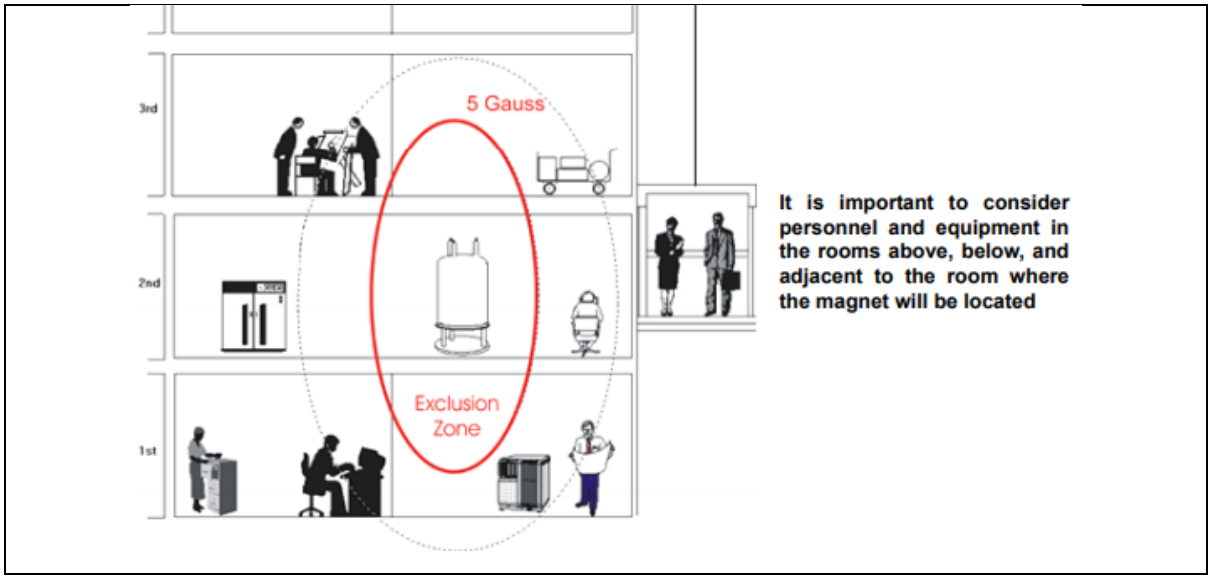


Figure 10. Exsiting stronger stray fields in vertical direction than horizontal direction of NMR setup
Source: http://www2.chem.uic.edu/nmr/downloads/BASHCD10/pdf/z31276.pdf

8.2 Equipment and Supplies

8.2.1 Major Equipment

Item No.	Equipment	Function
1.	 Fourier transform infrared spectrometer (FT-IR)	FT-IR is useful to obtain infrared spectrum of absorption, emission, and photoconductivity of solid, liquid and gas. It is useful for the detection different functional groups. The infrared absorption of the sample in frequency range 400–4000 cm^{-1} give information of the specific molecular groups prevailing in it through spectrum data

2.	 UV-Visible spectrophotometer	UV-Visible spectrophotometer is useful in analytical chemistry for the quantitative determination of different samples.
3.	 Nuclear Magnetic Resonance Spectroscopy (NMR)	Nuclear Magnetic Resonance Spectroscopy (NMR) is the key analytical tool capable of complete analysis and interpretation for determining structures of organic compounds. The spectral data such as 1D NMR data including proton, carbon and distortionless enhancement by polarization transfer (DEPT) and 2D data including homonuclear correlation spectroscopy (COSY), Heteronuclear Multiple Bond Coherence (gHMBC), gradient Heteronuclear Single Quantum Coherence (gHSQC) are used for structure elucidation of a compound. It has application in fields as chemistry, medicine, polymer chemistry, biochemistry, structural biology and materials science.
4.	 High-performance liquid chromatography (HPLC)	HPLC technique in analytical chemistry used for separation, identification, and quantification of each component in a mixture. The separation of components happen with pressurized liquid solvent passing through through a column filled with a solid adsorbent material. Depending on interaction of compounds with the adsorbent material and difference in flow rates separates components as they flow out of the column. The use of preparatory column can facilitate rapid, efficient separation and suitable yield of compounds.
5.	 Gas chromatography mass spectroscopy (GC-MS)	Gas chromatography mass spectrometer (GC-MS) Gas is used to analyze or separate volatile components. This is higly useful for research on essential oil as well as pesticide residue qualitative identification quantification.

6.	 Large Scale Rotary Vacuum Evaporator	Large scale rotary vacuum evaporator which can hold 10 liter flask for evaporation of extract in large quantity are highly useful to get concentrated extract.
7.	 Small Scale Rotary Vacuum Evaporator	Small scale rotary vacuum evaporator which can hold 1000-3000 liter flask for evaporation are highly useful to get concentrated extract after partition of crude extract as well as fractions obtained from column chromatography.
8.	 Digital Balance	Precise weight of compound and reagents are needed in laboratory for analytical purpose. The balance with option for tare, quick stabilization, fitted with sliding doors for easy access the weighing chamber in moisture free condition and provide reliable weight measurements within 0.001 are needed.
9.	 Normal  Retractable  Enclosed Fume hoods	All fume hood with exhaust suction will be appropriate. There can be different kinds of fume hoods stationary and with retractable arm and enclosed. Fume hood in enclosure are appropriate to place class column for column chromatography. Such facility will need rigid metallic frame to support glass columns.

10.	 UV chamber	The UV chamber is device with tubes for 254 and 365 nm wave length. This is used for Thin Layer Chromatography (TLC) plate visualization.
11.	 Sonicator	Ultrasonic waves sonicator is used for extraction procedure as well as useful for homogenization of solvent mixture. It is also useful for extracting out sticky substance from glassware.
12.	 Fraction collector	Fraction collector is needed for the collection of elution from column chromatography for fractionation.
13.	 Vacuum Solvent Purifier	The vacuum solvent purifier system will be useful for recycle of solvents used in laboratory.

14.	 Centrifugal Evaporator for Eppendroff tubes	Centrifugal Evaporator is useful for evaporation of solvent to prepare solvent free sample for storage of compounds as well as use for bioassay.
15.	 Soxhlet apparatus	Soxhlet apparatus is useful for extraction of essential oil from sample.
16.	 Polarimeter	Polarimeter measure the angle of rotation of polarized light through an optically active substance. It is necessary to find out levo-rotatory or dextro-rotatory enantiomers.
17.	 Melting point detector	Melting point detector is useful to find out melting point of isolated compounds.
18.	 Double distillation unit	Water used for preparation of solution of various ratio need distilled water.

19.	 Laboratory Refrigerator	Laboratory refrigerator is necessary to store heat sensitive reagents and compounds.
20.	 Hot air oven	Glassware used in natural products laboratory are needed to free of moisture, thus for drying purpose hot air oven is required.
21.	 Hot plate with electromagnetic stirrer	Hot plate with electromagnetic stirrer is needed to facilitate solvent preparation.
22.	 Liquid Chromatography with tandem mass spectrometry (LCMS/MS)	LCMS/MS equipment is useful for liquid chromatography (or HPLC) and mass analysis with mass spectrometry (MS). The liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity.
23.	 Freeze dryer	Freeze dyeing technology involves lyophilisation or cryodesiccation, which is a low temperature dehydration process involving lowering pressure. This equipment is useful for removal of water from extract as well as making compounds solvent free such as removal of Dimethyl sulfoxide (DMSO) to recover compound from sample used in NMR spectroscopy for obtaining spectrum.

8.2.2. Major Equipment Cost Estimation

S.N.	Name	Rate/Unit	Quantity	Estimated Cost '000 Rs.*
1.	Fourier transform infrared spectroscopy	2470	1	2470
2.	UV-Visible spectrophotometer	2311	1	2311
3.	Nuclear Magnetic Resonance Spectroscopy (NMR)	98721	1	98721
4.	High-performance liquid chromatography (HPLC)	4600	1	4600
5.	Gas chromatography mass spectroscopy (GC-MS)	31075	1	31075
6.	Large Scale Rotary Vacuum Evaporator	6280	1	6280
7.	Small Scale Rotary Vacuum Evaporator	1251	2	2502
8.	Digital Balance	125	1	125
9.	Fume hood	650	1	650
10.	UV chamber	30	1	30
11.	Sonicator	140	1	140
12.	Fraction collector	232	1	232
13.	Vacuum Solvent Purifier	256	1	256
14.	Centrifugal Evaporator for Eppendorf tubes	900	1	900
15.	Soxhlet apparatus	40	2	80
16.	Polarimeter	384	1	384
17.	Melting point detector	600	1	600
18.	Double distillation unit	220	1	220
19.	Laboratory Refrigerator	250	1	250
20.	Hot air oven	100	1	100
21.	Hot plate with electromagnetic stirrer	150	1	150
22.	Liquid Chromatography with tandem mass spectrometry (LCMS/MS)	37500	1	37500
23.	Freeze dryer	750	1	750
	Total			190326

8.2.3 Glassware

S. No.	Name	Rate Per unit	Quantity	Estimated Cost
1.	Separation funnel 3000ml capacity	6000	4	24000
2.	Separation funnel 500 ml capacity	2000	4	8000
3.	Separation funnel 100 ml capacity	1500	4	6000
4.	Measuring Cylinder (Mass Cylinder) 1000 ml	2200	2	4400
5.	Measuring Cylinder (Mass Cylinder) 100 ml	800	2	1600
6.	Measuring Cylinder (Mass Cylinder) 50 ml	500	2	1000
7.	Measuring Cylinder (Mass Cylinder) 10 ml	400	10	4000
8.	Funnel 30 cm diameter	3000	4	1200

9.	Funnel 10 cm diameter	300	10	3000
10.	Funnel 5 cm diameter	200	10	2000
11.	Development Chamber (10 cm height x 4cm breath x 12 cm length)	600	8 pc	4800
12.	Solvent bottles 1 L capacity	1000	10	10000
13.	Reagent bottles 200 ml capacity	500	10	5000
14.	Vials and caps (7 cm length x 2 cm diameter) i.e. 25 ml	200	1pk (100pc)	20000
15.	Vials and caps (3 cm length x 1 cm diameter)	100	1pk (100pc)	10000
16.	Test tubes (1cm x 7.5 cm)	7.5	1 box (100 pc)	1500
17.	Test tubes (1.5 cm x 15 cm)	30	1 box (100 pc)	3000
18.	Conical flask			
	2000 ml	1000	10 pc	10000
	1000 ml	500	10 pc	5000
	500 ml	300	20 pc	6000
	200ml	200	20 pc	4000
	100 ml	100	20 pc	1000
19.	Pasteur pipette 3ml (100 pc)	600	1 box	600
20.	Pipette 25 ml with rubber bulb	1400	2pc	2800
21.	Pipette 10 ml with rubber bulb	1300	2pc	2600
22.	Pipette 5 ml with rubber bulb	1200	1pc	2400
23.	Glass column 15 cm x 60 cm with stopcock	2000	2pc	
	Glass column 7 cm x 60 cm with stopcock	1500	2pc	
	Glass column 4 cm x 60 cm with stopcock	1200	2pc	
	Glass column 3 cm x 30 cm with stopcock	1000	2pc	
	Glass column 2 cm x 30 cm with stopcock	600	2pc	
	Glass column 1 cm x 20 cm with stopcock	500	2pc	
24.	Chromatography Solvent Reservoir Ball 1000 ml	1600	2pc	3200
25.	Chromatography Solvent Reservoir Ball 500 ml	1000	2pc	2000
26.	Sintered Buchner Funnel for ODS washing funnel 500 ml capacity			
	40-90 μm (G2)	4000	2pc	8000
	5-15 μm (G4)	4000	2pc	8000
27.	Bump trap 250 ml	3400	2pc	6800
28.	Round bottom flask 1000 ml	800	10pc	8000
29.	Round bottom flask 500 ml	500	10pc	5000
30.	Round bottom flask 250 ml	400	10pc	4000
31.	Round bottom flask 100 ml	200	10pc	2000
32.	Volumetric flask clear glass			
	10 ml	1400	2pc	2800
	100 ml	1600	2pc	3200
33.	Mortar and pestle big 15 cm internal diameter	1400	1pc	1400
34.	Mortar and pistil small 8.5 cm internal diameter	500	2pc	1000
35.	Petri plate with cover 10 cm diameter	200	5 pc	1000
36.	Glass membrane filter holder assembly 300 ml funnel and 1000 ml flask	5000	1 pc	5000
	Total			205300

8.2.4 Other Laboratory Accessories

S.No.	Other Accessories	Rate	Quantity	Estimated Cost
1.	Filter paper big (approx. 60 cm x 60 cm) 100 pc	15000	1pk	15000
2.	Filter paper small 15 cm	2000	5pk	10000
3.	Filter paper small 9 cm	1200	5pk	6000
4.	Grinder general purpose	6000	1	6000
5.	Levelling scale	500	1	500
6.	Clamps for column small (for 1 to 3 cm diameter column) and large (4 cm above diameter column)	200	4 pc	800
7.	Ring Clamps for separation funnel 15 cm diameter	300	4pc	1200
8.	Ring Clamps for separation funnel 8 or 10 cm	300	4 pc	1200
9.	Ring Clamps for separation funnel 5 cm	300	4 pc	1200
10.	Aquarium vibrator pump for low pressure application	1200	1pc	1200
11.	Carboy container with stopcock (low-density-polyethylene) 20 lts	5000	1 pc	5000
12.	TLC Silica gel 60F 254 TLC plates	17000	10 box with 25 units each	170000
13.	TLC Silica gel 60 RP-18 F_{254}s TLC plates	20000	10 box with 20 units each	200000
14.	Plastic vial rack for 25 ml	600	10 pc	6000
15.	Plastic vial rack for 3 ml	600	10 pc	6000
16.	1.5 ml Eppendorf tubes stand	600	2 pc	6000
17.	Eppendorf Lobind Microcentrifuge Tubes 1.5 ml capacity	1000	100 pc/1pk	1000
18.	Butter paper 10 cm x 10 cm	400	100 pc/1pk	400
19.	Aluminum foil (commonly used in food packing)	1000	1kg	1000
20.	Blue nitrile gloves, powder free sizes XL, L and S sizes	1000	1 pk each	3000
21.	Cotton	800	1 kg	800
22.	Kimwipe	3000	2 pk	6000
23.	Silicon solvent tubing (2 mm internal diameter)	1600	10 m	1600
24.	ELISA 96 well plates	250	10 pc	2500
25.	Micropipette tips 1000 µl 200 µl 100 µl 20 µl	 600 800 1200 1600	 1pk 1pk 1pk	 600 800 1200 1600
26.	Steel test tube wire rack (hole 10 mm diameter)	3400	5 pc	17000
27.	Steel test tube wire rack (hole 16 mm diameter)	4000	5 pc	20000
28.	Weight ring for flask	800	4 pc	32000
29.	Plastic 400 or 500 ml water dispenser bottle	100	6 pc	600
30.	Plastic funnel 10 cm diameter	50	4 pc	200
31.	Timer watch	900	2 pc	1800
32.	Forceps with flat tip	250	6 pc	1500
33.	Sprit lamp	100	2 pc	200
34.	Parafilm tape for sealing 4 inch x120 ft	4000	1 roll	4000
35.	Metal Ruler 60 cm length	200	1 pc	200
36.	Plastic ruler 30 cm length	100	2 pc	200

37.	Stainless steel container of 50 L	2000	1 pc	2000
38.	Dryer (Hair dryer) for drying TLC plates	1200	2 pc	1200
39.	Laboratory wiper e.g., (Kimberly-Clark 47000 WYPALL® L20 Textured Wipers, Tan, 12.5 x 13-Inch, 1/4-Fold, Eco-Pack	5000	20pack / case	5000
40.	Micropipette 1000 µl	10000	1	10000
41.	Micropipette 200 µl	10000	1	10000
42.	Micropipette 100 µl	10000	1	10000
43.	Micropipette 20 µl	10000	1	10000
44.	Micropipette 10 µl	10000	1	10000
45.	Multi Micropipette 300 µl	45000	1	45000
	Total			637500

8.2.5 Reagents and Solvents

S.N.	Chemicals	Specification	Quantity	Estimated Cost
8	Methanol	Laboratory grade 99.9% anhydrous	20 L can x 6	80000
9	Ethanol	Laboratory grade	20 L can x 6	160000
10	Ethyl Acetate	Laboratory grade 99.9% anhydrous	20 L can x 6	160000
11	Chloroform	Laboratory grade 99.9% anhydrous	20 L can x 5	176000
12	n-hexane	Laboratory grade 99.9% anhydrous	10 L can x 4	320000
13	n-butanol	Laboratory grade 99.9% anhydrous	20 L can x 2	12000
14.	Acetone	Laboratory grade 99.9% anhydrous	20 L can x 5	12000
15.	Acetonitrile	Laboratory grade 99.9% anhydrous	20 L can x 4	24000
16.	Dichloromethane	Laboratory grade 99.9% anhydrous	20 L can x 1	4000
17.	Pyridine	Analytical grade	500 ml	5000
18.	Acetic Acid (Glacial)	CAS Number 64-19-7 100%	500 ml	700
19.	Formic Acid	CAS Number 64-18-6 100%	500 ml	800
20.	Sulfuric Acid	CAS Number 7664-93-9 99.999%	500 ml	2500
21.	Nitric Acid	CAS Number 7697-37-2	500 ml	5000
22.	Hydrochloric Acid	CAS Number 7647-01-0 36.5-38.0%	500 ml	6000
23.	SiO_2 (Kiesel gel 60, Merck, Darmstadt, Germany)	CAS Number 112926-00-8	5 kg	150000
24.	Octadecyl silica gel (ODS) LiChroprep RP-18, 40-63 µm	CAS Number: 108688-10-4	500 g	318000
25.	Sephadex LH-20	CAS 9041-37-6	100 g	135000
26.	Sephadex G-15	CAS Number 11081-40-6	500 g	45000

27.	Diaion HP-20	CAS Number 9052-95-3	500 g	20000
28.	Dragendorff's reagent	CAS Number: 39775-75-2	100 ml	500
29.	Benedict's reagent	CAS Number 63126-89-6	250 ml	7000
30.	Ninhydrin	CAS Number 485-47-2	10 g	150
31.	Sodium hydroxide pellets (anhydrous)	CAS Number 1310-73-2	500 g	40000
32.	Gallic acid	CAS Number 149-91-7	100 g	23000
33.	Catechin	CAS Number 225937-10-0	500 mg	12500
34.	2,2-Diphenyl-1-picrylhydrazyl (DPPH)	CAS Number 1898-66-4	1 g	29000
35.	Fehling's solution A		500 ml	700
36.	Fehling's solution B		500 ml	1100
37.	Nessler's reagent	CAS Number 7783-33-7	100 ml	44000
38.	Picric acid	CAS Number: 88-89-1	100 g	13000
39.	Potassium hydroxide	CAS Number 1310-58-3	500 g	800
40.	Iodine	CAS Number 7553-56-2	100 g	5000
41.	Sodium nitroprusside	CAS Number 13755-38-9	100 g	15000
42.	Lead acetate	CAS Number 6080-56-4	500 g	12000
43.	Ferric chloride	CAS Number7705-08-0	100 g	11280
44.	Gelatin powder	CAS Number 9000-70-8	500 g	16000
45.	Acetic anhydride	CAS Number 108-24-7	100 ml	5600
	Total			1872630

8.2.6. Overall Cost

S.N.	Particulars	Estimated Cost Rs.*
1	Major Equipment	190326000
2	Glassware	205300
3	Other Laboratory Accessories	637500
4	Reagents and Solvents	1872630
	Total	19,30,41,430

* Some variation depending on brand name of equipment likely

8.3 Conclusion

Natural products laboratory equipment need is estimated as NRs. 19,30,41,430 which includes major equipment of NRs. 19,03,26,000. The costliest equipment needed is Nuclear Magnetic Resonance (NMR) with estimated cost NRs. 9,87,21,000. However, it has diverse fields of application including chemistry, biochemistry, polymer chemistry, organometallic chemistry, structural biology, materials science, and medicine. The state of the art world class facility with sophisticated instruments will not only benefit research activities of Madan Bhandari University of Science and Technology but also can provide service at certain cost to other institutions of Nepal by processing their samples and providing trainings which can bring ripple effect in expansion of research culture in Nepal.

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10. Appendices

Appendix I

Selected non-timber forest products and spices of Nepal their price list in Nepali markets (January 2020)

SN	Botanical Name	English Name	Nepali Name	Kathmandu NRS./Kg	Nepalgunj NRS./Kg
1.	*Zingiber officinale*	Fresh ginger / Dried ginger	Aduwa / Sutho	- /280	-/250
2.	*Amomum subulatum*	Large cardamom	Alaichi	600	-
3.	*Phyllanthus emblica*	Indian gooseberry	Amala	200	140
4.	*Rheum australe*	Himalayan rhubarb	Amalbed (Chulthi)	250	150
5.	*Aconitum heterophyllum*	Aconite	Atis	-	700
6..	*Terminalia bellirica*	Beleric myrabolan	Barro	80†	-
7.	*Selinum tennifolium*	Rugwort	Bhutkesh	150	170
8.	*Aconitum spicatum*	Nepali aconite	Bikhjara	350	350
9.	*Acorus calamus*	Sweetflag	Bojho	100	130
10.	*Swertia chirayita*	Chiretta	Chiraito	225	250
11.	*Cinnamomum zeyllanicum*	Cinnamon	Dalchini	130	130
12.	*Morchella conica*	Morel mushroom	Guchhichyau	11000	13000
13.	*Terminalia chebula*	Chebulic myrabolan	Harro	90†	-
14.	*Nardostachys grandiflora**	Spikenard	Jatamansi	825	750
15.	*Parmelia nepalensis***	Lichen	Jhyau	-	-
16.	*Pistacia chinensis*	Indian mastiche	Kakrasinghi	850	700
17.	*Picrorhiza scrophulariiflora**	Gentian	Kutki	1600	2000
18.	*Rubia manjith*	Madder	Majitho (red)	125	135
19.	*Delphinium denudatum*	Larkspur	Nirmansi (Jadwar)	6000	5000
20.	*Rheum australe*	Himalayan rhubarb	Padamchal (rhizome)	145	100
21.	*Bergenia ciliata*	Rock foil	Pakhanbed	75	70
22.	*Piper longum*	Long piper	Pipla (long)	375	550

23.	*Piper pedicellatum*	Round piper	Pipla (round)	325	-
24.	*Sapindus mukorossi*	Soap nut	Ritha	90	90
25.	*Asparagus racemosus*	Wild asparagus	Satawari (Kurilo)	425	350
26.	*Paris polyphylla*	Love apple	Satuwa (big)	-	2000
27.	*Acacia rugata*	Soap pod	Sikakai	70	75
28.	*Cinnamomum glaucescens*		Sugandhakokila*	170	270
29.	*Valeriana jatamansii*	Indian valerian	Sugandhawaal*	350	400
30.	*Cinnamomum tamala*	Indian cassia	Tejpat	70	50
31.	*Zanthoxylum armatum*	Prickly ash	Timur	375	490
32.	Silajit*	Rock exudate	Silajit	850	-

Source: Asia Network for Sustainable Agriculture and Bioresources (ANSAB), www.ansab.org.np

*Ban for export outside Nepal without processing (except Kutki) and prior permission from DoF required;

**Ban in Nepal for collection, trading and export;

†Prices of Harro and Barro of Kathmandu are without seed; for other markets, the prices are of with seed

Prices indicated above are for good quality dried parts offered by the buyers of a particular market. Prices for Delhi are from commission agents, a 6.5% to 8% commission is deducted from the supplier in the quoted price.

https://www.intracen.org/uploadedFiles/intracenorg/Content/Exporters/Market_Data_and_Information/Market_information/Market_Insider/Medicinal_plants/MI_Medicinal_Plants_Prices_2016_January.pdf

Appendix II

List of medicinal plants prioritized for research and development by Department of Plant Resource, Nepal Government

S.N.	Scientific name	Nepali name	Family
1.	*Aconitum spicatum* (Bruhl) Stapf	Bikh	Ranuncnlaceae
2.	*Acorus calamus* L.	Bojho	Araceae
3.	*Asparagus racemosus* Willd.	Kurilo	Asparagaceae
4.	*Azadirachta indica* A. Juss.	Neem	Meliaceae
5.	*Bergenia ciliata* (Haw.) Sternb.	Pakhan Ved	Saxifragaceae
6.	*Cinnamomum glaucescens* (Nees.)	Sugandhakokila	Lauraceae
7.	*Cinnamomum tamala* (Buch.-Ham.) Nees &	Tejpat	Lauraceae
8.	*Dioscorea deltoidea* Wall. ex Griseb.	Ban tarul	Dioscoreaceae
9.	*Gaultheria fragrantissima* Wall	Dhasingre	Ericaceae
10.	*Juglans regia* L.	Okhar	Juglandaceae
11.	Lichens	Jhyau	
12.	*Morchella conica* Pers.	Guchchhi chyau	Helvellaceae
13.	*Phyllanthus emblica* L.	Amala	Euphorbiaceae
14.	*Piper longum* L.	Pipla	Piperaceae
15.	*Rauvolfia serpentine* (L.) Benth ex Kurz	Sarpagandha	Apocynaceae
16.	*Rheum australe* D. Don	Padam chal	Polygonaceae
17.	*Rubia manjith* Roxb. ex Fleming	Majitho	Rubiaceae
18.	*Sapindus mukorossi* Gaertn.	Riththa	Sapindaceae
19.	*Swertia chirayita* (Roxb. ex Fleming) Karsten	Chiraito	Gentianaceae
20.	*Tagetes minuta* L	Jangali sayapatri	Asteraceae
21.	*Taxus wallichiana* Zucc.	Lauth sallo	Taxaceae
22.	*Tinospora sinensis* (Lour.) Merr.	Gurjo	Menispermaceae
23.	*Valeriana jatamansii* Jones	Sugandhawala	Valerianaceae
24.	*Zanthoxylum armatum* DC.	Timur	Rutaceae

Appendix III

I. Natural products research areas are associated with following research priority areas given by Yan (2020) for Master's and PhD program in "Forest Biomaterials Science and Engineering":

Technical Elective Courses:

1. Non-timber Forest Products Processing and Value Addition
2. Natural Products Chemistry

II. Natural products research areas associated with following research priority areas given for Master's and PhD Program in "Organic Agriculture" by Amstel (2020):

Cluster 1: Organic Plant Production

Supporting and facilitating the innovation in the field of novel pesticides suited for organic agriculture

Cluster 2: Livestock

Need for and alternatives to synthetic vitamins in organic animal husbandry

Cluster 4: Quality, Health and Food Security

Enhancing health promoting properties of organic food

Cluster 6: Novel Product Development for Organic Agriculture

Biopesticide production from various plant allelochemicals