Biodiversity and Bioprospecting

Proceedings of International Conference

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Editors

Sangeeta Rajbhandary

Ram Sundar Sah

Samjhana Pradhan

Seerjana Maharjan

Gaurav Parmar

Tirtha Raj Pandey

Pratiksha Shrestha

Government of Nepal

Ministry of Forests and Environment

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International Conference of Biodiversity and Bioprospecting

In Collaboration With

Foreword

Nepal is well established as a treasure of Biodiversity with about 12000 species of flowering and non-flowering plants. Included among these are some of the rarest, endemic and most valuable plants known for their ecological and socioeconomic roles. Despite this remarkable diversity and potentiality, the conservation and utilization part is still less explored. Moreover, climate induced and anthropogenic causes are posing a severe threat in the way of survivability of these valuable resources. In this context, assessing the prospect of development through the sensible use of the bioresources, selection of appropriate conservation, research and development strategies becomes more crucial. To achieve these goals, there should be a wider consensus and deeper interaction among the researchers, academia, managers and policy makers at national and international level.

Through sharing the knowledge on updated practices, techniques and outcomes, better strategies can be devised and the benefits can be multiplied. With these aims and with the objectives of developing broader understanding and networking among national and international perspectives in the field of biodiversity and bioprospecting, Department of Plant Resources (DPR) organized the "International Conference on Biodiversity and Bioprospecting (ICBB)" from June 22 to 24, 2022 in Kathmandu. The conference was first of its kind organized by DPR successfully at such level with 85 national and international verbal presentations along with 17 poster presentations. The conference provided an important platform for experts, researchers and managers to exchange the ideas and forge the possibilities of cooperation and collaboration at local and international level.

DPR documented the major highlights and outcomes of the program and includes the selected 10 special papers in this proceeding that were presented during various technical sessions of the conference. The papers included in this proceeding will be useful resources for the understanding of various contemporary issues and their solution in the field of biodiversity and bioprospecting.

Finally, on behalf of DPR, I would like to thank all those who have put their efforts to bring out this publication in present form.

Radha Wagle, Ph. D.
Director General

Acknowledgements

We would like to express our gratitude and appreciation to all those who contributed for the success of the International Conference on Biodiversity and Bioprospecting held on June 22-24, 2022 in Kathmandu, Nepal.

Firstly, we are highly indebted to the Honourable Minister, Mr. Ramsahay Prasad Yadav and Secretary, Dr. Pem Narayan Kandel, Ministry of Forests and Environment, Government of Nepal for their continuous encouragement and support for the successful organization of this International Conference.

We would also like to thank the keynote speakers Prof. Emeritus Dr. Ram Prasad Chaudhary and Prof. Emeritus Dr. Pramod Kumar Jha for their invaluable insight and presentation in the conference. Their expertise and knowledge inspired thought-provoking discussions and helped to broaden our perspectives on the latest research and advancements in the field.

We would also like to extend our special thanks to the plenary speakers Dr. Achyut Adhikari, Prof. Dr. Bharat Babu Shrestha, Prof. Dr. Hari Datta Bhattarai, Dr. Keshab Raj Rajbhandari, Prof. Dr. Krishna Kumar Shrestha, Dr. Rajendra Gyawali, Dr. Ripu M. Kunwar, Dr. Robbie Hart, Prof. Dr. Sangeeta Rajbhandary, Dr. Sanjay Kumar, Dr. Uttam Babu Shrestha, and the researchers Mr. Adarsha Subedi, Mr. Amrit K.C., Dr. Anju Sharma Poudel, Mr. Ashis Kumar Roy, Dr. Ashok Kumar Dhakad, Ms. Astha Chauhan, Ms. Babita Shrestha, Mr. Babu Lal Tiruwa, Mr. Baburam Nepali, Mr. Bal Bahadur Thapa Magar, Dr. Bhupal Govinda Shrestha, Dr. Bijendra Basnyat, Ms. Bishnu Sharma Gaire, Mr. Chandra Bahadur Thapa, Ms. Chandra Kumari Paudel, Dr. Chandra Mohini Nemkul, Ms. Chandrakala Thakur, Ms. Chetana Khanal, Dr. Chudamani Joshi, Mr. Damodar Gaire, Ms. Darshita Rawat, Prof. Dr. Devendra M. Bajracharya, Mr. Devi Prasad Bhandari, Mr. Dhan Raj Kandel, Ms. Eliza Acharya Siwakoti, Dr. Haneef Ur Rehman, Dr. Hari Prasad Aryal, Mr. Hom Nath Pathak, Dr. Imtiaz Khan, Ms. Januka Pathak, Ms. Jyoti Devi, Ms. Jyoti Singh, Ms. Kalpana Sharma (Dhakal), Ms. Kareena Panth, Ms. Kiran Chauhan, Ms. KM Uma Kumari, Ms. Krishna Kumari, Mr. Krishna Prasad Sigdel, Ms. Lajmina Joshi, Mr. Laxmi Raj Joshi, Ms. Laxmi Thapa, Dr. Madhu Shudan Thapa Magar, Prof. Dr. Man Dev Bhatta, Ms. Manisha Nagarkoti, Dr. Muhammad Ishfaq Khan, Ms. Neera Joshi Pradhan, Dr. Nirmala Joshi, Dr. Nirmala Phuyal, Ms. Nisha Kharel, Ms. Nisha Thakur, Ms. Nita Kumari Joshi, Mr. Paras Mani Yadav, Mr. Parashu Birat Rai, Mr. Piyush Bhalla, Mr. Prabin Bhandari, Ms. Pramila Gacchadar, Ms. Pratikshya Chalise, Mr. Prem Narayan Paudel, Mr. Pusp Raj Joshi, Ms. Radhika Khanna, Mr. Raghuram Parajuli, Mr. Rahul Kumar, Dr.

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Our sincere thanks also go to the reviewers and editors who dedicated their time and expertise to ensure the quality and relevance of the papers included in the proceedings. Your diligent efforts have been instrumental in making the conference a success.

Our whole hearted appreciation and gratitude also goes to the co-organizers NAST, Agriculture and Forestry University, Pokhara, Mid-Western University, Surkhet, Central Department of Botany TU, NTNC, ICIMOD, IUCN, WWF, and ZSL for their unconditional support and cooperation.

We would also like to acknowledge Herbs Production and Processing Co. Ltd., Evans Life Sciences Pvt. Ltd., Nature Nest Pvt. Ltd., Bhaskar Herbaceuticals Pvt. Ltd., Himalayan Bio Trade Pvt. Ltd., and Kanaya Herbs Store Pvt. Ltd. for providing insight into bioprospecting of medicinal plants by displaying their herbal products during the conference.

Finally, we would like to acknowledge the support of the Advisory Committee, Organizing committee, Scientific Sub-committee, Management Sub-committee, Technical Committee and the Conference staff who worked tirelessly to ensure the smooth running of the event. Your hard work and dedication have been essential in making the conference a memorable and rewarding experience for all.

Thank you once again for your contributions and support. We hope that the proceedings will serve as a valuable resource for future research and look forward to your continued involvement in the field.

Saroj Kumar Chaudhary
Deputy Director General

Preface

Biodiversity has economic, ecological, aesthetic as well as existence value. Therefore, biodiversity has been a common resource for the betterment of people’s lives. It demands constant policy reform for its long-term conservation, management and utilization. Hence, biodiversity, biotechnology and nano-biotechnology are the areas that need special attention from the perspectives of new knowledge-based industrial ventures, international trade and Intellectual Property Right (IPR) protection. Bioprospecting is the exploration of biological material for commercially valuable genetic and biochemical properties. These cover the general conducts of bioprospecting, sharing of financial benefits and research findings, IPRs, and conservation and sustainable use of biodiversity. Several scientific findings and innovations have been described and implemented in recent years, in the field of biodiversity and bioprospecting.

The International Conference on **Biodiversity and Bioprospecting** was organized in Kathmandu on June 22-24, 2022 to bring together academicians, scientists, researchers, experts, managers and policy makers to share and discuss existing problems, latest innovations, emerging opportunities and future priorities in the field of biodiversity and bioprospecting. To accomplish the conference’s objectives, the organizer was supported from government, non-government, Universities, and prominent national and international organizations.

This book is an output of the International Conference which includes informative and reviewed articles along with a few original research articles presented during the conference. We highly appreciate the intellectual contribution of all authors, without their support it would have been difficult to bring the publication on time. We also acknowledge our sincere thanks to all referees for reviewing the articles in a timely manner. We also acknowledge Mr. Mahesh Maharjan for layout and printing on time.

Editors

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Content

Plant Invasions in Nepal: Knowledge Gaps and Research Needs

Bharat Babu Shrestha
Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
Email: shresthabb@gmail.com

Abstract

Biological invasion is pervasive with range of direct and indirect negative impacts worldwide on most of the natural environments, production systems and human wellbeing. In Nepal, various global and local drivers have aggravated the problems of plant invasions, as indicated by continued detection of new invasive plants and expansion of established species to new areas. Although some policy documents of the Government of Nepal have identified plant invasions as one of the major drivers of biodiversity loss and environmental degradation, the policy and management responses at the national level is inadequate to prevent and control invasive plants. In last two decades, wealth of knowledge on diversity of alien and invasive plants, their spatial distribution, and ecological impacts have been generated. Current state of knowledge of plant invasions in Nepal, combined with global knowledge base, is sufficient to inform policy decisions and management interventions. However, it appears that the policy makers are not fully aware of the seriousness of the problems and future consequences of the lack of timely responses to prevent and control invasive plants. In addition, poor knowledge base on some of the aspects of the plant invasions such as the introduction pathways, economic cost, impacts on human wellbeing, and habitat/ecosystem specific control measures might have also contributed to the inadequate policy and management responses. Prioritization of these data poor topics in future research through interdisciplinary approaches can effectively inform policy and management decisions for the prevention and control of invasive plants and other alien species in Nepal.

Keywords: Control measures, Economic cost, Invasive alien species, Introduction pathways

Introduction

Direct and indirect human activities have led to the redistribution of organisms globally. Distribution of species is naturally confined to a particular region, often referred to as native range, due to biogeographic barriers such as ocean, high mountains, desert for their dispersal (Brown & Lomolino, 1998; Rahel, 2007). However, humans have been introducing species outside of their native distribution range by crossing the biogeographic barriers where they may establish self-replacing population and spread rapidly in to a large geographic area (Seebens et al., 2021). This human mediated introduction of species outside of their native distribution range is called biological invasions and the introduced species are called alien (or exotic) species in the introduced range. While several alien species have contributed positively to human food security and economy, a small fraction of alien species often spread rapidly in a large geographic area with range of direct and indirect negative impacts on natural environment (e.g., ecosystem, biodiversity), production system and human wellbeing. Such alien species with negative impacts are referred to as invasive alien species (https://www.cbd.int/idb/2009/about/what/; accessed on 5 December 2022). Globally, biological invasion is one of the five most prominent negative impacts that humanity have to earth's natural environment (IPBES, 2019).

With increasing trade and travel, the number of alien species including invasive ones have been increasing in all geographic (island to continent) and political levels (sub national to regional block such as European Union) (Seebens et al., 2017; Hulme et al., 2021). Major impacts of invasive alien species include biodiversity loss (population to species extinction), altered evolutionary course (e.g., genomic extinction to origin of new species by hybridization), modified ecological processes (e.g., nutrient dynamics, fire regime, hydrology), reduced nature's contribution to people (e.g., degradation of valuable organism's habitat, reduced food productions) and increasing economic burden (e.g., increasing cost of invasive species management) (Davis, 2009; Diagne et al., 2021). According to a recent estimate, economic cost of biological invasions was US$ 162.7 billion in 2017 (Diagne et al., 2021). The study also revealed that the cost associated with direct damage by invasive alien species has been increasing at a rate faster than the cost associated with their management. Importantly, the current level of biological invasions, impacts of invasive alien species and the associated economic costs have been predicted to increase further in future (Bellard et al., 2013; Seebens et al., 2015). Therefore, prioritization of invasive alien species management at different levels of governance (subnational, national, regional to global) will reduce biological invasions (introduction rate, number, impact) and the associated economic costs, which will ultimately help to meet ten of seventeen Sustainable Development Goals of the United Nations (IUCN, 2018) and Target 6 of Kunming-Montreal 2030 Targets (Kunming-Montreal Global Biodiversity Framework) of the Convention on Biological Diversity (https://www.cbd.int/doc/c/e6d3/cd1d/daf663719a03902a9b116c34/cop-15-l-25-en.pdf; accessed on 12 January 2023).

As in other parts of the world, biological invasions are an emerging threat to environment and people's livelihood in Nepal (Shrestha, 2019; Shrestha & Shrestha, 2021). In particular, invasive alien plant species (IAPS) of Nepal have been reported to inflict diverse ecological and socio-economic impacts (see below for a brief account). Some of the biodiversity-related policy documents of Nepal have identified IAPS as a threat to native biodiversity and ecosystems, and mentioned various preventive (e.g., strengthening quarantine inspection at international border) and control measures (e.g., biological control) (Siwakoti & Shrestha, 2015; MFE, 2018). However, implementation of such measures has remained very low. Additionally, there are some data and knowledge gaps in key aspects of plant invasions, undermining informed policy and management decisions. In this communication, major data and knowledge gaps of plant invasions in Nepal have been identified with the hope that they will be prioritized by researchers in their future researches.

Rising threats

More than 180 species of alien flowering plants are naturalized in Nepal and, at least 28 of them are invasive (Shrestha & Shrestha, 2021; Shrestha et al., 2021; Adhikari et al., 2022). Nearly two decades ago, 166 alien plant species were reported as naturalized and 21 species as invasive (Tiwari et al., 2005). Among globally worst 34 invasive flowering plants, five species are invading different ecosystems in Nepal; they are *Chromolaena odorata* (Syn. *Eupatorium odoratum*), *Lantana camara*, *Mikania micrantha*, *Pontederia crassipes* (Syn. *Eichhornia crassipes*) and *Sphagneticola trilobata*. Currently, invasion of *S. trilobata* is limited to Kavrepalanchwok district in central Nepal (Shrestha et al., 2021). Remaining four species are already widespread from east to west in Tarai, Siwalik and Middle Mountain regions (Adhikari et al., 2022). In recent years, one or more naturalized and invasive alien plant species have been reported every year. For example, a noxious invasive weed *Mimosa diplotricha* was reported from eastern Nepal in 2020 (Sharma et al., 2020) and globally worst invasive weed *Sphagneticola trilobata* was reported from Kavrepalanchwok district in 2021 (Shrestha et al., 2021). Naturalization of several previously introduced species have been also reported in recent years – *Senna alata* (Pathak & Chhetri, 2017), *Tradescantia fluminensis* (Rajbhandari et al., 2019), *Aeschynomene americana* (Pandey et al., 2021). In addition to a rise in number, areas invaded by the IAPS have also increased over the time. For example, *Mikania micrantha* was previously reported only in Tarai and Siwalik regions of eastern and central Nepal (Tiwari et al., 2005) but currently the species has also invaded Middle Mountain region (hilly region) (e.g., Pokhara valley) and western Nepal (Dang district) (Adhikari et al., 2022). These reports suggest that the number of IAPS and the areas invaded by them have been increasing over the time and this trend may continue in future unless stringent prevention and control measures are not implemented.

Diverse ecological and socio-economic impacts of the IAPS have been reported in Nepal. Ecological impacts include loss of biodiversity (Bhatt et al., 2020), change in plant community structure and soil nutrient content (Timsina et al., 2010; Khatri-Chhetri et al., 2022), reduced tree regeneration (Thapa et al., 2016) and degradation of wildlife habitats (Murphy et al., 2013; Basaula et al., 2021; Adhikari et al., 2022). Similarly, the socio-economic impacts primarily include reduced nature’s contribution to people such as fodder supply, agriculture production and wetland resources (Rai & Scarborough, 2015; Shrestha et al., 2019a; Pathak et al., 2021). However, the reported impacts of IAPS are likely an underestimate of the real impacts because of geographic and taxonomic biases in the past studies (Pandey et al., 2020).

It is highly likely that various global and local drivers will further aggravate the problems of plant invasions in Nepal. For example, climatically suitable areas of majority IAPS have been predicted to increase under future climate scenarios (Shrestha & Shrestha, 2019). Due to increasing dependency to imported agriculture and other goods, and low national capacity to intercept species at borders, threats of invasive alien species to Nepal's agriculture sector is exceptionally high (3rd most threatened among 124 countries, Paini et al., 2016). Local drivers such as agriculture lands abandonment, forest degradation and road constructions have also facilitated establishment and spread of the IAPS (Jaquet et al., 2015; Shrestha et al., 2019b).

Inadequate policy and management response

Some policy documents of the Government of Nepal related to biodiversity and environment have identified plant invasions as one of the major drivers of biodiversity loss and habitat degradation (MFSC, 2014; Siwakoti & Shrestha, 2015; MFE, 2018). For example, National Ramsar Strategy and Action Plan (2018-2024) identified invasive alien species as one of the three major threats to the Ramsar sites of Nepal (MFE, 2018). Given that, the policy and management responses at the national level remain inadequate to prevent and control invasive plants (Shrestha, 2019). There is no national strategic plan to prevent and control invasive alien species in Nepal though this is deemed essential and about 39% of the countries have already developed such national strategy (Pagad et al., 2020). For example, a South-East Asian country, Philippine has prepared and implemented National Invasive Species Strategy and Action Plan (NISSAP) 2016-2026 (DENR-BMP, 2016). As envisaged in the National Biodiversity Strategy and Action Plan 2014-2020 (MFSC, 2014), there has been significant recent progress on spatial distribution mapping of the IAPS (e.g., Shrestha et al., 2019b; Shrestha & Shrestha, 2020), reporting new IAPS (e.g., Sharma et al., 2020; Shrestha et al., 2021) and publication of community education materials (e.g., Bisht et al., 2016; Ghimire et al., 2020; Adhikari et al., 2022). However, progress towards the management of IAPS has remained very poor. For example, Nepal's National Biodiversity Strategy and Action Plan for the period of 2014 to 2020 has envisaged strengthening border quarantine and initiating biological control program (MFSC, 2014), but they have never been translated into action.

It appears that the policy makers and management planners are not fully aware of the seriousness of the problems and future consequences of the lack of timely responses for preventing and controlling invasive alien species to biodiversity, environment, economy and people's livelihood. It can be anticipated that the NISSAP of Nepal be prepared jointly by the Ministry of Forests and Environment and the Ministry of Agriculture and Livestock Development. In line with the NISSAP, the sectoral plans can be prepared and implemented by the various government institutions such as the Departments of National Park and Wildlife Conservation, Department of Forest and Soil Conservation and Department of Agriculture.

Knowledge gaps and research needs

In spite of the wealth of knowledge on invasive plants generated during last two decades (Shrestha, 2019; Pandey et al., 2020; Shrestha & Shrestha, 2021), there are some prominent knowledge gaps which need to be prioritized in future research. Poor knowledge base on some of the aspects of the plant invasions such as the introduction pathways, invasion risk of cultivated species, impacts of ecological processes, economic cost, and site and species specific control measures might have contributed to the inadequate policy and management responses mentioned in the previous section. Prioritization of these data poor topics in future research through interdisciplinary and trans-disciplinary approaches can effectively inform policy and management decisions for the prevention and control of plants and other invasive alien species in Nepal. A brief account of these data poor topics has been presented below.

Introduction pathways

Introduction pathways of invasive and other naturalized plant species of Nepal have not been investigated though some inferences have been made based on the use value (e.g., ornamental) of plant and research done elsewhere (Shrestha & Shrestha, 2021). Given that the prevention of species introduction through pathway management is the most effective and a priority approach for invasive alien species management globally (Hulme et al., 2008; Aichi Biodiversity Target 9, https://www.cbd.int/sp/

targets/rationale/target-9), generation of such knowledge is of paramount importance to make informed policy and management decisions.

Risk assessment

Majority of the alien plant species have been intentionally introduced globally for one or another uses which escape to the wild, establish (being naturalized) and some of them become invasive (Saul et al., 2016). Hundreds of alien plants were introduced to Nepal as crop, garden flower, livestock fodder and for agroforestry and timber production (Shrestha et al., 2022). Crop species are less likely to escape from the cultivation, and naturalized in wild. However, species introduced for other purpose may escape and naturalize in wild. For example, *Tithonia diversifolia* was previously reported as a cultivated plant (Hara et al., 1982), but currently the naturalized population of this species can be found at several locations along Koshi highway in eastern hills of Nepal (Ilam, Panchthar, Taplejung districts) (personal observations of author, October 2020) and along Araniko Highway (Sindhupalchwok district) in central Nepal (personal communication with Dipak Lamichhane, National Botanical Garden, Godawari on December 8, 2022). Similarly, *Hypoestes phyllostachya* was introduced as garden plant but now it is naturalized (Rajbhandari et al., 2012). These examples suggest that a number of cultivated alien plant species of Nepal have risks of naturalization in future. The risk assessment of these cultivated alien plants will help to identify species with high potential to naturalize and becoming invasive in future. When such data is available, appropriate measures can be implemented to prevent their naturalization and further spread.

Ecological impacts

A few previous studies have examined impacts of IAPS on plant community composition, soil seed bank, soil nutrient content, tree regeneration and wildlife habitat quality (e.g., Timisina et al., 2011; Murphy et al., 2013; Thapa et al. 2016; Khatri-Chhetri et al., 2022). However, the IAPS impacts on additional ecological attributes such as pollination, productivity, nutrient dynamics, competition and hydrology remain unexplored in

Nepal. Future research on these topics will complement current state of knowledge for holistic understanding of ecological impacts by the IAPS.

Economic cost

Economic cost of invasive alien species includes direct damage/loss (e.g., reduced agriculture production due to weed and pests) and management cost (e.g., cost of removal, herbicide uses). Impacts on non-material nature's contribution to people (e.g., recreational value of lake) are also accounted for while estimating the economic costs. Global estimates have revealed that the invasive alien species cost billions of dollars to global economy and it is ever increasing over the time (Diagne et al., 2021). The invasive alien species cost at least US$ 137 to 183 billion dollars to Indian economy between 1960 and 2020 (Bang et al., 2022). The economic cost estimates of this kind provide strong basis for resources allocation to invasive species control through appropriate policy and management responses. Additionally, cost-benefit analysis of different management options provides opportunities for managers to select cost-effective measures for invasive species management. Economic cost estimation requires interdisciplinary and trans-disciplinary approach in which researchers from different disciplines (ecology, resources economy to governance) work closely with managers and policy makers.

Site and species specific control measures

The IAPS have invaded diverse habitats and land use types, ranging from cultivated lands to protected areas. Control measures that are customary in one land use type may no longer be appropriate in another land use type. For example, among various control measures (e.g., physical, chemical and biological control), chemical methods involving the use herbicide may be implemented to control invasive weeds in cultivated land but use of such chemicals is not permissible inside the protected areas. In natural areas, ecosystem based approaches (e.g. reducing disturbances, suppression of IAPS by promoting native species) can be better than physical and chemical methods. However, biological control measures can be implemented in both natural and modified ecosystems. Similarly, control measures are highly species-specific due to differences in life form, mode of reproduction, dispersal method, among others. Field based action researches are needed to optimize site and species specific control measures before such methods are implemented in a large geographic areas.

Conclusions

In spite of high threats and rising problems of the IAPS in Nepal, current policy and management responses remain inadequate, undermining national progress towards biodiversity conservation and sustainable development goals. While substantial data related to IAPS problems have been generated in last two decades, some aspects of plant invasions remain under-studied which may be an impediment for appropriate policy and management responses. Prioritization of these aspects such as identification of introduction pathways, ecological impact studies (particularly focusing to ecological processes), economic cost estimation and action research to optimize site and species-specific control measures in future research will generate adequate data for informed policy and management decisions.

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Pteridophytes of Bhutan: A Checklist

C.R. Fraser-Jenkins
Rua de São Mateus 485 S/C Dt., Fontainhas 2750-142, Cascais, Portugal
Email: chrisophilus@yahoo.co.uk

Abstract

The pteridophytes of Bhutan have been studied in detail by the author for over 20 years, including three detailed collection-visits to west and east Bhutan and study and re-identification of all specimens by the author in 16 relevant herbaria with Bhutan collections. While parts of the resulting checklist have been unofficially published in Bhutan from early stages of the author's work under other's names without permission, they were inaccurate and incomplete due to failure to consult with the author as had previously been agreed. The actual checklist and specimen-list, for which purpose the present author was officially invited by the National Biodiversity Centre (NBC), Ministry of Agriculture and Forests, Bhutan, is far more advanced and detailed, with many more species identified, and replaces the secondary and fragmentary paper and website copied by staff of the NBC. Preliminary details of the real checklist (Fraser-Jenkins, C.R., et al. 2022, in press) are given here. It contains full specimen-lists, brief diagnostic descriptions and nomenclatural details, along with details of one new species. Many species previously unknown from Bhutan have been identified or collected and are listed and the Bhutanese specimens in THIM, ASSAM, CAL, BM, K, UC and other herbaria have all been re-identified and listed to vouch for the reported species present in Bhutan. The present paper provides the condensed species and subspecies list, organised and classified by a combination of taxonomic and molecular cladonomic considerations as outlined by Fraser-Jenkins et al. (2016-2020), in the *Annotated Checklist of Indian Pteridophytes* vols. 1 to 3. Two new combinations are made and a new species validated: *Dryopteris bhutanica* (Fraser-Jenk. & Pariyar) Fraser-Jenk., *Dryopteris obtusipinnula* (Fraser-Jenk.) Fraser-Jenk. and *Arachniodes fraserjenkinsii* L.Zhang & N.T.Lu.

Keywords: Checklist, ferns, herbaria, new combination

Introduction

Bhutan contains various very distinct climatic zones and pteridophytic floristic regions, including:

(i) In the west a high-altitude dry Tibetan-type region, with many hitherto unnoticed high Sino-Himalayan Tibetan-type species of ferns.

(ii) Normal low-, mid- and upper Sino-Himalayan forest zones in the mid-parts of W., C. and E. Bhutan.

(iii) Rich tropical rain-forest flora in the S.E. and S. Centre, bordering Assam State, with many Malaysian or S.E. Asian species, richer in species-diversity than Darjeeling and Nepal to its west.

(iv) The high-Himalayan main-range snow-peaks and meadows running across the north of the country from west to east and still very little known.

But unfortunately, much restriction placed on foreign botanical visits and collection and years of isolation have resulted in local botanists being unable to develop knowledge of the species and taxonomy of Bhutanese pteridophytes and at the same time have prevented many species from becoming known from the country. A list provided in the full check-list, of expected species that must be present but have never been collected, is disproportionally larger for Bhutan compared to Nepal, Darjeeling and Sikkim to the west and even Arunachal Pradesh to the east.

Nevertheless, collections by the author, partly with Tandin Wangdi and Sagun Pariyar, in 2005, 2009 and 2013, have revealed many previously unnoticed species. In addition, the author re-identified the earlier Griffith, Cooper and Ludlow & Sherriff collections; followed by the Botanical Survey of India collections; the Japanese 3rd Himalayan Expedition collections; the Edinburgh Botanic Garden collections of Grierson, Long, Bigger & Broad, for their *Flora of Bhutan* (with some ferns listed). He has also revised many interesting high-altitude collections made by G. & S. Miehe at Marburg, of which most were effectively unidentified until re-studied by the author; and many useful collections, unidentified, made by Tandin Wangdi and colleagues then at the NBC, including by Dr. S. Matsumoto, Tsukuba, all identified and listed by the present author. But since then, only minimal work or collection of common lowland species of little interest and without knowing the species and even genera to any reasonable extent have been carried out there to date. Though one single new finding, the low altitude and Critically Endangered (in both India and Bhutan) *Pteris griffithii* Hook. was identified for R. Dorji by the present author (Dorji's claim to have identified it at THIM was untrue) and details of its riparian habitat etc. were supplied to Dorji by him, first reported by Fraser-Jenkins et al (2016). But the population was probably exterminated, or nearly so, by Dorji's collecting all the plants he saw, which promptly died at the N.B.C. at high altitude near Thimphu.

It has been a refreshing contrast for the present author to be able to work with Dr. Phuntsho Wangdi, Forest Ranger, Trashigang, who is actively making many good collections, identified by the author and learning to recognise the species. He has found a number of species previously "missing" and unknown from Bhutan. A new species of his discovery is also named after him, *Diplazium phuntshoi* Fraser-Jenk.

As a result of the present author's study the current checklist contains 568 verified species or subspecies (i.e., significant taxa), 150 more than recently listed on the Bhutan Biodiversity Portal, which was gleaned from the present author's early and incomplete preliminary list provided to the N.B.C. staff as agreed, but then reproduced unauthorisedly and without notice as if under Dorji's name. Additionally, a further seven sterile hybrids are known. A further 140 taxa are placed on the expected species list, known from each side. The majority of the known species are Sino-Himalayan elements and the approximately one quarter to one third remaining is S.E. Asian species. The total number of species in Bhutan compares with 583 species and subspecies known from Nepal (which has an additional European element in west Nepal) (Fraser-Jenkins et al. 2015, Fraser-Jenkins & Kandel 2019, Fraser-Jenkins et al. 2020, Kandel & Fraser-Jenkins 2020), 460 from the small State of Sikkim (Kholia, 2011, 2014), and 659 species and subspecies known from Arunachal Pradesh, with more expected (Fraser-Jenkins & Baishya, 2020).

Many of the species in Bhutan are known from only one or two collections and 72 Bhutanese species have been identified by Fraser-Jenkins (2012) as endangered under various IUCN categories (from near threatened upwards) in adjacent India and a new in Bhutan. Bhutanese forests are still in much better state than in India, but even so timber-cutting and lower-level deforestation is gradually occurring. Only four species are endemic to Bhutan (*Asplenium rebeccae* Fraser-Jenk & Wangdi, *Diplazium bouffordii* Fraser-Jenk. & Pariyar, *Diplazium phuntshoi* Fraser-Jenk. and *Pichisermollodes major* Fraser-Jenk.), but all presumably only temporarily so as there are no natural barriers between Bhutan, N.E. India and Myanmar or China (Fraser-Jenkins 2008) and they are to be expected there.

Further details of all Indian region species were given by Fraser-Jenkins et al. (2016, 2018, & 2020).

Condensed checklist of Bhutanese pteridophytes, from verified collections

Lycopodiaceae
Huperzia arunachalensis (D.D.Pant & P.S.Pandey) Fraser-Jenk., *H. cancellata* (Spring) Trevis., *H. hamiltonii* (Spreng.) Trevis., *H. herteriana* (Kümmerle) T.Sen & U.Sen, *H. javanica* (Sw.) Fraser-Jenk., *H. phlegmaria* (L.) Rothm., *H. pulcherrima* (Wall. ex Hook. & Grev.) Pic.Serm., *H. ulicifolia* (Vent. ex Sw.) Trevis., *H. squarrosa* (G.Forst.) Trevis.

Lycopodium annotinum L. subsp. *alpestre* (Hartm.) Á.Löve & D.Löve, *L. casuarinoides* Spring, *L. complanatum* L. subsp. *complanatum*, *L. dendroideum* Michx., *L. japonicum* Thunb., *L. veitchii* Christ.
Palhinhaea cernua (L.) Franco & Vasc.

Selaginellaceae
Selaginella bisulcata Spring, *S. bryopteris* (L.) Baker, *S. chrysocaulos* (Hook. & Grev.) Spring, *S. chrysorrhizos* Spring, *S. ciliaris* (Retz.) Spring, *S. indica*R.M.Tryon, *S. involvens* (Sw.) Spring, *S. megaphylla* Baker, *S. monospora* Spring (two forms or taxa), *S. pallida* (Hook. & Grev.) Spring, *S. pennata* (D.Don) Spring, *S. remotifolia* Spring, *S. repanda* (Desv. ex Poir.) Spring, *S. reticulata* (Hook. & Grev.) Spring, *S. subdiaphana* (Wall. ex Hook. & Grev.) Spring, *S. tenuifolia* Spring, *S. vaginata* Spring, *S. wallichii* (Hook. & Grev.) Spring.

Equisetaceae
Equisetum arvense L. subsp. *diffusum* (D.Don) Fraser-Jenk., *E. ramosissimum* Desf.

Psilotaceae
Psilotum nudum (L.) P.Beauv.

Ophioglossaceae

Botrychium daucifolium Wall. ex Hook. & Grev., *B. lanuginosum* Wall. ex Hook. & Grev., *B. lunaria* (L.) Sw., *B. multifidum* (S.G.Gmel.) Rupr. subsp. *robustum* (Rupr. ex Milde) Clausen, *B. simplex* E.Hitchc. subsp. *simplex*, *B. ternatum* (Thunb.) Sw., *B. virginianum* (L.) Sw.
Helminthostachys zeylanica (L.) Hook.
Ophioglossum petiolatum Hook., *O. reticulatum* L.

Marattiaceae

Angiopteris crassipes Wall. ex C.Presl, *A. evecta* (G.Forst.) Hoffm., *A. helferiana* C.Presl.

Osmundaceae

Osmunda cinnamomea L. subsp. *asiatica* (Fernald) Fraser-Jenk., *O. claytoniana* L. subsp. *vestita* (Wall. ex Milde) Á.Löve & D.Löve, *O. japonica* Thunb.

Plagiogyriaceae

Plagiogyria adnata (Blume) Bedd., *P. pycnophylla* (Kunze) Mett.

Dipteridaceae

Dipteris wallichii (R.Br.) T.Moore.

Gleicheniaceae

Dicranopteris lanigera (D.Don) Fraser-Jenk., *D. linearis* (Burm.f.) Underw., *D. splendida* (Hand.-Mazz.) Ching, *D. taiwanensis* Ching & P.S.Chiu.
Diplopterygium giganteum (Wall. ex Hook.) Nakai.

Lygodiaceae

Lygodium flexuosum (L.) Sw., *L. japonicum* (Thunb.) Sw., *L. salicifolium* C.Presl.

Marsileaceae

Marsilea minuta L.

Salviniaceae

Azolla pinnata R.Br. subsp. *asiatica* R.M.K.Saunders & K.Fowler.

Cyatheaceae

Cyathea brunoniana (Wall. ex Hook.) C.B.Clarke & Baker, *C. chinensis* Copel., *C. gammiei* R.D.Dixit, *C. khasyana* (T.Moore ex Kuhn) Domin, *C. spinulosa* Wall. ex Hook.

Hymenophyllaceae

Hymenophyllum badium Hook. & Grev., *H. barbatum* (Bosch) Baker, *H. exsertum* Wall. ex Hook., *H. levingei* C.B.Clarke, *H. simonsianum* Hook., *H. tenellum* D.Don.

Trichomanes auriculatum Blume, *T. birmanicum* Bedd., T. campanulatum Roxb., *T. latealatum* Bosch, *T. saxifragoides* C.Presl, *T. schmidianum* Zenker ex Taschner, *T. striatum* D.Don, *T. sublimbatum* Müll.Berol.

Dennstaedtiaceae

Dennstaedtia appendiculata (Wall. ex Hook.) J.Sm., *D. zeylanica* (Sw.) Zink ex Fraser-Jenk. & Kandel.

Histiopteris incisa (Thunb.) J.Sm.

Hypolepis polypodioides (Blume) Hook.

Microlepia caudigera T.Moore, *M. firma* Mett. ex Kuhn, *M. hallbergii* d'Almeida, *M.marginata* (Houtt. ex Panz.) C.Chr., *M. nepalensis* (Spreng.) Fraser-Jenk., *M. platyphylla* (D.Don) J.Sm., *M. rhomboidea* (Wall. ex Kunze) Prantl, *M. setosa* (Sm.) Alston, *M. speluncae* (L.) T.Moore, *M. strigosa* (Thunb.) C.Presl.

Monachosorum henryi Christ.

Pteridium revolutum (Blume) Nakai.

Lindsaeaceae

Lindsaea himalaica K.U.Kramer, *L. odorata* Roxb.

Odontosoria chinensis (L.) J.Sm. subsp. *chinensis*, subsp. *tenuifolia* (Lam.) Fraser-Jenk.

Pteridaceae

Actiniopteris radiata (Sw.) Link.

Adiantum capillus-veneris L., *A. caudatum* L., *A. edgeworthii* Hook., *A. incisum*Forssk. subsp. *incisum*, *A. myriosorum* Baker, *A. pedatum* L. subsp. *pedatum*, *A. philippense* L. subsp. *philippense*, subsp. *intermedium* S.C.Verma & Fraser-Jenk., subsp. *teestae* S.C.Verma & Fraser-Jenk., *A.*

tibeticum Ching & Y.X.Lin, *A. venustum* D.Don.

Aleuritopteris albomarginata (C.B.Clarke) Ching, *A. argentea* (S.G.Gmel.) Fée, *A. bicolor* (Roxb.) Fraser-Jenk., *A. chrysophylla* (Hook.) Ching, *A. dealbata* (C.Presl) Fée, *A. dubia* (C.Hope) Ching, *A. formosana* (Hayata) Tagawa, *A. grisea* (Blanf.) Panigrahi, *A. leptolepis* (Fraser-Jenk.) Fraser-Jenk., *A. stenochlamys* Ching & S.K.Wu.

Ceratopteris pteridoides (Hook.) Hieron.

Cerosora microphylla (Hook.) R.M.Tryon.

*Coniogramme affinis*Hieron., *C. fraxinea* (D.Don) Fée, *C. intermedia* Hieron., *C. procera* Fée, *C. pubescens* Hieron., *C. serrulata* (Blume) Fée.

Cryptogramma brunoniana Wall. ex Hook. & Grev. subsp. *brunoniana*, *C. stelleri* (S.G.Gmel.) Prantl.

Notholaena/Paragymnopteris borealisinensis (Kitag.) Fraser-Jenk., *N. delavayi* (Baker) C.Chr., *N. himalaica* Fraser-Jenk., *N. marantae* (L.) R.Br.

Oeosporangium duthiei (Baker) Fraser-Jenk. & Ph.Wangdi, *O. hancockii* (Baker) Fraser-Jenk., *O. nitidulum* (Hook.) Fraser-Jenk., *O. stramineum* (Ching) Fraser-Jenk., *O. subvillosum* (Hook.) Fraser-Jenk. & Pariyar, *O. yunnanense* (Ching) Fraser-Jenk. & Pariyar.

Onychium cryptogrammoides Christ subsp. *cryptogrammoides*, subsp. *fragile* (S.C.Verma & Khullar) Fraser-Jenk., *O. lucidum* (D.Don) Spreng., *O.moupinense* Ching, *O. siliculosum* (Desv.) C.Chr., *O. tenuifrons* Ching, *O. vermae* Fraser-Jenk. & Khullar.

Pityrogramma calomelanos (L.) Link.

Pteris arisanensis Tagawa, *P. aspericaulis* Wall. ex J.Agardh, *P. assamica* Fraser-Jenk. & T.G.Walker, *P. biaurita* L. subsp. *fornicata* Fraser-Jenk., subsp. *walkeriana* Fraser-Jenk. & Rajkumar, *P. cretica* L. subsp. *cretica*, subsp. *laeta* (Wall. ex Ettingsh.) Fraser-Jenk., *P. dactylina* Hook., *P. dixitii* Fraser-Jenk. & Pariyar, *P. emodi* (Fraser-Jenk.) Fraser-Jenk. & Khullar, *P. ensiformis* Burm.f., *P. griffithii* Hook., *P. hirtula* (C.Chr.) C.V.Morton, *P. khasiana* (C.B.Clarke) Hieron., *P. medogensis* Ching & S.K.Wu, *P. normalis* D.Don, *P. pellucens* J.Agardh, *P. pseudopellucida* Ching, *P. puberula* Ching, *P. scabririgens* Fraser-Jenk., S.C.Verma & T.G.Walker, *P. semipinnata* L., *P. spinescens* C.Presl, *P. subindivisa* C.B.Clarke, *P. subquinata* Wall. ex J.Agardh, *P. taiwanensis* Ching, *P. terminalis* Wall. ex J.Agardh, *P. venusta* Kunze subsp. *matsudae* (Masam.) Fraser-Jenk.

& Kandel, *P. vermae* (Fraser-Jenk.) Fraser-Jenk. & Khullar, *P.vittata* L., *P. wallichiana* J.Agardh.

Vittariaceae

Antrophyum obovatum Baker, *A. plantagineum* Kaulf., *A. reticulatum* (G.Forst.) Kaulf.

Vittaria amboinensis Fée, *V. doniana* Mett. ex Hieron., *V. elongata* Sw., *V. flexuosa* Fée, *V. mediosora* Hayata, *V. sikkimensis* Kuhn, *V. taeniophylla* Copel., *V. zosterifolia* Willd.

Aspleniaceae

Asplenium adiantum-nigrum L. subsp. *adiantum-nigrum, A. amoenum* C.Presl ex Mett., A. *bullatum* Wall. ex Mett., *A. capillipes* Makino subsp. *capillipes*, subsp. *fugax* (Christ) Fraser-Jenk., *A. cheilosorum* Kunze ex Mett., *A. daghestanicum* Christ subsp. *aitchisonii* (Fraser-Jenk. & Reichst.) Fraser-Jenk., *A. dalhousiae* Hook., *A. delavayi* (Franch.) Copel., *A. ensiforme* Wall. ex Hook. & Grev., *A. excisum* C.Presl, *A. exiguum* Bedd. subsp. *lushanense* (C.Chr.) Fraser-Jenk., Pangtey & Khullar, subsp. *yunnanense* (Franch.) Fraser-Jenk., Pangtey & Khullar, *A. finlaysonianum* Wall. ex Hook., *A. hondoense* N.Murak. & Hatan., *A. khullarii* Reichst. & Rasbach ex Fraser-Jenk., *A. laciniatum* D.Don subsp. *laciniatum*, subsp. *kukkonenii* (Reichst.) Fraser-Jenk., subsp. *tenuicaule* (Hayata) Fraser-Jenk., *A. lacinioides* Fraser-Jenk., Pangtey & Khullar, *A. nidoides* Fraser-Jenk. & Kandel, *A. nitidum* Sw., *A. normale* D.Don, *A. obliquissimum* (Hayata) Sugim. & Sa.Kurata, *A. obscurum* Blume, *A. paucivenosum* (Ching) Bir subsp. *paucivenosum*, subsp. *birii* Á.Löve & D.Löve, *A. phyllitidis* D.Don, *A. prolongatum* Hook., *A. rebeccae* Fraser-Jenk., *A. rockii* C.Chr., *A. septentrionale* (L.) Hoffm. subsp. *septentrionale*, *A. shimurae* (H.It) Nakaike, *A. sikkimbirii*Fraser-Jenk., *A. simile* Blume, *A. simonsianum* Hook., *A. tenuifolium* D.Don, *A. tienshanense* Ching, *A. trichomanes* L. subsp. *trichomanes*, subsp. *humistratum* (Ching ex H.S.Kung) Fraser-Jenk., subsp. *quadrivalens* D.E.Mey., *A. yoshinagae* Makino subsp. *yoshinagae*, subsp. *indicum* (Sledge) Fraser-Jenk. [Hybrids: *A.×capillicaule* Fraser-Jenk., *A. exiguum* Bedd. nothosubsp. ×*luyunense* Z.R.Wang ex Fraser-Jenk., Pangtey & Khullar, *A. laciniatum* nothosubsp. ×*laciniocaule* Z.R.Wang & W.M.Chu ex Fraser-Jenk., Pangtey & Khullar, *A.* ×*mustangense* Fraser-Jenk., Pangtey & Khullar, *A.*×*tandinii* Fraser-Jenk.]. *A.* ×*chingii* Fraser-Jenk.was mistakenly listed by Fraser-Jenkins (2022) in error for *A.* ×*mustangense*.

Thelypteridaceae

Thelypteris arida (D.Don) C.V.Morton, *T. auriculata* (J.Sm.) K.Iwats., *T. aurita* (Hook.) Ching, *T. cana* (J.Sm.) Ching, *T. clarkei* (Bedd.) C.F.Reed, *T. crinipes* (Hook.) K.Iwats., *T. dentata* (Forssk.) E.P.St.John, *T. erubescens* (Wall. ex Hook.) Ching, *T. esquirolii* (Christ) Ching, *T. evoluta* (C.B.Clarke & Baker) Tagawa & K.Iwats., *T. flaccida* (Blume) Ching, *T. glanduligera* (Kunze) Ching, ?*T. griffithii* (T.Moore) C.F.Reed, *T. jaculosa* (Christ) Panigrahi, *T. lakhimpurensis* (Rosenst.) K.Iwats., *T. levingei* (C.Hope) Ching, *T. loyalii* (Holttum) Fraser-Jenk., *T. microstegia* (Hook.) Fraser-Jenk. subsp. *microstegia*, subsp. *hirtirachis* (C.Chr.) Fraser-Jenk., subsp. *laterepens* (E.W.Trotter) Fraser-Jenk., *T. mollissima* (Fisch. ex Kunze) Thapa, *T. nudata* (Roxb.) C.V.Morton, *T. oblancifolia* (Tagawa) Fraser-Jenk., *T. ornata* (J.Sm.) Ching, *T. papilio* (C.Hope) K.Iwats., *T. penangiana* (Hook.) C.F.Reed, *T. phegopteris* (L.) Sloss. ex Rydb., *T. procera* (D.Don) Fraser-Jenk., *T. prolifera* (Retz.) C.F.Reed, *T. rectangularis* (Zoll.) K.Iwats., *T. squamaestipes* (C.B.Clarke) Ching, *T. subpubescens* (Blume) K.Iwats., *T. tenera* (Roxb.) C.V.Morton, *T. torresiana* (Gaudich.) Alston, T. tylodes (Kunze) Ching [Hybrid:*T.*×*nareshii* Fraser-Jenk.].

Woodsiaceae

Acystopteris tenuisecta (Blume) Tagawa.

Athyrium anisopterum Christ, *A. atkinsonii* Bedd., *A. contingens* Ching & S.K.Wu, *A. davidii* (Franch.) Christ, *A. decorum* Ching, *A. devolii* Ching, *A. distans* (D.Don) T.Moore, *A. drepanopterum* (Kunze) A.Braun ex Milde, *A. dubium* Ching, *A. eburneum* (J.Sm. ex Mett.) J.Sm., *A. fangii* Ching, *A. fimbriatum* T.Moore, *A. flabellulatum* (C.B.Clarke) Tardieu, *A.foliolosum* T.Moore ex R.Sim, *A. himalaicum* Ching ex Mehra & Bir, *A. kandelii* Fraser-Jenk., *A. mehrae* Bir, *A. micropterum* Fraser-Jenk., *A. nakanoi* Makino, *A. pectinatum* (Wall. ex Mett.) C.Presl ex T.Moore, *A. repens* (Ching) Fraser-Jenk., *A. rupicola* (Edgew. ex C.Hope) C.Chr., *A. schimperi* Moug. ex Fée subsp. *biserrulatum* (Christ) Fraser-Jenk., *A. schizochlamys* (Ching) K.Iwats., *A. setiferum* C.Chr., *A. spinulosum*

(Maxim.) Milde, *A. strigillosum* (T.Moore ex E.J.Lowe) Salom., *A. vermae* Fraser-Jenk., Khullar & Pangtey, *A. wallichianum* Ching [Hybrid: *A.* ×*lobulosoimpolitum* Fraser-Jenk.]
Cornopteris badia Ching, *C. banajaoensis* (C.Chr.) K.Iwats. & M.G.Price, *C.decurrentialata* (Hook.) Nakai, *C. opaca* (D.Don) Tagawa.
Cystopteris fragilis (L.) Bernh. subsp. *kansuana* (C.Chr.) Fraser-Jenk., *C. montana* (Lam.) Bernh. ex Desv., *C. moupinensis* Franch.
Deparia allantodioides (Bedd.) M.Kato, *D. boryana* (Willd.) M.Kato subsp. *boryana*, *D. japonica* (Thunb.) M.Kato subsp. *japonica*, subsp. *petersenii* (Kunze) Fraser-Jenk., *D. lancea* (Thunb.) Fraser-Jenk., *D. membranacea* (Ching & Z.Y.Liu) Fraser-Jenk., *D. subsimilis* (Christ) Fraser-Jenk.
Diplazium bellum (C.B.Clarke) Bir, *D. bouffordii* Fraser-Jenk. & Pariyar, *D. dilatatum* Blume, *D. doederleinii* (Luerss.) Makino, *D. esculentum* (Retz.) Sw., *D. himalayense* (Ching) Panigrahi, *D. javanicum* (Blume) Makino, *D. kawakamii* Hayata, *D. latifolium* T.Moore, *D.laxifrons* Rosenst., *D. longifolium* T.Moore, *D. maximum* (D.Don) C.Chr., *D. phuntshoi* Fraser-Jenk., *D. procumbens* Holttum, *D. pseudosetigerum* (Christ) Fraser-Jenk., *D. sikkimense* (C.B.Clarke) Ching, *D. spectabile* (Wall. ex Mett.) Ching, *D. stoliczkae* Bedd., *D.subspectabile* (Ching & W.M.Chu) Z.R.He, D. succulentum (C.B.Clarke) C.Chr., *D. sylvaticum* (Bory) Sw., *D. tibeticum* (Ching & S.K.Wu) Z.R.He.
Gymnocarpium jessoense (Koidz.) Koidz.
Woodsia andersonii (Bedd.) Christ, *W. cycloloba* Hand.-Mazz., *W. elongata* Hook., *W. lanosa* Hook., *W. rosthorniana* Diels.

Onocleaceae
Onoclea orientalis (Hook.) Hook.

Blechnaceae
Blechnum melanopus Hook., *B. orientale* L.
Woodwardia unigemmata Makino.

Dryopteridaceae
Arachniodes coniifolia (T.Moore) Ching, *A. cornucervi* (D.Don) Fraser-Jenk., *A. foeniculacea* Ching, *A. fraserjenkinsii* L.Zhang & N.T.Lu, *A.*

simulans Ching, *A. superba* Fraser-Jenk.
?*Ctenitis subglandulosa* (Hance) Ching.
Cyrtomium anomophyllum (Zenker) Fraser-Jenk., *C. caryotideum* (Wall. ex hook. & Grev.) C.Presl, *C. fortunei* J.Sm., *C. macrophyllum* (Makino) Tagawa.
?*Didymochlaena truncatula* (Sw.) J.Sm.
Dryopsis apiciflora (Wall. ex Mett.) Holttum & P.J.Edwards, *D. clarkei* (Baker) Holttum & P.J.Edwards, *D. manipurensis* (Bedd.) Holttum & P.J.Edwards, *D. nidus* (Baker) Holttum & P.J.Edwards.
Dryopteris acutodentata Ching, *D. atrata* (Kunze) Ching, *D. barbigera* (T.Moore ex Hook.) Kuntze, *D. basisora* Christ, *D. bhutanica* (Fraser-Jenk. & Pariyar) Fraser-Jenk., *D. carolihopei* Fraser-Jenk., *D. chrysocoma* (Christ) C.Chr., *D. cochleata* (D.Don) C.Chr., *D. conjugata* Ching, *D. fangii* Ching, *D. flemingii* Fraser-Jenk., *D. fructuosa* (Christ) C.Chr., *D. gamblei* (C.Hope) C.Chr., *D. juxtaposita* Christ, *D. komarovii* Kossinsky, *D. lepidopoda* Hayata, *D. lunanensis* (Christ) C.Chr., *D. marginata* (C.B.Clarke) Christ, *D. nobilis* Ching, *D. obtusipinnula* (Fraser-Jenk.) Fraser-Jenk., *D. panda* (C.B.Clarke) Christ, *D. pulvinulifera* (Bedd.) Kuntze, *D. redactopinnata* Soumen K.Basu & Panigrahi, *D. scottii* (Bedd.) Ching, *D. serratodentata* (Bedd.) Hayata, *D. sikkimensis* (Bedd.) Kuntze, *D. sinonepalensis* Z.Y.Zuo & Fraser-Jenk., *D. sparsa* (D.Don) Kuntze subsp. *sparsa*, subsp. *rectipinnula* Fraser-Jenk., *D. splendens* (Hook.) Kuntze, *D. stenolepis* (Baker) C.Chr., *D. subimpressa* Loyal, *D. sublacera* Christ, *D. wallichiana* (Spreng.) Hyl. subsp. *wallichiana*, subsp. *himalaica* Fraser-Jenk., subsp. *nepalensis* Fraser-Jenk., subsp. *pachyphylla* Fraser-Jenk. & R.Knapp, *D. woodsiisora* Hayata, *D. xanthomelas* (Christ) C.Chr., *D. yigongensis* Ching & S.K.Wu, *D. zayuensis* Ching & S.K.Wu. *D. edwardsii* Fraser-Jenk., was mistakenly listed by Fraser-Jenkins (2022) in error for *D. yigongensis*.
Hypodematium crenatum (Forssk.) Kuhn subsp. *crenatum*, subsp. *loyalii* Fraser-Jenk. & Khullar.
Nothoperanema hendersonii (Bedd.) Ching, *N. squamisetum* (Hook.) Ching.
Peranema aspidioides (Blume) Mett., *P. cyatheoides* D.Don, *P. paleolatum* (Pic.Serm.) Fraser-Jenk.

Pleocnemia submembranacea (Hayata) Tagawa & K.Iwats.
Polystichum acutidens Christ, *P. atkinsonii* Bedd., *P. attenuatum* Tagawa & K.Iwats., *P. bakerianum* (Atk. ex C.B.Clarke) Diels, *P. cyclolobum* C.Chr., *P. discretum* (D.Don) J.Sm., *P. glaciale* Christ, *P. hookerianum* (C.Presl) C.Chr., *P. lachenense* (Hook.) Bedd., *P. lentum* (D.Don) T.Moore, *P. levingei* C.Hope ex Christ, *P. longipaleatum* Christ, *P. makinoi* (Tagawa) Tagawa, *P. manmeiense* (Christ) Nakaike, *P. mehrae* Fraser-Jenk. & Khullar, *P. mucronifolium* (Blume) C.Presl, *P. neolobatum* Nakai, *P. nepalense* (Spreng.) C.Chr., *P. obliquum* (D.Don) T.Moore, *P. piceopaleaceum* Tagawa, *P. prescottianum* (Wall. ex Mett.) T.Moore, *P. pseudotsus-simense* Ching, *P. punctiferum* C.Chr., *P. scariosum* (Roxb.) C.V.Morton, *P. semifertile* (C.B.Clarke) Ching, *P. shensiense* Christ, *P. sinense* (Christ) Christ, *P. squarrosum* (D.Don) Fée, *P. stimulans* (Kunze ex Mett.) Bedd., *P. thomsonii* (Hook.f.) Bedd., *P. woodsioides* Christ, *P. yunnanense* Christ.
Tectaria coadunata (J.Sm.) C.Chr., *T. fuscipes* (Wall. ex Bedd.) C.Chr., *T. heterocarpa* (Bedd.) C.V.Morton, *T. ingens* (Atk. ex C.B.Clarke) Holttum, *T. morata* Fraser-Jenk., Kandel & Andreis, *T. polymorpha* (Wall. ex Hook.) Copel., *T. pseudosiifolia* Fraser-Jenk. & Wangdi, *T. subconfluens* (Bedd.) Ching.

Lomariopsidaceae
Bolbitis angustpinna (Hayata) H.It, *B. appendiculata* (Willd.) K.Iwats., *B. costata* (C.Presl) Ching, *B. deltigera* (Hook.) C.Chr., *B. heteroclita* (C.Presl) Ching, *B. major* (Bedd.) Hennipman, *B. sinensis* (Baker) K.Iwats., *B. tibetica* Ching & S.K.Wu.
Elaphoglossum marginatum T.Moore, *E. stelligerum* (Wall. ex Baker) T.Moore ex Salom.

Oleandraceae
Oleandra pistillaris (Sw.) C.Chr., *O. wallichii* (Hook.) C.Presl.

Nephrolepidaceae
Nephrolepis cordifolia (L.) C.Presl.

Davalliaceae
Araiostegiella hookeri (T.Moore ex Bedd.) Fraser-Jenk., *A. perdurans*

(Christ) M.Kato.
Davallia assamica (Bedd.) Baker, *D. bullata* Wall. ex Hook., *D. griffithiana* Hook., *D. repens* (L.f.) Kuhn.
Davallodes membranulosa (Wall. ex Hook.) Copel.
Katoella beddomei (C.Hope) Fraser-Jenk., *K. pulchra* (D.Don) Fraser-Jenk., Kandel & Pariyar, *K. squamata* (Decne.) Fraser-Jenk., Kandel & Pariyar, *K. yunnanensis* (Christ) Fraser-Jenk. & Kholia.
Leucostegia immersa C.Presl.
Paradavallodes multidentata (Hook.) Ching.

Polypodiaceae
Arthromeris himalovata Fraser-Jenk. & Kandel, *A. lehmannii* (Mett.) Ching, ?*A. moulmeinensis* (Bedd.) Fraser-Jenk., *A. tatsienensis* (Franch. & Bureau) Ching, *A. tomentosa* W.M.Chu, *A. wallichiana* (Spreng.) Ching, *A. wardii* (C.B.Clarke) Ching.
Drynaria coronans (Wall. ex Mett.) J.Sm. ex T.Moore, *D. delavayi* Christ, *D. mollis* Bedd., *D. propinqua* (Wall. ex Mett.) J.Sm., *D. quercifolia* (L.) J.Sm.
Goniophlebium argutum (Wall. ex Hook.) Bedd.
Gymnogrammitis dareiformis (Hook.) Ching ex Tardieu & C.Chr..
Lemmaphyllum carnosum (Hook.) C.Presl subsp. *carnosum*, *L. rostratum* (Bedd.) Tagawa.
Lepisorus bicolor (Takeda) Ching, *L. clathratus* (C.B.Clarke) Ching, *L. contortus* (Christ) Ching, *L. henryi* (Hieron. ex C.Chr) Li Wang, *L. jakonensis* (Blanf.) Fraser-Jenk. & J.Krieg., *L. loriformis* (Wall. ex Mett.) Ching, *L. macrosphaerus* (Baker) Ching, *L. mehrae* Fraser-Jenk., *L. morrisonensis* (Hayata) H.It, *L. nudus* (Hook.) Ching, *L. scolopendrium* (Ching) Mehra & Bir, *L. sublinearis* (Baker ex Takeda) Ching, *L. thunbergianus* (Kaulf.) Ching, *L. tricholepis* K.H.Shing & Y.X.Lin.
Leptochilus decurrens Blume subsp. *decurrens*, subsp. *hemionitideus* (C.Presl) Fraser-Jenk., *L. ellipticus* (Thunb.) Noot., *L. pedunculatus* (Hook. & Grev.) Fraser-Jenk., *L. pothifolius* (D.Don) Fraser-Jenk., *L. pteropus* (Blume) Fraser-Jenk. subsp. *pteropus*.
Loxogramme chinensis Ching, *L. cuspidata* (Zenker) M.G.Price, *L. grammitoides* (Baker) C.Chr., ?*L. involuta* (D.Don) C.Presl, *L. porcata*

M.G.Price.
Microsorum cuspidatum(D.Don) Tagawa subsp. *cuspidatum*, *M. membranaceum* (D.Don) Ching, *M. punctatum* (L.) Copel.
Neocheiropteris chinensis (Mett. ex Kuhn) Fraser-Jenk., *N. maculosa* (Christ) Fraser-Jenk. & Kholia, *N. normalis* (D.Don) Tagawa, *N. ovata* (Fée) Fraser-Jenk., *N. subhemionitidea* (Christ) Fraser-Jenk., *N. superficialis* (Blume) Bosman, *N. venosa* (Ching) Fraser-Jenk., *N. zippelii* (Blume) Bosman.
Pichisermollodes crenatopinnata (C.B.Clarke) Fraser-Jenk., *P. ebenipes* (Hook.) Fraser-Jenk., *P. erythrocarpa* (Mett. ex Kuhn) Fraser-Jenk., *P. glaucopsis* (Franch.) Fraser-Jenk., *P. major* Fraser-Jenk., *P. malacodon* (Hook.) Fraser-Jenk., *P. nepalensis* (Nakaike) Fraser-Jenk., *P. nigrovenia* (Christ) Fraser-Jenk., *P. quasidivaricata* (Hayata) Fraser-Jenk., *P. stewartii* (Bedd.) Fraser-Jenk.
Polypodiodes amoena (Wall. ex Mett.) Ching subsp. *amoena*, subsp. *yunnanensis* (Franch.) Fraser-Jenk., *P. fieldingiana* (Kunze ex Mett.) Fraser-Jenk., *P. hendersonii* (Bedd.) Fraser-Jenk., *P. lachnopus* (Wall. ex Hook.) Ching.
Pyrrosia adnascens (Sw.) Ching, *P. boothii* (Hook.) Ching, *P. costata* (Wall. ex C.Presl) Tagawa & K.Iwats., *P. flocculosa* (D.Don) Ching, *P. glabra* (Desv.) Fraser-Jenk., *P. heteractis* (Mett. ex Kuhn) Ching, *P. lanceolata* (L.) Farw., *P. mannii* (Giesenh.) Ching, *P. nummulariifolia* (Sw.) Ching, *P. porosa* (C.Presl) Hovenkamp, *P. stenophylla* (Bedd.) Ching, *P. subfurfuracea* (Hook.) Ching.
Selliguea chrysotricha (C.Chr.) Fraser-Jenk., *S. griffithiana* (Hook.) Fraser-Jenk., *S. majoensis* (C.Chr.) Fraser-Jenk., *S. oxyloba* (Wall. ex Kunze) Fraser-Jenk., *S. rhynchophylla* (Hook.) Fraser-Jenk.

Grammitidaceae
Micropolypodium sikkimense (Hieron.) X.C.Zhang.
Tomophyllum donianum (Spreng.) Fraser-Jenk. & Parris.

New Combinations and Species

Dryopteris bhutanica (Fraser-Jenk. & Pariyar) Fraser-Jenk., comb. et stat. nov. (basionym: *Dryopteris wallichiana* (Spreng.) Hyl. subsp. *bhutanica*

Fraser-Jenk. & Pariyar, in Fraser-Jenkins, Kandel & Pariyar, *Ferns Fern-Allies Nepal* **1**: 29. 2015).

Dryopteris obtusipinnula (Fraser-Jenk.) Fraser-Jenk., comb. et stat. nov. (basionym: *Dryopteris sparsa* (D.Don) Kuntze subsp. *obtusipinnula* Fraser-Jenk., *Indian J. Forestry* **45**(1): 13 [as "9"]. 2022).

Arachniodes fraserjenkinsii L.Zhang & N.T.Lu, sp. nov. (misapplied name: *A.* ?*spectabilis* sensu Fraser-Jenkins, in herb., non (Ching) Ching [from China]).

This distinctive species was first identified by Dr. N.T. Lu from its different molecular sequences, obtained from a plant from near Daman, Makawanpur, C. Nepal. They named it after the present author. It was long-known to CRFJ from West Bengal (Darjeeling) and Meghalaya (Shillong Peak and Sohra) as a distinctive species that he mistakenly thought tentatively to be possible *A. spectabilis.*

Rhizoma crassum, breve erectum, fasciatum; *stipes sparse paleaceus, et paleas longas linearas fuscas sparsas ad basses*; *lamina tripinnata, lobi pinnularum bene stipitati, plerumque adamas formibus, leviter lati, a dentibus parvis acutis ad apices*; *sori approximati ad venas medialis segmenti*. Holotypus: Nepal, Makawanpur, Daman (Thaha), north side of pass, *X.F.Gao, J.D. Ya, Q.R. Zhang, G.S. Cui, W.L. Zhao et al.* QTP-II08-T05-000655, 15.12.2017, KATH. It also occurs in West Bengal (Darjeeling), Sikkim and Bhutan.

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Flora of Nepal: A Taxonomical Discourse

Keshab R. Rajbhandari
Kathmandu, Nepal
Email: krrajbhandari@yahoo.com

Abstract

The taxonomic studies of the flowering plants of Nepal started when J. E. Smith published in 1805 a plant collected by Buchanan-Hamilton from Nepal in 1802. Then Wallich's and Hooker's taxonomic studies added many plants from Nepal while D. Don's '*Prodromus Florae Nepalensis*' became the first flora of Nepal. In this paper the taxonomic studies of the flowering plants of Nepal carried out since the beginning of the nineteenth century based on published literature are discussed. The discourse is divided into three groups, British (European) contributions, Japanese contributions and Nepalese contributions. The activities for publications of '*Flora of Nepal*' and future activities on the taxonomic studies of flowering plants are also discussed.

Keywords: British contributions, Floral diversity, Japanese contributions, Nepalese contributions

Introduction

Flora means the plants characteristic of a region, period, or special environment. Flora of Nepal or plants found within Nepal is a reflection of its unique geographic position and altitudinal and climatic variations. Nepal's location in the central portion of the Himalayas places it in the transitional zone between the eastern and western Himalayan floras and thus Nepal has tremendous diversity of plants. The history of taxonomical studies on flora of Nepal has now passed more than 200 years (Rajbhandari, 1976, 2002a). Taxonomy means knowing plants (identification, nomenclature and classification). About 225 taxonomical papers on Nepalese plants have been published up to the end of the twentieth century (Rajbhandari, 2002b). The objective of this paper is to make a taxonomical discourse for understanding the activities carried out in the taxonomical studies of the flowering plants of Nepal and to show the activities for the publication of flora of Nepal. The studies of the Nepalese flowering plants are divided into three contributions made by British (European), Japanese and Nepalese groups.

British (European) contributions to the study of Nepalese flowering plants

In the U. K. two Institutions, Natural History Museum, London and Royal Botanic Garden Edinburgh, are interested in flora of Nepal. Since 1842 Natural History Museum, London (formerly, British Museum Natural History) was engaged working on flora of Nepal. About 50000 herbarium specimens of the flowering plants of Nepal are preserved in that Institution.

Work on the Himalayan flora were being carried out since long time back in the Royal Botanic Garden Edinburgh and since 1920s interest on Nepal flora has been shown. About 60000 herbarium specimens of the flowering plants of Nepal are preserved in that Institution. The third important institution in the U. K., where Nepalese specimens are preserved, is the Royal Botanic Garden, Kew. In that institution specimens collected by Nathaniel Wallich and Joseph Dalton Hooker from Nepal are preserved.

The first systematic plant collection in Nepal was done by Francis Buchanan (later Hamilton) in 1802-1803 travelling from Raxaul to Kathmandu. That was followed by Nathaniel Wallich in 1820-1821. Based on these collections David Don prepared a first book on flora of Nepal 'Prodromus Florae Nepalensis' published in 1825, where he recorded some 712 species, 321 genera and 92 families from Nepal. Some largest families in that book containing Nepalese plants are Compositae (33 genera and 59 species), Orchideae (23 genera and 49 species), Labiatae (11 genera and 30 species), Polygoneae (5 genera and 25 species), and Leguminosae (10 genera and 24 species). In the same way some largest genera are Polygonum (Polygonaceae, 21 species), Rubus (Rosaceae, 10 species), Carex (Cyperaceae, 9 species), and Potentilla (Rosaceae, 9 species). The collection of Buchanan Hamilton, on which Don's work was largely based, came into possession of the British Museum in 1842 (Stearn, 1978). Before this the collection was in the possession of Aylmer Bourke Lambert. David Don worked in the library of Lambert. The specimens were sold in the auction and British Museum bought them for £ 9. Hamilton had also given one set of Nepalese plant specimens along with their drawings to his friend James Edward Smith who published new species in Rees's Cyclopedia based on that material (Stearn, 1978).

Hamilton's collections given to Smith were incorporated in the Herbarium now in the keeping of the Linnean Society of London. Smith published in 1805 *Begonia picta* in Exotic Botany. That plant was collected in Nepal by Buchanan Hamilton on July 21, 1802. This might be the first scientific publication on the flowering plant of Nepal.

Nathaniel Wallich went to Kathmandu in 1820 and stayed there for about a year. His own collections were more extensive than Hamilton's and he himself based descriptions of many new plants from them, notably in his contributions to the first edition of Roxburgh's *Flora Indica* (1820-1824), his *Tentamen Florae Nepalensis* (1824-26) and *Plantae Asiaticae Rariores* (1830) and many other publications. Catalogued and numbered in his Numerical List of dried specimens of plants (1829-49), commonly cited as 'Wall. Cat.', Wallich's specimens were made widely available through their distribution by the East India Company, and for a long period were the only Nepal specimens available to botanists.

Joseph Dalton Hooker collected plants in east Nepal in 1848. Hooker and T. Thomson published '*Flora Indica volume 1*' in 1855. The book includes 50 species belonging to 23 genera and 11 families of Nepalese flowering plants collected mainly by Hooker and some previously collected plants in Nepal by Hamilton and Wallich. Hooker worked out on all his collections of Nepalese plants and incorporated them in his '*Flora of British India Volumes 1-7'* (1872-1897). This book contains 845 species of Nepalese flowering plants in 439 genera and 104 families. Some largest families of the flowering plants of Nepal in Hooker's books are Orchideae (42 genera and 90 species), Compositae (17 genera and 47 species), Gramineae (26 genera and 41 species), Leguminosae (26 genera and 35 species), and Labiatae (18 genera and 28 species). In the same way some largest genera are *Polygonum* (Polygonaceae, 13 species), *Senecio* (Asteraceae, 13 species), *Impatiens* (Balsaminaceae, 11 species), *Bulbophyllum* (Orchidaceae, 10 species), *Carex* (Cyperaceae, 9 species), and *Dendrobium* (Orchidaceae, 9 species).

In 1907, I. H. Burkill made a visit to Kathmandu following the route of Wallich and also went up to Nuwakot, north of Kathmandu to collect plant specimens (Burkill, 1910). Burkill's collections of Nepalese plants are deposited in the Herbarium of the Botanical Survey of India, Kolkata, India.

In 1928, a list of flowering plants and ferns of Nepal was published as an appendix in Landon's Nepal. This list included 1679 species, 748 genera, and 130 families and was based on the plants published up to that period.

No major botanical collection was carried out in Nepal from 1908 until 1927-37 when Lall Dhwoj, an officer in the Nepal army, and then Khadanand Sharma made collections of herbarium specimens and seeds for presentation to King George V in the U. K. Their activities were arranged through Clive Wigram, then private secretary to King George V, on behalf of T. Hay, then in charge of the royal parks in London, who handled the seeds, and George Taylor of the British Museum (Natural History), into which the herbarium specimens passed, supplementing the old Buchanan Hamilton collection already in the Museum (Stearn, 1978). Based on these collections revisions of genera were published, such as *Gentiana* (Gentianaceae) by Marquand (1928, 1931), *Primula* (Primulaceae) by Smith (1931, 1936) and Smith and Fletcher (1942-1946), and *Meconopsis* (Papaveraceae) by Taylor (1934).

Since 1949 many plant explorations were carried out in Nepal, such as O. Polunin in 1949, D. G. Lowndes in 1950, the British Museum (Natural History) plant exploration teams to west Nepal with O. Polunin, W. R. Sykes and L. H. J. Williams as members in 1952 and to central Nepal with J. D. A. Stainton, Sykes & Williams as members in 1954. The contribution of J. D. A. Stainton from 1954 to 1972 is noteworthy for his extensive collections of plants from east to west Nepal. From such expeditions great and small the British Museum (Natural History) has acquired about 50000 Nepalese specimens. Study of those materials has led to the publication of a series of memoirs on individual genera published in the *Bulletin of the British Museum, Natural History (Botany)*, i. e., *Allium* and *Milula* by Stearn (1960); *Epilobium* by Raven (1962); *Pedicularis* by Tsoong (1955); *Saussurea* and *Corydalis* by Ludlow and Stearn (1956, 1975); *Saxifraga* and *Swertia* by Smith (1956, 1960, 1970); *Taraxacum* by Van Soest (1961); and so on.

The collections of plants in Nepal by D. G. Long of Royal Botanic Garden Edinburgh and others from the U. K. have enriched its herbarium to a great extent recently from 1989 onwards. Flora of Bhutan was initiated there in 1974 (Noltie, 2002) and completed publications between 1984 and 2002 in 3 volumes and 9 parts (Grierson and Long, 1984a, 1984b, 1987b, 1991, 1999, 2001; Noltie, 1994c, 2000b; Pearce and Cribb, 2002). While working on flora of Bhutan Nepalese specimens were also examined and many new species and records were added to the flora of Nepal. These records were published as notes relating to the flora of Bhutan (numbers 1-44) in *Edinburgh Journal of Botany* (formerly, *Notes from the Royal Botanic Garden Edinburgh*). Some of them, which deal with Nepalese plants also are by Aitken and Long (1996) on Gentianaceae; Grierson and Long (1978, 1979, 1982, 1987a) on Mandragora, Leguminosae, Pentasacme, Elatostema, Rosaceae; Grierson and Springate (2000) on Compositae; Long (1984a, 1984b, 1986) on Lauraceae, *Corydalis*, Euphorbiaceae; Mill (1996a, 1996b, 2001) on Boraginaceae, Convolvulaceae, *Pedicularis*; Noltie (1993a, 1993b, 1993c, 1994a, 1994b, 1999a, 1999b, 2000a) on *Kobresia*, *Lloydia*, *Carex*, *Juncus*, *Smilax*, Gramineae; Pearce et al. (2001) on Orchidaceae; Springate et al. (1996) on Rubiaceae; Stapleton (1994a, 1994b, 1994c, 1994d) on bamboos; Watson (1996, 1998) on Umbelliferae; Wood (1994) on Acanthaceae.

Japanese contributions to the study of Nepalese flowering plants

In Japan two Institutions, University of Kyoto and University of Tokyo, are interested in flora of Nepal. Since 1952 the University of Kyoto was involved in flora of Nepal. Kitamura (1953, 1954a, 1954b, 1955a, 1955b) published new species of flowering plants from the collections of S. Nakao in central Nepal and also in Kihara's '*Fauna and flora of Nepal Himalaya*'. About 30000 herbarium specimens of the flowering plants of Nepal are preserved in that Institution.

Since 1963 the University of Tokyo was involved in flora of Nepal. About 100000 herbarium specimens of the flowering plants of Nepal are preserved in that Institution.

Several Japanese expedition teams from the University of Tokyo in 1963, 1972 and onward had extensively explored the country from east to west. Contributions of H. Hara (from 1963-1972), H. Kanai (from 1963-1983) and H. Ohashi (from 1969-1977) are significant and noteworthy in exploring the flora of Nepal. Three valuable volumes edited by Hara and by Ohashi, *The Flora of Eastern Himalaya* (in 1966, *Second Report* in 1971, and *Third Report* in 1975) gave systematic enumerations of the plants collected, together with numerous critical notes and revisions educidating problems of nomenclature and taxonomy. H. Hara and other Japanese scientists worked out on their collections and many new species and records were noted for Nepal. Besides revisionary works of *Arisaema* (Hara, 1971b), Hara worked out on the plants of the Himalaya and published his first article on Nepalese plant (Hara, 1954) and later a series of papers '*new or noteworthy flowering plants from eastern Himalaya*' up to 21 numbers published in *Journal of Japanese Botany*, of which numbers 3, 5, and 9 to 21 also deal with the Nepalese plants (Hara, 1965-1978). In the same way, Ohashi (1965, 1971b, 1973, 1974, 1975b, 1976, 1984) and Ohashi and Tateishi (1975) worked on Leguminosae, Ohba (1974a, 1974b, 1975, 1976, 1977, 1978, 1980, 1981a, 1981b) on Crassulaceae, Yamazaki (1954, 1963, 1970a, 1970b, 1971a, 1971b, 1978, 1980) on Scrophulariaceae and other Gamopetalous plants, Satake (1973) on *Eriocaulon*, Momiyama & Hara (1973) on *Elaeagnus*, Kitamura (1968a, 1968b, 1969a, 1969b, 1974, 1979, 1981) on Compositae, and so on.

Hideaki Ohba of the University of Tokyo and several of his colleagues collected plant specimens extensively in Nepal from 1983 onwards up to 2012 almost every year. As a result of the collections of plants from 1983 to 1996 and studies carried out by specialists more than 55 new species of flowering plants have been discovered from Nepal (Rajbhandari, 2002a). Many important publications related to flora of Nepal appeared. Some of them are '*Himalayan plants Vol. 1-2*' edited by H. Ohba and S. B. Malla published in 1988 and 1991 and '*Himalayan plants Vol. 3-4*' edited by H. Ohba and published in 1999 and 2006, '*Alpine flora of Jaljale Himal*' edited by H. Ohba and S. Akiyama and published in 1992, '*Flora of Ganesh Himal*' edited by H. Ohba and H. Ikeda and published in 1999, '*Flora of Hinku and Hunku valleys, east Nepal*' edited by H. Ohba and H. Ikeda and published in 2000. '*Name list of the flowering plants and gymnosperms of Nepal*' compiled by H. Koba et al. in 1994 is an alphabetical list of all seed plants recorded in '*An enumeration of the flowering plants of Nepal*'.

A detailed references on the taxonomical works on flora of Nepal are available in Rajbhandari (1994, 2001). Some of the works were carried out by Akiyama (1987, 2021a, 2021b), Akiyama and Ohba (1993), and Akiyama et al. (1991, 1992) on *Impatiens*; Fujikawa (2002) and Fujikawa and Ohba (2002) on *Saussurea*; Hara (1984, 1987, 1988) on *Disporum* and *Smilacina*; Ikeda and Ohba (1992, 1993a, 1993b, 1996a, 1996b, 1999) on *Potentilla*; Iokawa and Ohashi (2002a, 2002b) on *Campylotropis*; Kadota (1991, 1997, 1999) on *Aconitum* and *Ranunculus*; Kurosawa (1998, 2002) on Euphorbiaceae; Miyamoto (2002), Miyamoto and Ohba (1993, 1995, 1997, 2002), and Miyamoto et al. (2003) on *Juncus*; Ohashi (1984) on *Desmodium*; Ohba (1984, 2003) on *Saxifraga* and *Kalanchoe*; Ohba and Akiyama (1992, 1999) on *Bistorta* and *Saxifraga*; Ohba and Rajbhandari (1986) on *Rhodiola*; Ohba (1984) and Ohba and Wakabayashi (1987) on *Saxifraga*; Yamazaki (1986a, 1986b, 1988, 2000) on *Pedicularis*; Yonekura and Ohashi (1999, 2002) on *Bistorta*; and so on.

Nepalese contributions to the study of Nepalese flowering plants

In Nepal three Institutions, Department of Plant Resources (DPR) (formerly, Department of Medicinal Plants, Ministry of Forests and Soil Conservation), Central Department of Botany (CDB) (Tribhuvan University) and Nepal Academy of Science and Technology (NAST, formerly, Royal Nepal Academy of Science and Technology), are interested in flora of Nepal. Since its establishment in 1961 Department of Plant Resources was engaged in flora of Nepal and started preserving herbarium specimens. Several local floras including flora of Kathmandu valley and fascicles of flora of Nepal were worked out in that Institution. About 160000 herbarium specimens of the flowering plants of Nepal are preserved in that Institution.

The National Herbarium (KATH) belonging to the Department of Plant Resources is now editorial center of Flora of Nepal, and works to publish online floras are being carried out there. In this regard a few online floras, such as Iridaceae, *Habenaria* and *Peristylus,* were published in July 2022. The National Herbarium (KATH) also published specimens preserved in that Institution in its website. More than 110000 specimens of KATH have been digitized, 90000 of them are available online. From 2009 to 2022 Department of Plant Resources published many books, such as '*Endemic flowering plants of Nepal Parts 1-3'* (Rajbhandari & Adhikari, 2009, Rajbhandari & Dhungana, 2010, 2011) and '*Catalogue of Nepalese flowering plants parts 1-3'* and their '*Supplement*' (Rajbhandari & Baral, 2010, Rajbhandari et al., 2011, 2012, 2015). Recently, Department of Plant Resources published some books related to the flowering plants of Nepal, such as '*A handbook of the orchids of Nepal*' (Rajbhandari, 2015), '*Plant resources of Kailali, west Nepal'* (Rajbhandari et al., 2016), '*Plant diversity of Salhesh Phulbari, Siraha***'** (Bhatt and Khatri, 2016), '*Flowering plants of Makwanpur'* (Chapagain et al., 2016), '*Plant diversity in Central Chure Region, Nepal***'** (Chapagain et al., 2017), '*Plants of Ramapithecus Botanical Garden*' (in Nepali) (Bhatt et al., 2017), '*Flowering plants of Nepal: An introduction'* (Rajbhandari et al., 2017), and '*Flowering plants discovered from Nepal'* (Rajbhandari et al., 2019).

Based on the herbarium specimens of the flowering plants collected in Nepal the Department of Plant Resources published '*A handbook of the flowering plants of Nepal vol. 1 & 2*' (Rajbhandari & Rai, 2017, 2019), and '*vol. 3 & 4*' (Rajbhandari et al., 2021, 2022) to update the status and nomenclature of the checklist of the flowering plants of Nepal, such as '*An enumeration of the flowering plants of Nepal vol. 1-3*' by Hara and others published in 1978-1982.

Taxonomy on flora of Nepal has been taught in the Central Department of Botany and Amrit Science College of the Tribhuvan University. Several students carried out revisionary works on Nepalese plants for Master's and doctoral degrees. About 25000 herbarium specimens of the flowering and non-flowering plants of Nepal are preserved in the herbarium (TUCH) of Central Department of Botany. Several M. Sc. and Ph. D. thesis on Nepalese flowering plants were prepared by the students studying in the Central Department of Botany and Amrit Science College. Some of the M.

Sc. theses on the revisions of the genera and families of the flowering plants are *Crotalaria* (Fabaceae) by Pant in 1996), *Thalictrum* (Ranunculaceae) by Yadav in 1996, Malvaceae by Joshi in 1998, Hypericaceae by Shrestha in 1999), *Aconitum* (Ranunculaceae) by Shahi in 1999, Melastomataceae by Sharma in 1999, Violaceae by Dani in 2001, Geraniaceae by Yadav in 2002, *Smilax* (Smilacaceae) (Subedi, 2003), Hydrangeaceae (Shrestha, 2005), *Hedychium* (Zingiberaceae) (Mishra, 2007), Commelinaceae (Gajurel, 2008), *Ipomoea* (Convolvulaceae) (Shrestha, 2009), *Indigofera* (Fabaceae) (Thapa, 2014), Iridaceae, Colchicaceae, Melanthiaceae (Thapa, 2014), Liliaceae (Rana, 2014), *Dioscorea* (Dioscoreaceae) (Gurung, 2016), *Aster* (Asteraceae) (Shrestha, 2017), *Panicum* (Poaceae) (Bhatt, 2018), *Setaria* (Poaceae) (Joshi, 2018), *Thalictrum (*Ranunculaceae*)* (Neupane. 2018), *Swertia* (Gentianaceae) (Khatri, 2019), *Hoya* (Apocynaceae*)* (Chhetri, 2020), *Codonopsis* (Campanulaceae) **(**Timalsina, 2022), *Cyperus* (*Cyperaceae*) (Basukala, 2022), *Cremanthodium* (Asteraceae) (Pathak, 2018), *Cirsium* (Asteraceae) (Gyawali, 2019), *Taraxacum* (Ranunculaceae), (Luintel, 2019). In the same way, Ph. D. thesis are *Oberonia* (Orchidaceae) (Shakya, 1999), *Eria* (Orchidaceae) (Bajracharya, 2005), *Begonia* (Begoniaceae) (Rajbhandary, 2010), *Anaphalis* (Asteraceae) (Vaidya, 2015), *Senecio* (Asteraceae) (Joshi, 2015), and *Dendrobium* (Orchidaceae) (Baba, 2022). The Central Department of Botany also published a catalogue, '*Catalogue of the Nepalese flowering plants preserved in the Tribhuvan University Central Herbarium Part 1: Monocotyledons*' (Rajbhandari et al., 2016), which contains 388 species in 22 families. Nepal Academy of Science and Technology works as a facilitator for flora of Nepal. There is no herbarium specimen preserved in that Institution.

Ph. D. theses on some genera and families of the flowering plants of Nepal were prepared by the Nepalese botanists in the foreign universities. Some of them are Orchidaceae by Pradhan in 1981, *Anemone* (Ranunculaceae) by Chaudhary in 1987, *Poa* (Poaceae) by Rajbhandari in 1989, *Cyananthus* (Campanulaceae) by Shrestha in 1992, *Boehmeria* (Urticaceae) by Acharya in 2003, Loranthaceae by Devkota in 2003, *Berberis* (Berberidaceae) by Adhikari in 2010, *Herminium (*Orchidaceae*)* by Raskoti in 2015, and *Habenaria* (Orchidaceae) by Pandey in 2021.

Recently, some other books on the flowering plants of Nepal have been published, such as '*Plant diversity of eastern Nepal*' (Siwakoti and Varma, 1999), '*Handbook of flowering plants of Nepal Volume 1*' (Shrestha et al., 2018), '*Flowering plants of Province No. 1 (East Nepal)* (Rajbhandari et al., 2020), '*Flora of Kailash Sacred Landscape Nepal: An annotated checklist volume 1*' (Chaudhary, 2021).

Besides these studies of revisions of several genera and families of Nepalese flowering plants have been undertaken by Nepalese, Indian and other scientists. Some of them are *Rhododendron* (Rajbhandari & Watson, 2005), *Koenigia* (Hedberg, 1997), *Aconogonon* (Hong, 1992), *Anemone* (Chaudhary, 1988), *Cyananthus* (Shrestha, 1997), *Poa* (Rajbhandari, 1991), *Pegaeophyton* (Al-Shehbaz, 2000a), *Taphrospermum* (Al-Shehbaz, 2000b), *Lignariella* (Al-Shehbaz et al., 2000; Arai et al., 2002), *Kobresia* (Rajbhandari, and Ohba, 1991), Orchids (Raskoti, 2009; White & Sharma, 2000), *Cynoglossum* (Koenig et al., 2015), *Chenopodiaceae* (Sukhorukov & Kushunina, 2014), *Potentilla* (Sojak, 1966, 1987, 1988a, 1988b, 1991, 1994), *Astragalus* (Chaudhary & Siwakoti, 2015), and so on.

Activities on the publication of flora of Nepal

After 1966 it became evident that taxonomic work on Nepal plants by Japanese botanists based upon material in Tokyo collected on their expeditions in the previous few years, and by European botanists upon material in London was likely to result in much unnecessarily duplicated effort and possibly in competing names (Stearn, 1978). It became obvious that science would be best served by co-operation rather than by competition. William Stearn of British Museum met (at the Seattle International Botanical Congress, U. S. A. in 1969) Professor Hiroshi Hara of the University of Tokyo and initiated the Anglo-Japanese enumeration of the flora of Nepal. Thus, a very important publication of the checklist of the flowering plants of Nepal was published, '*An enumeration of the flowering plants of Nepal Vol. 1-3*' (Hara et al, 1978, Hara and Williams, 1979, Hara et al., 1982).

Nepal has no '*Flora of Nepal*', yet. In fact, '*Nepal is one of the last countries in the northern hemisphere yet to produce a completed Flora*'

(Watson, 2018). At present, RBGE (UK), Society Himalayan Botany (Japan) and Nepal Academy of Science and Technology (NAST) are the international collaborators for preparing flora of Nepal. RBGE (UK) is the international editorial centre for flora of Nepal. Up to now only one volume of flora of Nepal, '*Flora of Nepal Volume 3*', had been published in 2011 by the Royal Botanic Garden Edinburgh (Watson et al., 2011). This book includes the descriptions of 21 families of Nepalese flowering plants. The editors of this book are Shinobu Akiyama, Hiroshi Ikeda, Mark F. Watson, Colin A. Pendry, Keshab R. Rajbhandari and Krishna K. Shrestha. As planned in the editorial meeting held in NAST in 2019 the remaining nine volumes of Flora of Nepal are to be completed by 2030. Works on Volume 4 (in Japan), Volume 7 (in U. K.), and Volume 10 (in Nepal) are being carried out. Recently, DPR has established Flora of Nepal office and published flora of Nepal online on its website.

Floral diversity of Nepal

The estimated number of flowering plants in Nepal is 6500 species. Recent publication on flora of Nepal, '*An enumeration of the flowering plants of Nepal*' (Hara & Williams, 1979; Hara et al., 1978, 1982), has given a list of 5036 species under 1494 genera and 212 families. After the publication of this more than 150 species of the flowering plants have been added to the flora of Nepal (Rajbhandari, 2002-2003, 2003; Rajbhandari and Joshi, 2001; Rajbhandari et al., 2003). By checking the available references on the flowering plants of Nepal it is found that there are 5309 species under 1515 genera and 193 families (revised from Rajbhandari et al., 2017).

Ten big families of the flowering plants of Nepal are given in Table 1. All these families have more than 100 species each.

The most diversified family is Orchidaceae followed by Asteraceae and Poaceae. Poaceae, Asteraceae and Orchidaceae, which have more than 300 species each followed by Fabaceae and Cyperaceae with more than 200 species each (Table 1).

Table 1: Big families of the flowering plants of Nepal having 100 or more than 100 species

Family	Number of genera	Number of species
Poaceae	126	406
Asteraceae	118	394
Orchidaceae	96	381
Fabaceae	93	281
Cyperaceae	21	210
Lamiaceae	59	175
Ranunculaceae	19	159
Rosaceae	26	155
Rubiaceae	45	115
Apiaceae	42	114
Gentianaceae	15	113
Saxifragaceae	8	113
Brassicaceae	33	101
Primulaceae	8	100

Big genera of the flowering plants of Nepal are shown in Table 2. All these genera contain 30 or more than 30 species each. The most diversified genera in the flora of Nepal are *Carex*, *Saxifraga*, *Pedicularis*, and *Primula* having more than 60 species each followed by *Gentiana*, *Impatiens*, *Juncus*, and *Corydalis* having more than 40 species each.

Table 2: Big genera of the flowering plants of Nepal having 10 or more than 10 species

Genus	Family	Number of species
Carex	Cyperaceae	110
Saxifraga	Saxifragaceae	87
Pedicularis	Orobanchaceae	66
Primula	Primulaceae	61
Gentiana	Gentianaceae	49
Impatiens	Balsaminaceae	48
Juncus	Juncaceae	45
Corydalis	Papaveraceae	44
Bulbophyllum	Orchidaceae	35
Cyperus	Cyperaceae	35
Ficus	Moraceae	34
Potentilla	Rosaceae	34
Rubus	Rosaceae	32

Saussurea	Asteraceae	32
Rhododendron	Ericaceae	31
Salix	Salicaceae	31

Flora of Nepal: Tomorrow

Taxonomical studies of Nepalese flowering plants are still continuing and newly discovered species and new records for flora of Nepal are being added. Revisions of the Nepalese flowering plants especially of the alpine region are largely missing. Herbarium in Nepal should be strengthened by addition of not only newly described species and new additions to Nepal but of the species already reported from Nepal but not represented in the Herbarium. Digitization of more herbarium specimens preserved in KATH and TUCH should be continued. Manpower (experts) for flora of Nepal should be trained. More M. Sc. and Ph. D. scholars from T. U. (Nepal) and other international universities of the U. K., Japan, China, and other universities should be invited to work on the revisions of Nepalese plants. Flora of Nepal (FON) office in DPR should be strengthened, and floral data base should be established in Nepal (in FON office, DPR). The young and enthusiastic botanists (one from DPR and one from CDB, TU) should join as editors of flora of Nepal. The present organization for publishing flora of Nepal, since it has already experienced 20 years (from its first international editorial meeting in 2002) should be revisited. The remaining nine volumes of flora of Nepal should be completed in eight years by 2030 as discussed in the eight international editorial meeting held in NAST in Nepal in 2019. The need to publish the flora of Nepal at the earliest was also felt by many participants and speakers in the international conference on biodiversity and bioprospecting organized by DPR and held in Kathmandu from June 22 to 24, 2022.

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Securing Economic Benefits of Nepal through Product Development from Locally Available Medicinal Plants

Rajendra Gyawali [1]*, Prem Narayan Paudel [2], Ajaya Acharya [1], Anjila Shrestha [1], Namuna Adhikari [1], Sujata Mahat [1], Ujwola Suwal [1] and Aarogya Gyawali [3]

[1] Department of Pharmacy, Herbal Research Laboratory, Kathmandu University, Dhulikhel, Nepal

[2] Department of Chemical Science and Engineering, Kathmandu University, Dhulikhel, Nepal

[3] School of Computing Sciences and Computer Engineering, The University of Southern Mississippi, Hattiesburg, MS, USA

***Email: ragyawali@gmail.com, gyawali@ku.edu.np**

Abstract

Many traditional Nepalese herbs have been shown to have medicinal, cosmetic, cultural, and nutritional values among different ethnic communities. The extracts, oils, resins, ash or plant parts are used to prepare the medicines, supplements and cosmetics due to their properties for various ailments of the skin, hair, stomach, liver and dental care. The prospect of herbal product development in Nepal in this era is very challenging due to limited technology, policy issues, research funds, experts and market values. But current initiation can generate new opportunities for future directions of international standard product development within the country. The private sectors, particularly the Ayurvedic industries of Nepal, are heavily involved in developing value-added products that claim to be highly effective for daily usage and avoid side effects. Out of the highly traded 300 medicinal plants of Nepal, approximately 85% plants are exported in crude form to more than 50 countries worldwide. Bulk amounts of medicinal plants are mostly exported to India. It is agreed that, improving traditional medicine, standardizing raw materials quality, isolation of active molecules, or new innovations opens the doors to improved financial avenues. Pharmaceutical, agribusiness, cosmetics, personal care, fragrance, botanicals, food and beverage industries are currently doing bio-prospecting of Nepalese medicinal plants. However, there is a significant gap in terms of patenting and sharing the knowledge gained from the specific domain. Most of the academic institutions in Nepal are working on medicinal plant-based research in different perspectives. Only a few are focusing on herbal product development, commercialization, and revenue generation through the finished products. National level trainings, industry-academia linkage, development of Nepalese herbal pharmacopoeia, medicinal plants export policy, biotechnology, research and development (R&D) facility, formulation development, and product standardization are few key components that should be prioritized for promotion of herbal products within the country. To accomplish this target, researchers, scientists, academicians, industry professionals, and students should collectively work on a common platform from the conceptualization of value-addition ideas to detailed revenue collection from both domestic and international markets. Local medicinal plants can be developed into various dosage forms such as tablets, capsules, ointments, creams, gels, microspheres, transdermal patches, and so on using ethno-botany knowledge from Nepal's indigenous cultures. The phytochemical and common pharmacological strategies such as antimicrobial, antioxidant, wound healing, analgesic, antidiabetic, anticancer, anti-anxiety, anti-inflammatory etc. of plants might lead to the early discovery and commercialization of local herbs.

Keywords: Bioprospecting, Ethnobotany, Herbal formulation, Plant biology, Technology

Introduction

Nepal is a treasure trove of medicinal herbs and a hotspot of biodiversity based traditional knowledge. More than thirteen thousand species of plants are found in Nepal, while 312 species are endemic (Tiwari et al., 2019). A major volume of medicinal plants comes from wild collection. The extracts, oils, resins, ash or plant parts are used to prepare medicine, supplement and cosmetic formulations for various ailments of the skin, hair, stomach, liver and dental diseases. Now with increasing marketing trend, many of the traditionally used products are gradually entering into the high value products. The new product development process from these herbs needs entrepreneurs' characteristics, business analysis, technical feasibility, government policy, and product R&D. Experienced and renowned teams from around the world should be recruited to establish the production sector at the very beginning. There is also an alternative method to prepare the products using locally available knowledge and the application of Western research methodologies to the evidence-based evaluation of herbs.

Conceptualization of ideas beginning from detailed market survey, financial projections, plant layout, operational convenience, validation and regulatory requirements are other important points for product development. Industry-academia collaboration initiatives in the country are gradually improving. Inclusion of traditional practitioners in the national health care system protects their knowledge of a variety of products. The World Health Organization (WHO) also recommends collaborative efforts between researchers and traditional healers (Gureje et al., 2020).

Universities are also introducing the traditional knowledge-based curriculum to promote the empirical knowledge of herbal-based medicines.

Nepali herbs have immense potential to secure export as Himalayan herbs. Government is also assisting botanical researchers through several channels but did not find much input on phytochemical based drug discovery. The country can consume this potential for national revenue with the development of quality standardization, labelling and providing detail analysis report to buyers. As the scope of natural products has been dramatically increased, particularly after COVID-19, more and more people in developed countries have become attracted to herbal medicines and cosmetics prepared from naturally found in herbs (Gyawali et al., 2022). The rising demand for herbal products is further boosting the growth of the herbal industry. In this circumstance, the identification of a suitable entrepreneur, suitable enterprise, and appropriate assistance are very important for Nepalese herbal product commercialization. But the prospect of new herbal product development in Nepal in this era is very challenging. However, the current initiation can generate new opportunities for future directions of international standard product development within the country. The reasons could be the high cost of collection and processing, the complex nature of plants, the lack of fingerprints, and the convenience of standardization protocol of local formulations. Poly-herbal multi-component formulations are more complex to standardize.

The private sector, particularly the Ayurvedic industries of Nepal, is heavily involved in developing value-added products that claim to be highly effective for daily usage and avoid side effects. In Nepal, government-owned manufacturing facilities such as Singha Durbar Baidhya Khana, Herbs Production and Processing Co. Ltd. (HPPCL), and a number of private manufacturers are present at various levels, consuming medicinal plants and facilitating the production and trade of related products. Department of plant resources (DPR) under the Ministry of Forests and Environment is playing key role in product development from local herbs. However, this effort is not enough to accelerate successful innovative products. Thus, the government should emphasize the standardized mass production of herbal materials and quality certification of these materials for industrial use. The new product development process in general includes the conceptualization, concept feasibility, product development, testing, and launch of the product. Researchers have to adopt the participation of traditional practitioners in the entire process of drug development to gain trust from the market. Pharmaceutical, agribusiness, cosmetics, personal care, fragrance, botanicals, food and beverage industries are currently doing bioprospecting of Nepalese medicinal plants. However, there is a significant gap in terms of patenting and sharing the knowledge gained from the specific domain.

Most of the academic institutions in Nepal are also working on medicinal plant-based research in different perspectives. Only a few institutions in the country are focusing on herbal product development, commercialization, and revenue generation through the finished products within the country. National level training, industry-academia collaboration, the development of Nepalese herbal pharmacopoeias, medicinal plant export policy, biotechnology and R&D, innovation and formulation development, and traditional product standardization are a few key components that should be prioritized for the production and commercialization of herbal products within the country. To accomplish this target, researchers, scientists, academicians, industry professionals, and students should collectively work on a common platform from the conceptualization of value-addition ideas to detailed revenue collection from both domestic and international markets (Gyawali et al., 2020a, 2020b; Poudel et al., 2022; Gyawali & Poudel, 2022).

Biological activities of several medicinal plants should be carried out before production. Different dosage forms, such as tablets, capsules, ointment, creams, gels, microspheres, transdermal patches, etc., should be developed by taking knowledge of ethnobotany in the indigenous cultures of Nepal. The general consideration of the WHO on herbal drugs is that each country or area should adopt a regulatory system that can help ensure that herbal medicines have acceptable quality, safety, and efficacy. The treatment of modern disease has also become increasingly difficult, and sometimes patients have to be left helplessness. It is estimated that a single herbal preparation may contain more than 100 active compounds, and their bioactivities and/or drug-like properties are difficult to be accurately identified and evaluated by conventional pharmacological methods or drug screening approaches.

The major pharmacological strategies, such as phytochemical and antimicrobial screenings, antioxidant, wound healing, analgesic, antidiabetic, anticancer, anti-anxiety, anti-inflammatory etc. properties of plants might lead to the high economic benefit from plants (Gyawali et al., 2014, 2015, 2016, 2020c; Pant et al., 2019). Different forms of herbal products (Figure 1) can be obtained from plants.

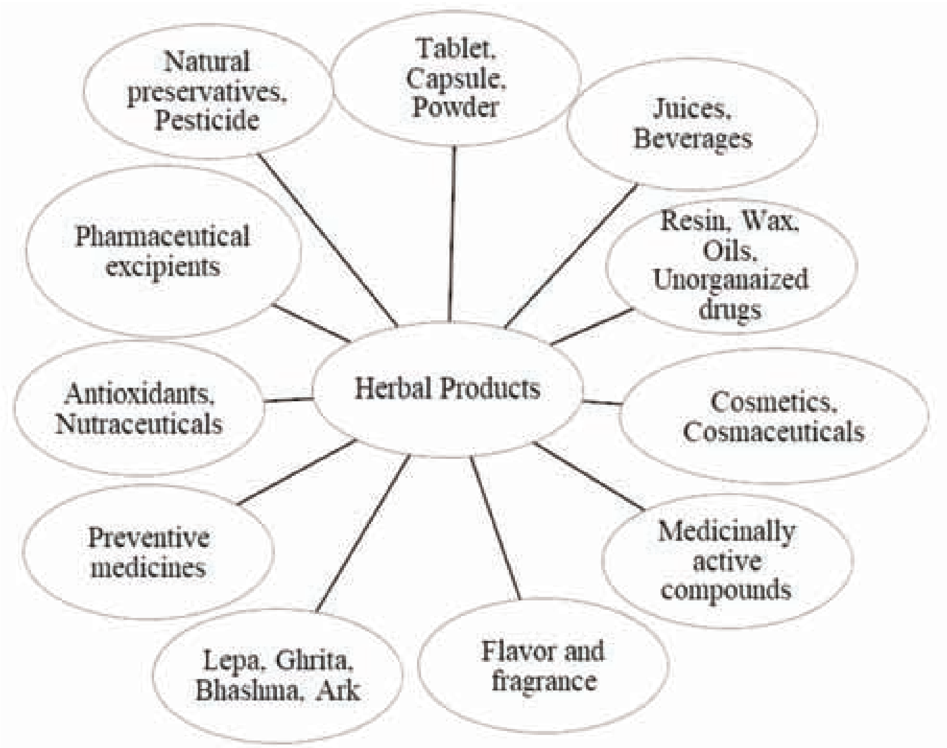

Figure 1: Different forms of herbal products

There are some limitations for new drug discovery from Nepalese medicinal plants. However, the ethnobotanical approach to pharmaceutical drug discovery from Nepalese medicinal plants may enhance the probability of identifying new bioactive molecules from the Himalayas. This requires interdisciplinary or multidisciplinary studies. However, the available research to make better use of these products is limited, and low success rate and huge capital investment in single drug development is another challenge. On the other hand, there is huge potential for Nepalese medicinal plants to go for commercial drug preparations rather than discovery. Phytochemicals from these plants have already provided pharmacologically active compounds targeted against a multitude of conditions of diseases (Table 1). Therefore, these materials remain a solid niche as a source from which several therapies can be derived. There are numerous other reports which also provide evidence of drugs as traditional medicines (Song et al., 2015). Despite this, a vast number of unidentified natural products have the potential to serve as the promising sources for medical applications.

Table 1: Drug molecules possible from Nepalese medicinal plants

Drug Molecule	**Nepalese Medicinal Plant**	**Biological property/Clinical use**	**References**
Artemisinin	*Artemisia annua*	Antimalarial	Maude et al., 2010
Atropine	*Atropa belladonna*	Anticholinergic	McLendon & Preuss, 2022
Berberine	*Berberis aristata*	Bacillary dysentery	Imenshahidi & Hosseinzadeh, 2019
Caffeine	*Camellia sinensis*	CNS stimulant	Trexler & Smith-Ryan, 2015
Ephedrine	*Ephedra sinica*	Branchodialator, antihistamine	Stohs et al., 2020
Podophyllotoxin	*Podophyllum peltatum*	Antitumor agent	Shah et al., 2021
Hyoscyamine	*Hyoscyamus niger*	Anticholinergic	Kohnen-Johannsen & Kayser, 2019
Menthol	*Mentha piperata*	Rubefacient	Kamatou et al., 2013
Methyl salicylate	*Gaultheria fragrantissima*	Rubefacient	Boyd et al., 2019
Reserpine	*Rauvolfia serpentina*	Antihypertensive, tranquillizer	Griebenow et al., 1997

Salicin	*Salix alba*	Analgesic, antipyretic	Shara & Stohs, 2015
Scopolamine	*Datura stramonium*	Sedative	McLendon & Preuss, 2022
Sennosides	*Cassia fistula*	Laxative	Le et al., 2021
Taxol	*Taxus wallichiana*	Antitumor agent	Runowicz et al., 1993
Vinblastine, Vincristine	*Catharanthus roseus*	Antitumor, antileukemic agent	Barnett et al., 2022
Curcumin	*Curcuma longa*	Anti-inflammatory, anticancer	Giordano & Tommonaro, 2019

Herbal Products Manufacturing Practices in Nepal

In Nepal, Ayurvedic medicine production started during the regime of Pratap Malla (Khanal, 2018). Later on, the large scale manufacturing unit started with the name of Singha Durbar Vaidhya Khana. Currently, more than 70 Ayurveda industries are in operation, and more than 100 herbal companies are manufacturing a variety of herbal products (Department of Industry, Nepal, 2021). Several indigenous manufacturers in village area prepare products for local consumption and the knowledge is transferred from generation to generation. In the production houses, the herbs are analyzed for their morphological, physiochemical, phytochemical, and pharmacological properties. Only upon passing the above inspections for quality, the herbs are accepted into production processing. Once the herbs are inspected, they go through a meticulous cleaning process until dirt and foreign particles are removed. Some of the companies' manufacturing procedures are in accordance with good manufacturing practices (GMP). Some companies are also performing chromatographic and spectroscopic applications for the qualitative and semi-quantitative information about the main active constituents present in the crude drug as chemical markers in the thin layer chromatography (TLC) fingerprint evaluation of herbals. However, in some companies, products are manufactured by general factories, small workshops, or traditional medical practitioners, which may not meet the GMP standard.

Major pharmaceutical companies of Nepal are currently unable to conduct extensive research on various species plants for their potential bioactive compounds and medicinal properties. As Nepalese companies produce small volumes, the unit cost of production is also higher and local products are unable to compete with the cheap imports of WHO GMP standard products. It is urgent to upgrade these companies in accordance with GMP requirements within some timeframe. From the manufacturer's point of view, the problem is to have a raw material in bulk quantity and quality from a single origin. It leads to breakage in the continuous production cycle of herbal products. Raw materials obtained from different geographical origins with varying climates cannot be treated as the same as there is chance of difference in herbal potency. Different regulatory procedures should be applied to inspect the plant materials, processed materials and various types of products. Depending on human resources and laboratory facilities, more comprehensive regulatory procedures are being implemented in order to achieve a higher level of safety, quality, and efficacy. Government authorities have also initiated developing prototypes of herbal products and coordinating with the stakeholders and investment parties for technology transfer. The companies are utilizing the herbs, particularly in the following sectors:

Ayurvedic companies

The Ayurvedic companies of Nepal are utilizing a small fraction of the locally available medicinal plants to process them into medicine. Companies are manufacturing herbal tablets, capsules, syrups, oils, and classical preparations. During the product quality analysis, a high degree of testing and evaluation is done, as well as the processing, finishing, and packing are maintained by Nepalese companies. Their formulations are based on classical preparations, as well as their own formulated proprietary products. Some of the large manufacturers of herbal drugs or value-added products in Nepal have now started to go in for contract farming of herbs, guaranteeing the growers to buy their crops. But regular supply of quality medicinal plants to herbal industry is an important requisite for enabling their commercial use in the production which maintains the economic sustainability.

Traditional medical system

Traditional medical systems such as Ayurveda, Traditional Chinese Medicine, Unani, Siddha, Tibetan, etc., utilize plant resources to produce traditional medicines. Traditional Chinese medicine has been reported to use up to 6000 medicinal plants; however, the most commonly used plants range from 500 to 600 (Pyakurel et al., 2019; Tiwari et al., 2019). In Ayurveda, 125 to1400 plant species are used. Similarly, in Tibetan medicine, there are 1106 to 3600 species (Gewali, 2008). In Unani, 342 species and in Siddha, 328 species are likely to be used. They are preparing their own traditional system of dosage forms, but mostly in crude form. Traditional medicines are mostly formulated and prepared locally because they are hardly regulated in Nepal.

Herbal extraction companies

Medicinal plants are also consumed in the locally available herbal processing companies. They have installed plants in different parts of Nepal; some are designed as per good manufacturing practices and relevant requirements of pharmacopoeias. An extraction and a distillation plant for Lemongrass oils, Patchouli oil, Wintergreen oil, Calamus oil, Chamomile oil, Turpentine resin, and dried powder of *Curcuma* and *Moringa*, are preparing the products and exporting them. But the same companies have not focused much on the formulation development of new products by using the same raw materials. Reproducibility of extracts is also assured by standardizing each batch with a defined amount of the active ingredients.

Consumer product sector

Demands of the herbal market come under the category of natural & health food shops, such as herbal teas, medicated drinks, tonics, etc. Besides, the cosmetics and perfumery industries also use essential oils such as Jatamansi and Sugandhawal oils for this purpose. Consumer product manufacturers such as, toothpaste company, food company, soap company and cosmetic company are utilizing the local medicinal herbs for developing personal care products, flavoring agents, food additives and natural dyes (Gyawali et al., 2020a; Gyawali & Aryal, 2022).

Installation of Recent Technology in Nepal

The current upsurge in herbal medicines involves both modern tools and technologies. The new biotechnological tools, good agriculture practice, successful chromatography, quantification methods, major constituents, reproducibility, clinical advancement, pharmacopoeial guidelines, are the key to develop standard herbal products. Suitable extraction procedure, and use of qualified standard references in the facility with WHO good manufacturing facility to produce high quality products remain an important objective to ensure high quality products and their therapeutic performances. The ‘omics technologies are very much useful but it costs high for the start-up companies. Many low- and middle-income countries are unlikely to be able to provide such facilities. However, public-private partnerships, government scientific training, lease of facilities, and optimized use of existing infrastructure can be a good model to secure long-lasting advantage without huge investment in the technology. The use of recent technology in herbal product development has great potential to create novel chemical constructions and to be used as an innovative product in food and medicine.

Special attention has to be paid to innovate or import the different machineries for the preparation and processing of the herbs to maximize the quality and export of finished goods. Though herbal products have become increasingly popular throughout the world, one of the impediments to its acceptance is the lack of a standard quality control profile. Hence, for herbal drugs and products, standardization should encompass, in this respect, internationally recognized guidelines for their quality assessment and quality control. Safety and quality surveillance must coincide with herbal preparations as medicinal plants can be used directly for therapeutic agents and they are a better source for pharmacological drug research and development (Liu et al., 2015; Paudel et al., 2022). In contrast, modern pharmaceutical technology is equally playing a precise role in quality (Li & Weng, 2017), and appropriate technology is being established for drug discovery, isolation, and purification of bioactive compounds (Song et al., 2016). Herbal medicine drug discovery remains difficult in Nepal, but mass spectrometry strategies, pharmacophore-guided knockout/knock in chromatography, and precise chemical screening technology appear to be useful for advanced identification and discovery of natural products chemistry (Song et al., 2016).

Bioactivity-directed fractionation and isolation technology and high throughput screening techniques are most common to discover the combinatorial active chemistry of plants from their extracts (Younes et al., 2007; Itokawa et al., 2008), which should be adopted without further delay. Medicinal chemistry discovery, pharmacognosy, phytochemistry, and drug development research into natural products has begun in recent years, but technology used is still limited and expensive (Li & Vederas, 2009; Gyawali et al., 2020b), which should be concerning given that medicinal plants are a leading source for the development of new chemotherapeutic agents (McChesney et al., 2007). However, Ayurvedic medicines in classical form or herbal products in suitable form are being prepared in Nepal by using modern pharmaceutical technology without harming the theme of Ayurveda. Such products are obtaining better therapeutic efficacy through improved bioavailability. Some techniques, such as liposome formation, micronization, nanotechnology, amorphous solid products, etc., can enhance the absorption of the active compounds.

Collaboration for Herbal Product Development in Nepal

Over the previous few decades, the importance of medicinal plants has been realized for enhancing the expansion of the national economy. A wide range of stakeholders, including community-based organizations, NGOs, government agencies, local traders, exporters, traditional healers, and professional practitioners, as well as small and large-scale manufacturers should collaborate on this. The government should include production, marketing, and business related programs, seminars, and techno-fairs, annually to develop desired success from the herbal sector in Nepal. In rural areas, the Government can provide an environment for small enterprises, especially by training, business strengthening as well as infrastructure development regarding the promotion of products.

The research on product development from Nepalese medicinal plants has been carried out honorably by both national and international researchers mostly for their academic degree projects. Their findings clearly demonstrate the value of these plants, which supports the traditional claims for treating various diseases. The local government should know this potentiality and provide funds to support entrepreneurship, R&D, trade related products, to enhance the abilities of promotion networking. The "National Medicinal Plants Board" is equally important to formulate which can play an important role in overseeing activities related to the R&D and promotion of herbal products. Additionally, the government and other funding agencies are supporting small businesses in rural areas to create a favorable economic environment. Many organizations such as the Department of Plant Resources, Singha Durbar Baidhya Khana, HPPCL, International Union for Conservation of Nature (IUCN) Nepal, National Ayurveda Research and Training Center (NARTC), etc. are involved in promoting sustainable management of medicinal plants. But there are still gaps in inter-institutional collaboration on product development and technology transfer.

Additionally, the institutions in Nepal are restricted in their ability to conduct research into medicinal plants due to a lack of funding and advanced equipment. The government of Nepal must establish a supportive environment for innovation, research, and development aimed at enhancing traditional medicines and adding value to offer new opportunities for funding. A clear policy to protect traditional knowledge and local product development would benefit not only economic activity but also local knowledge protection. The following sectors are prioritized to promote herbal-based product development and economic benefits.

Education and training sector collaboration

Very small fraction of research fund is allocated for herbal research so that many academics, including Kathmandu University, Tribhuvan University, Pokhara University, and research institutes in Nepal like the Nepal Academy of Science and Technology (NAST), Department of Plant Resources, NARTC and Agriculture and Forest University, are also conducting few scientific researches on plant product development. The provision of providing funding as well as training like national level training, conducting symposiums, national and international conferences regarding various medicinal plants, traditional medicinal systems, etc. by skilled and trained professionals is very useful for product development.

Collaboration for value-addition

Institutional collaboration, cooperation, goal identification, and plan formation are of the utmost urgency for the promotion of local herbs in Nepal. Developing industry-academia linkages is very important for R&D. The financial subsidization policy may encourage collaboration for the value-addition of specific products from both local companies and researchers, scientists, academicians, industry experts, and students. The policy can outline approaches for how Nepal, in collaboration with research and academic institutions, can be actively involved in the R&D of medicinal plants. Screening program led to the discovery of many novel agents of active pharmaceutical ingredients (API) can help in the development of high value products. The value addition stage involves numerous activities including drying, extraction, packaging, and storage which enhance the price and shelf life, and assist the marketing of high value (pharmaceutical) products (Figure 2).

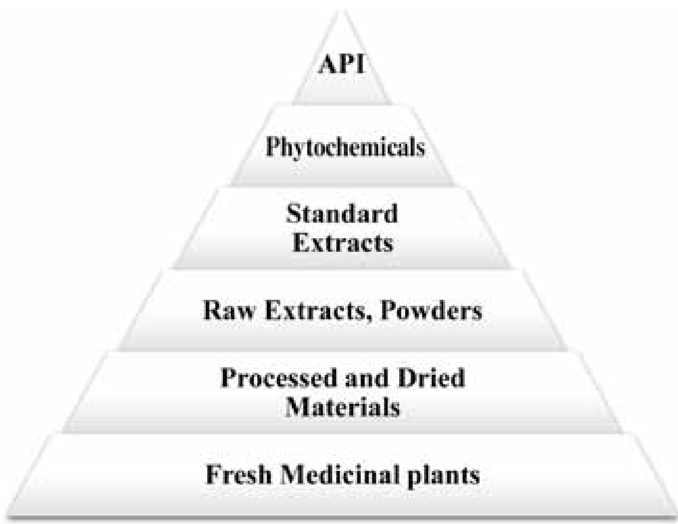

Figure 2: Value-addition of medicinal plants

Collaboration for entrepreneurship

Entrepreneurship is considered one of the sources of economic growth. Entrepreneurial training for herbal professionals provides the ability to recognize commercial opportunities of local herbs and initiate business venture. Financial resources, such as low-interest government loans, credit counseling, and technical assistance for entrepreneurs, should be made a policy to encourage local entrepreneurship and may inspire people to seek out new opportunities. Herbal based rural entrepreneurship is recognized as important activity in the local economy in Nepal. Government has made plan to promote the herbal based enterprises, but this effort should be increased to promote the herbal business.

Collaboration for promotion of traditional knowledge

Traditional medicinal plant knowledge should be protected because it is valuable information that is passed down from generation to generation. Therefore, the government should collaborate with local communities so that they are capable of developing their own products from various medicinal plants. Inventions based on genetic resources associated with traditional knowledge should be patented or protected by inventors of Nepal. Principles of the Convention for Biological Diversity (CBD) to genetic resources and equitable sharing of benefits from their utilization have not been translated into reality. The products from practitioners are not well legislated for just as the indigenous knowledge and not legally taken care of. This has also to an extent affected the development of herbal products in Nepal. Government can collaborate with community, and harmonize on legislative framework for marketing of traditional herbal products, training on crude drug preparation, encourage with simplified registration procedure, and facilitate the analysis on efficacy and safety for market assurance.

Cultivation collaboration

In order to meet the escalating demand for medicinal plants, herbal agriculture or farming of these plants is imperative and the development of agro-technology related to herbal production has become an area of intensive research (Pan et al., 2013). Many people are interested in cultivating medicinal plants either on private land or in community forest but they are not getting suitable propagation methods, full technical assistance, a buy-back guarantee, and value-addition techniques. The commercial cultivation of valuable medicinal plants is promoted by the Department of Plant Resources with herbal nurseries at several locations in the country for the production of medicinal plants. Large scale cultivation of a few medicinal plants is also practiced by the HPPCL as well as Dabur Nepal in collaboration with community user groups and local stakeholders. They also provide farmers with training in the production and handling of herbal materials. Lemongrass, Palmarosa, Citronella, Mentha, Chamomile, Basil, Zingiber, Turmeric, Eucalyptus, etc. are common plants cultivated for industrial purposes.

Trained Human Resources for Herbal Sector Conclusion

Human capital operating machines in industrial settings, entering information in an office setting, selling products, and providing services are critical for the development of herbal products in Nepal. However, there is some lack of natural product drug specialists to meet international specifications, which has held us back in this sector. This is the time to focus more on developing experts by providing them with training to prepare drug molecules such as taxol, curcumin, podophyllotoxin, vincristine, etc. from locally available plants. Trained specialists can develop methods to add value to the product through extraction, purification, appropriate packaging, along with new product development using their skills and innovative ideas. Each level of human resources brings something useful to product development. Trained human capital like ethno-botanists, ethno-pharmacologists, specialists in pharmacognosy, phytochemistry, product development, molecular biology, biotechnology, plant conservation, agro-technologies, biodiversity, systematics, herbarium, environmental technologies, research and development, etc., add value and help in the socio-economic development of herbal products with their specialized knowledge and experience. Trained people who landed in Nepal are there as a qualified human resource and they have the potential to bring new innovative ideas to develop herbal products in entrepreneurship. They can also provide technical guidance and consult the farmers by establishing testing facilities, agri-clinics, and local collection and extraction centers. Trained human capital with knowledge of natural products and their medicinal uses can become the backbone for the development of herbal product development (Aminullah et al., 2017; Rathore & Mathur, 2018). Nepal should invite a diaspora of Nepali experts in the national institutions to carry out more research in the herbal formulation that will economically benefit the country.

Market Status of Herbal Products Conclusion

The herbal products industry is one of the fastest growing industries in the world. Herbs and medicinal plants are the products which Nepal has comparative advantage thanks to its geography. Nepal is a significant provider of raw materials to the Chinese and Indian medicinal plant industries. The trade involves many species, and the annual shipment is thought to be between 7000 and 27,000 tons at a cost of between USD 11 and USD 48 million (Pyakurel et al., 2019; Caporale et al., 2020). The government also has plans to work with the private sector to establish industries based on herbal and forest materials. The domestic market for herbal products is also growing every year. Building on Trade Policy, 2015, the National Trade Integration Strategy, 2016 has identified medicinal and aromatic plants together with other products and services as having high export potential. Development of these products, along with other potential products, has been prioritized. Therefore, it is highly essential to develop a strategy to promote the herbal products in local market and discourage the import of products from neighboring countries.

Nepal is also seeking to develop new products and expand its market coverage. This is because of the preferred health benefits of herbal products, as well as such products' being more cost-effective than pharmaceutical drugs. To improve the economic sector through herbal resources, the development of the business sector is necessary. Currently, the fashion of returning to nature has become a trending lifestyle. Herbal product marketers educate consumers on both the holistic and physical health benefits of herbal products. In the current era of the COVID-19 pandemic, every country has another opportunity to promote herbal products. One of the major reasons for the increased interest in herbal products is the perception by consumers that conventional pharmaceutical products are expensive and of high risk in contrast to natural product based preparations that are obtained from root of traditional knowledge. So, the business actors are trying to work harder to develop their local product-based businesses. Various steps are being taken to expand market segments, such as developing a research strategy, identifying new herbal products, and removing market barriers.

The view of modern consumers is different due to the wide availability of online information, databases, and networks about advanced types of products. These kinds of developments have created huge pressure on marketers to switch from local product marketing approaches towards more fancy types of finishing. Such approaches can enable them to increase sales with their target groups. But also, it is necessary to identify the constantly changing and evolving customer needs, respond quickly to competitive movements and predict market trends early and accurately (Ganesan, 2008; Egbuna et al., 2019).

Conclusion

For a long time, herbs and spices have been used for flavor as well as for medicinal purposes in Nepal. Ayurveda and other traditional systems of medicine, and associated ethnomedical knowledge are key components for sustainable bioprospecting and value-addition processes. Herbal products associated with health benefits need to be incorporated into suitable dosage forms such as tablets, capsules, powders, essential oils, extracts etc. The quality control of herbal products is very important to monitor the bioactive constituents and potency between batch to batch and also to avoid possible toxic contaminants. As evident from the above discussion, natural product compounds discovered from medicinal plants have provided numerous clinically useful drugs. *Artemisia annua, Atropa belladonna, Berberis aristata, Camellia sinensis, Ephedra sinica, Podophyllum peltatum, Hyoscyamus niger, Mentha piperata, Gaultheria* *fragrantissima, Rauvolfia serpentina, Salix alba, Datura stramonium, Cassia fistula, Taxus wallichiana, Catharanthus roseus,* and *Curcuma longa* can be immediately utilized to isolate the active pharmaceutical ingredients and commercialized. Proper utilization of these resources and tools in bio-prospecting will certainly help in discovering novel drugs. This is the right time to devote to new innovations for herbal-based products to support the economy of the Government of Nepal.

Author Contributions

Rajendra Gyawali has conceptualized the paper, worked to draft the manuscript, reviewed and submitted the paper. Prem Narayan Paudel and Aarogya Gyawali reviewed thoroughly and edited the paper. Ajaya Acharya, Anjila Shrestha, Namuna Adhikari, Sujata Mahat, Ujwola Suwal wrote the small units of the paper.

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An Overview of Plant Diversity and Conservation in Nepal

Sangeeta Rajbhandary
Central Department of Botany
Tribhuvan University, Kathmandu, Nepal
Email: s.rajbhandary3@gmail.com; imogine3@gmail.com

Abstract

The diversity of life forms, ranging from microorganisms to plants and animals, diversity of genetic materials within them, and the diverse ecosystems and habitats where they live, is called biological diversity or biodiversity. Biodiversity is the variation of life forms within a given ecosystem, biome, or the entire earth (biosphere). It is quite evident that the number of described or catalogued species and estimated number of species or predicted species varies widely. The Himalaya is one of the most dramatic examples of plate-tectonic forces in the world. Sandwiched between Tibetan plateau and Gangetic plains, Nepal Himalaya offers many niche climates with its very high altitudinal variation within short geographical distance. Although Nepal covers less than 0.1% of the earth's land area, it is disproportionately a species rich country with number of endemic species. Nepal with this rich plant diversity is the major resource that has been a part of subsistence living of majority of the Nepalese people. They also have greater role in maintaining and/or improving the environmental quality against its degradation. At the same there are several factors causing loss of plant diversity or threatening extinction also influence long-term evolutionary processes. Hence there is an urgent need to conserve the valuable but fast disappearing species for the sake of establishing ecological balance and saving the natural resources which have potential for discovery of novel molecules and new sources of active compounds for drug developments.

Keywords: Endemic, Exploration, Resources, Threats, Uses

Introduction

The Himalaya is one of the most dramatic examples of plate-tectonic forces in the world. The Himalaya is an immense series of mountain ranges that extends some 3200 km, from the Pamir Knot on the Afghan border eastwards through Jammu and Kashmir, Himachal Pradesh, Arunachal, Nepal, Sikkim, Chumbi Valley, Bhutan, Arunachal Pradesh and the northern tip of Burma. They encompass a pronounced variation in natural habitat and are one of the world's most prominent mountain ranges and contain rich plant diversity. The high species diversity in the Himalayas is due to species turnover associated with elevational variation in habitat, as well as variation in species composition along the range (Martens & Eck 1995; Price et al., 2003). Within the Himalaya the eastern parts (from east Nepal through to northern Myanmar) are wetter and contain the most biological diversity being at the crossroads of several floristic regions: Indo-Malayan, Indo-Chinese, Sino-Himalayan, and East Asiatic (Takhtajan, 1988). Nepal, almost centrally located in the long sweep of Himalaya, is also known as "the natural showroom of biodiversity" with its geological, ecological and climatic variation that has created a unique wealth of plant life.

Due to the crossroads of several floristic regions, not only the plant diversity is rich but the landscape diversity is equally unique. Within this general framework there are many varieties and complexities of landforms. The altitudinal gradients covering cis and trans Himalayan region covers all shades of climate from tropical to alpine and even to dry desertic in trans Himalayan region. This has resulted in environmental diversity giving Nepal's ecosystem a unique wealth and variety. Nepal's landscape is predominantly composed of hills and mountains, covering about 83% of the total land area. Nepal has the highest mountain in the world, Sagarmatha (Mount Everest) with an elevation of 8,849 m. Ten of the world's 14 peaks over 8,000m found in the Himalayas. 127 peaks over 7,000m and 1,311 others above 6,000m are also found in the Himalayas (Pandey et al., 1995). Snowline in the east is at 5,000m and in the west is at 4,000m. Geo-morphologically, the high Himalaya is a cold desert where coarse debris, rocks and snow dominate (Jha, 1992).

Presence of diverse and complex landforms the diversity of life forms, ranges from microorganisms to plants and animals, diversity of genetic materials within them, and the diverse ecosystems and habitats where they live, is also diverse. In the global scenario Nepal occupies about 0.1 percent of global land area, but harbours 3.2 percent and 1.1 percent of the world's known flora and fauna, respectively (Government of Nepal, 2014). Among the 3.2 percent known flora it includes the flowering and non flowering plant. This has made the country ranks the 31st richest country in the world and 10th in Asia in terms of biodiversity (Ministry of Agricultural Development, 2017).

History of botanical exploration, the initiation of plant diversity in Nepal

The first scientific exploration of the plants of Nepal dates back to the first twenty-five years of the nineteenth century, starting with Francis Buchanan-Hamilton's year in the Kathmandu valley in 1802-3, and culminating in Nathaniel Wallich's own year-long visit in 1820-21. The collections made during this time provide the basis for the description of well over a thousand new plant names and so they are scientifically important and highly regarded as laying the foundation for Himalayan botany. Out of the thousand plant collection *Begonia picta* was the first plant described from Nepal in the book *Exotic Botany* by James Edwards Smith (Smith, 1805).

According to the herbarium specimen the type *of Begonia picta* was collected from 'Simbu', Nepal, (Swayambhu, Kathmandu), on the date 21.07.1802 by Buchanan-Hamilton s.n.. The type is a Holotype and deposited at BM (Natural history Museum, London). Collections of Buchanan-Hamilton were made from Makwanpur (Indian border) to Kathmandu. Few years later Nathalien Wallich (1820-21) visited Nepal and collected hundreds of new and interesting plant specimens from Central Nepal (Terai, Kathmandu, Gosainkund). But before the visit of Wallich Edward Gardner (1818-1819), the second British Resident (1816-1829) collected many plant specimens from 1817, 1818 and 1819 for Wallich and possibly also in 1820 when Wallich himself came to Nepal (Rajbhandari, 2016).

All these collections deposited at Natural History Museum was an assets for David Don who compiled the information and published the book *Prodromus Florae Nepalensis* in 1825. This book contends 806 Nepalese species, where 77% of the species was new. Out of 806 species 595 were new names and 24 new combinations of flowering plants and ferns (Don, 1825). This book is actually the compilation of the plants of Nepal collected by Buchanan-Hamilton and has also included some of plants collected by Wallich from Nepal. Drawings of Nepalese plants collected by Wallich has been published in his two books *Tentamen florae Napalensis illustratae*, *Plantae Asiaticae rariores* 3 volume and a third book on *A Numerical List of dried specimens of Plants in the East India Company & Museum.* Another important publication regarding Nepalese plants is the first edition of Roxburgh's '*Flora Indica*', since many plants from Nepal got their place first time in this book. According to Rajbhandari (2016) about 700 type species were collected in Nepal by Wallich or his collectors he employed, of which more than 40 species bear the epithet *wallichii* or *wallichiana* after him. These early expedition and publication is an initiation and eye opening for Nepali people regarding the Flora of Nepal, otherwise it might have taken long time to understand the importance of flora.

Thereafter, several expeditions took place from 1848-1949. After 1949 till date and several British & Japanese expeditions has been taking place. Similarly, after establishment of Department of Medicinal Plants (later Department of Plant Resources) in 1960 many expeditions have been carried out from 1961 till date. An article by Rajbhandari (2016) has given detail information on 'History of Botanical explorations in Nepal: 1802-2015', and it is worth reading it and there is no point repeating about the exploration here. These botanical explorations then and now have received not only multinational but multi-continental contribution from Asia, Europe, and America.

Most of the specimens collected during the expedition by the British and the Japanese and some other collections till 1972 were deposited in Natural History Museum London, U. K. Firstly, L. H. J. Williams than working in the museum started compiling the Nepalese plant between 1969 and 1974 (Hara et al., 1979). Later "A Joint Project of the British Museum (Natural History) and the University of Tokyo" with the assistance of specialists from Nepal and others specialists from UK and Japan have all made contributions and has compiled the account of plant collecting in Nepal in the form of three volume "An Enumeration of the Flowering Plants of Nepal (Hara et al. 1978, 1982). This is one of the most important books which are highly used by the researchers and students.

Plant Diversity in Nepal

Along with international expeditions, several expeditions from Department of Plant Resources and other Nepalese research work have contributed many publications for non-flowering plant groups as mushrooms, algae, bryophyte, pteridophytes and recently three volumes of fern allies. Likewise, several publications have also been published for the flowering plants of Nepal. This has helped to known the status of plant diversity of Nepal. These publications have helped to get the current estimations of plant diversity species of both flowering and non-flowering plants which indicates that there are 998 species of algae (Rai & Ghimire, 2020), 2,467 species of fungi (Adhikari, 2016), 1078 species of lichens (Baniya et al., 2022), 1,217 species of bryophytes (Pradhan, 2020), 550 species and 33 subspecies of pteridophytes (Fraser-Jenkins et al., 2015), and 23 species 3 varieties gymnosperms (Rajbhandari et al., 2020). Regarding the flowering plants, Rajbhandari et al. (2017) reported 5309 species under 1515 genera and 193 families and recently Shrestha et al. (2022) reported 5,606 species and 214 infra-species under 1541 genera and 200 families.

However, the total number of species in Nepal is expected to increase as groups are revised for the Flora of Nepal. Similarly, extensive fieldwork in unexplored areas for the collection of previously recorded species in Nepal may result into new record or completely new species to science.

Endemic plants of Nepal

Endemic is applied to any species or other taxonomic unit which is distributed in a confined area in particular country or geographical region (Good, 1974; Rajbhandari, 2013). Research and exploration in Nepal and the publication have shown the increase in number of flowering as well as that of the non-flowering plants, along with new records and new species as well. The findings of new species have definitely increased the number of endemic species.

Endemic Plants

The recent updated list showed that there are 302 species and 49 infra-specific taxa of endemic flowering plant species (Shrestha et al., 2022) in Nepal. Likewise, the endemic species number of non-flowering plants are as follows:

Algae: 29 species (Rai & Ghimire, 2020)
Lichens: 55 species of (Baniya et al., 2010)
Fungi: 131 species (Adhikari, 2016)
Bryophytes: 30 species (Pradhan, 2020)
Pteridophytes: 3 species (Kandel, 2020)

According to Tiwari et al. (2019) highest percentage of endemism is recorded at a higher elevation between 3800-4200m. In other words, taxa, which occur only in single restricted geographical area, are known as endemics and the phenomenon is known as endemism. This might be due to exploration been done more on the higher altitude than on the lower belt of the country. If the explorations are done on the lower belt and the data obtained from these field works and publication of new species from these areas might change the scenario of endemism.

Useful Plant Resources and Threat to Plants Diversity

The rich plant diversity of Nepal has always been the major resource that has been a part of subsistence living of majority of the Nepalese people. Plant resources have both consumptive and non-consumptive values and are harvested and used in many ways, for example, they are used as herbal drugs for the treatment of different disease, as edible plants, veterinary medicine, construction materials for agricultural tools and house hold goods, as well as other uses such as fuel, fodder, fibre, oil yielding, spices, religious or ceremonial material, animal sheds, as wild genetic resources for improving crop plants, etc. (Rajbhandary & Winkler, 2015).

These plant resources have played important role in different ethnic communities residing in different parts of Nepal. The information obtained through all the ethnobotanical studies has demonstrated the richness of knowledge among different ethnic groups about the useful plant resources of Nepal. Apart from the useful plant resources consumption, a significant proportion of the population of remote areas of the country depends on the collection of these resources for trade, especially medicinal and aromatic plants (MAPs), for the upliftment of their livelihood. At the same time plants also purify air and/or balance oxygen and carbon dioxide ratio, protect soil and increase soil fertility, enrich the nutrient level and microbes decompose the dead bodies. They also have greater role in maintaining and/or improving the environmental quality against its degradation (Rajbhandary et al., 2020).

However, with several development projects throughout the country and tremendous pressure of socio-economic change along with the ecological knowledge and cultural traditions, which have been continuously developed and transferred from generation to generation, are getting lost. Hence, documentation of the ethno-botanical knowledge of these plant resources is very important as it serves as a useful information tool in the conservation of commercially threatened species.

Loss of habitat by human actions is a dominant cause of threat to plant diversity. Like most developing countries, threats to biodiversity in Nepal are initiated by the human activities. Deforestation, over grazing, biological invasions, natural disasters, construction activity, and increasing human intervention are the major threats to plant diversity (Rajbhandary et al., 2020). Grazing in the pastures is one important threat in the highlands, as animal husbandry is one of the important occupations in Nepal, this has exerted lot of pressure on the plant resources. Similarly, in the recent trend climate change impact is another threat for plant diversity. It is predicted that in the future this threat will exert the most severe impact on biodiversity by altering the weather conditions and increasing natural disasters.

Biological invasions have been recognized as one of the most serious global processes causing serious threats to biodiversity. They affect not only the species diversity of native ecosystems but also threaten their biological integrity and increasing number of invasive species in Nepal is definitely threatening our plant diversity. There are 179 species of naturalized alien plant species in Nepal (Shrestha, 2019), and among these, 27 species are invasive (Adhikari et al., 2022). Among them, four species (*Mikania micrantha, Chromolaena odorata, Lantana camara and Pontederia crassipes*) are in the list of 100 of the world's worst invasive alien plant species.

Why Conservation of plant resources is needed?

If the factors causing loss of plant diversity or threatening extinction is not controlled on time it will influence long-term evolutionary processes along with the immediate loss. If the present trends of habitat destruction and over-exploitation practices persist, majority of the endemic and other species will be lost forever resulting in an ecologically simple but biologically unstable ecosystem (Rajbhandary et al., 2020). These threats will exert serious consequences on the ecosystem services if not addressed by urgent based efforts and effective management. Hence there is an urgent need to conserve the valuable but fast disappearing plant species for the sake of establishing ecological balance. One of the important research projects in Nepal should now be to quantify the threatened and develop resources to save some important Red Listed species (Rajbhandary, 2008).

To protect and conserve the Plant diversity, one should understand the status of those resources through inventory (what they are), distribution (where they are located), abundance (how many there are), productivity (their capacity to produce), health (their well-being, resilience), and variables change over time and space. This requires committed, trained manpower for research along with modern equipment and opportunity to collaborate with national and international research institutions.

For the conservation of plant diversity emphasis should be given to:

- Initiation of **Provincial and protected areas flora**
- Initiation of **Herbarium** in each Province
- Prioritization of Biodiversity Exploration in **Less Explored Area**
- Collaborate and build network with international **taxonomic initiatives**
- Conservation Assessments for Red List Categorization of Floral

diversity to prepare **Plant Red Data Book**

- Conservation Strategies for **Endemic and Threatened plants** and their habitats
- Protection of specimen (herbarium) is as **important as collection is the asset** of the nation. Sharing of digitized material is equally important as this is also a kind of registration of plants in world scenario
- Effort to **publish data in GBIF** will increase the use value of the collected plant specimens as this is also a registration of our resources that will let rest of the world know about it
- *in-situ* and *ex-situ* conservation of prioritize species
- Urgent need to conserve the valuable but **fast disappearing species** for the sake of establishing ecological balance
- Framework to highlight lineages of the Nepalese flora to be prioritised in local **ethnopharmacological** studies
- **Building local capacity to list and assess** these resources as they are considered to be important steps for achieving conservation of these valuable plant resources
- Integrated approach for the **effective and proper management** of different drivers of global environmental changes

Conclusion

Plant diversity of Nepal is not only rich but an useful resources which is being threatend due to both ecological and an anthopogenic activities. It is therefore, high time to conserve the resources for our future generation. We must find new ways of thinking - an integrated multidimensional approach to the problem of global sustainability and this require the conservation of the plant resources, whether to aquire data for plant diversity number, endemic species, Red list, and for the bioprospective uses. Hence there is an urgent need to conserve the valuable but fast disappearing species for the sake of establishing ecological balance and saving the natural resources which have potential for discovery of novel molecules and new sources of active compounds for drug developments.

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Assessment of a Highly Nutritive Medicinal Plant, *Moringa Oleifera* Germplasm for Nutritional Adaptability under Subtropical Climatic Conditions

Ashok Kumar Dhakad[1*], Harpreet Kaur Oberoi[2] and Karamdeep Singh[1]
[1]Department of Forestry and Natural Resources
[2]Forage and Millet Section, Punjab Agricultural University, Ludhiana -141004, Punjab (India)
[*]Email: ashokdhakad@pau.edu

Abstract

The present study aims to assess the adaptability of *Moringa oleifera* by comparing the nutritional and fodder values of procured germplasm from diverse geographical regions of India with the locally available germplasm grown under subtropical conditions. For this, genotypic variability, effect of maturity stage and different phenophases were assessed using the standard protocols formulated by Association of Official Analytical Chemists (AOAC) (2005). Tender leaves, mature leaves and twigs were collected after rainy season. Significant differences were observed for all traits studied and were found to be affected by genotype as well. Maximum dry matter, protein and energy was recorded in S13 (Punjab Agricultural University (PAU) local germplasm; subtropical source), S9 (ODC-3, tropical source) and S14 (PAU local; subtropical source) ecotypes, respectively. Maximum dry matter intake and digestibility were observed in S5 (PAU local source). Tender leaves were observed best for carbohydrates, neutral and acid detergent fibres, lignin content, dry matter intake, digestible nutrients, relative feed value, and metabolized energy. However, mature leaves were observed best for crude protein, crude fats, crude fibre, total energy value and *in vitro* dry matter digestibility, while twigs were found to be best for dry matter and total soluble sugars. Thus, ecotypes were found adaptive and showed well performance since the procured sources had unique nutritional and fodder values as compared to the locally grown sources. This supports the prospect of cultivating S14 and S9 germplasm of *Moringa oleifera* as food and feed crops, respectively under subtropical conditions of north-western India.

Keywords: Adaptability, Dry matter digestibility, Fodder quality traits, *Moringa oleifera*, Nutritional values

Introduction

Moringa oleifera Lam. [syn. *Moringa pterygosperma* Gaertn. Nom. Illeg, *Guilandina moringa* L. and *Hyperanthera moringa* (L.) Vahl] belongs to monogeneric family, Moringaceae which is native to the north-western India and also at the foot hills of Himalayan tracts including Nepal, Pakistan, Bangladesh and Afghanistan. The family has one genus, *Moringa*, which has 13 species (Dangi et al., 2002), of which, *Moringa oleifera* is well known and widely distributed and adapted in tropics (Lalas & Tsaknis, 2002). In the Dravidian language (South Indian), it is known with several vernacular names but they are all found in the common root "Morunga". In local language, it is called as Sahjan (Hindi). In English, it is well-known by several names, of which drumstick, horseradish and West Indian ben tree are common (Ramachandran et al., 1980). *Moringa oleifera* is an indigenous tree and is often cultivated in hedges and home yards for household uses. *Moringa* is believed to be an important tree species in view of protection from dry spell and dry climatic conditions due to their tuberous roots (Padayachee & Baijnath, 2012). *Moringa oleifera* is adapted to various types of soils, temperatures and annual precipitation. It grows well from sea level to 1000 meters above mean sea level (Dalla, 1993).

Among the 13 species of *Moringa* genus, *Moringa oleifera* is observed as a store house of almost all type of nutrients like proteins, fats, fibres, carbohydrates, sugars, starches and essential amino acids (Stadtlander & Becker, 2017). All parts of *M. oleifera* showed high amount of nutrients, of which tender leaves and immature fruits have maximum nutrients (Verma & Nigam, 2014; Sohani, 2018). Recently, researchers have proven that *M. oleifera* tree has highly digestible nutrients and carotenoids suitable to undernourishment in low economic countries (Olagbemide & Alikwe, 2014 and Gopalakrishnan et al., 2016). All essential vitamins like vitamin B, folic acid, pyridoxine and nicotinic acid, vitamin A, C, D and E which are crucial for body growth and development are present in *M. oleifera* (Mbikay, 2012). Levels of nutrients in leaves of *M. oleifera* was quite high, for instance 27.51% crude protein, 19.25% crude fibre, 2.23% crude fat, 43.88% carbohydrate content and 305.62 cal/g energy value (Oduro et al., 2008). Yameogo et al. (2011) observed that plants grown under dry environment had similar amount of crude protein (27.2%) and 38.6% carbohydrates while crude fat (17.1%) was very high.

In addition, a nutritious diet plays an important role in livestock to increase the milk yield. Amino acids, fatty acids, minerals and vitamins are important in the daily diet of animals. The fibre components of fodder affect feed digestion which directly or indirectly affects animal feed consumption. It has been reported that the nutrient content in *Moringa* leaves vary from place to place (Anjorin et al., 2010). Several studies carried out by Richter et al. (2003), Sanchez et al. (2006) and Mendieta-Araica et al. (2011) examined the practices of growing *Moringa* and its use as animal feed and fish feed. They have shown that *Moringa* is as potential crop for animal feed and silage making. *M. oleifera* leaves possessed excellent nutritive and fodder values like crude protein (21.8%), carbohydrates (211.2 g kg^{-1}), acid detergent fibre (22.8%), neutral detergent fibre (30.8%), crude seed oil (412.0 g kg^{-1}), and less amount of inorganic compounds like ash (44.3 g kg^{-1}) (Oliveira et al.,1999 and Sanchez et al., 2006).

It is an established fact that nutrient composition of different plant parts of *M. oleifera* varies with the geographical location due to varying factors of locality. The nutritional composition varies with sources, cultural practices, environment, fertigation techniques, drying methods and storage conditions of plant parts (Dhakad et al., 2019). Huge difference for the nutritional values were observed among the leaves of *M. oleifera* planted in three different countries (Leone et al., 2015). Similar kind of results was obtained by Chelliah et al. (2017) for the nutritional and phytochemical variability among the leaf samples collected from the two different regions. Fuglie (2005) mentioned in his study that nutrient concentrations fluctuate with the seasons or environmental conditions. In summer-rainy season, vitamin A has more concentration, whereas, vitamin C and iron content were observed more in cool season (Yang et al., 2006). The high concentration of major bioactive ingredients reported in different plant parts during summer season may be responsible cause of higher usage of *M. oleifera* in summer season (Safaeian et al., 2015), of which the concentration of phenolic compounds was observed more in winter season (Shih et al., 2011). These results are in agreement of the facts reported by Moyo et al. (2011) and revealed that the variability in nutritional value is considerably attributed by the factors of locality and ecology. Iqbal and Bhanger (2006), Joshi and Mehta (2010) stated that the variations could be due to several other factors like genotypes, cultivation techniques, harvesting time, storage conditions and drying methods.

Several studies have been carried out to assess the variability in nutritional profile and concentration of mineral elements from different parts of *M. oleifera,* however, phenophase variations have not been reported from the Punjab area yet. It is an established fact that variations in nutritional, mineral and fodder traits are due to the environmental conditions (Forster et al., 2015). Nonetheless, these salient findings haven't been confirmed and reported from the field plantation areas of *M. oleifera* from North India. Likewise, *Moringa* is getting well known as a significant food commodity considering its present status as the natural nutrition of the tropics. There is sufficient scope for characterization of wild land races and varieties have been released at national level, based on their nutritional and fodder traits. The wide genetic variability found in the *Moringa* cultivars show their high level of biodiversity. However, no complete study could be found among the seed sources of *M. oleifera* that could allow the comparisons of nutritive, mineral and fodder values for different phenophases like tender leaves, mature leaves and twigs. Keeping in view the above realities and facts, the current study has aimed to assess the suitability of *Moringa oleifera* germplasm collected and procured from different geographical regions of India for nutritional adaptability under subtropical climatic conditions.

Materials and Methods

Study site

The study was conducted at Punjab Agricultural University, India. The experimental site is located at an elevation of 247 m amsl (30°54'N and 75°48'E). The area falls in the central zone of Punjab. The area is characterized by sub-tropical to tropical, semi-arid type of climate. This area experiences very hot summers during April to June and cold winters from December to January. The minimum temperature may go down to 4°C or even less while the maximum temperature may be as high as 46°C during the summer season. The average temperature of study period was 6.1°C to 40.4°C. Occurrence of frost is not common. The soils are deep, well drained, sandy loam in texture with low humus content. The pH of soil is neutral. The average annual rainfall of study site is 760 mm, about three-fourth of which is contributed by the south-west monsoon during June to September (Figure 1).

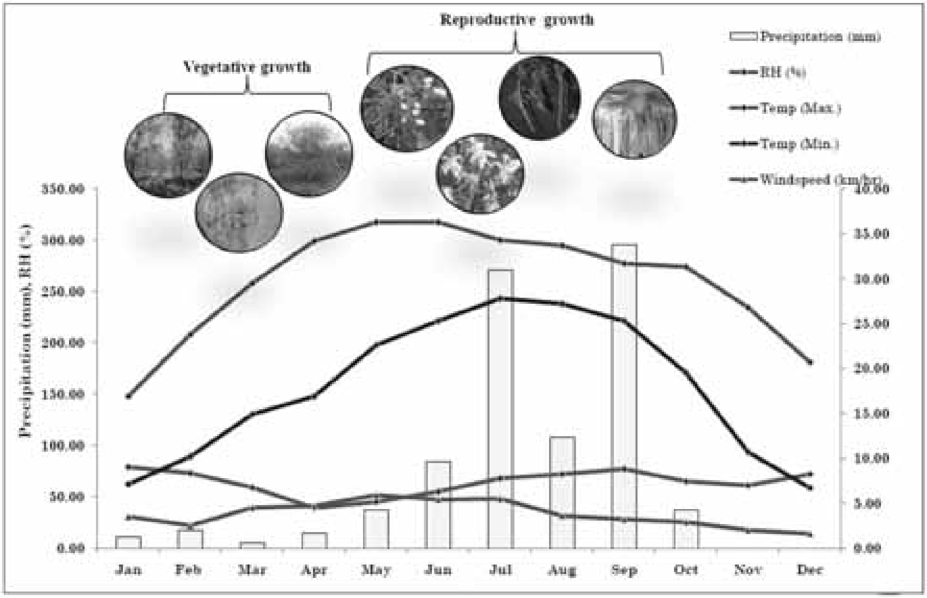


Figure 1: Seasonal appearance of phenological stages of *Moringa oleifera* under the Punjab conditions

Experimental details and cultural practices

Seeds of five recommended varieties (Bhagya, Konkan Ruchira, PKM-1, PKM-2 and ODC-3) adopted at national level and four landraces of *Moringa oleifera* were procured from different geographical regions of India. Besides, five land races were maintained at PAU, Ludhiana. These moringa seed sources represent ecotypes collected from different agro-climatic regions of India (Table 1). Five ecotypes belong to tropical region (Southern India), four from semi-arid to arid region (Western India) and three from subtropical region (Northern India). Fully mature seeds were sown about 3 to 5 cm deep in 9×6 inches poly bags containing the mixture of sand, soil and farm yard manure (FYM) at 1:1:1 in first week of July. Seeds were germinated within 3-12 days after sowing (DAS) and showed 75-90% germination success uniformly (data not presented here). The seedlings were ready to transplant in field after 35-45 DAS and transplanted out during first week of August 2017 at spacing of 3.5m × 2.0 m in three blocks with 5 plants per plot following the complete randomized block Design. The blocking was made based on the soil variations. The plants were irrigated and fertilized regularly as per the standard package of practices followed for *M. oleifera* (Anonymous, 2013). Plants were pollarded at the height of 6 feet during November 2018 for induction of a greater number of shoots to maximize the fodder biomass. The plants were then frequently pollarded in every summer and winter.

Table 1: Details of *Moringa oleifera* ecotypes used in study

Climate	Code	Seed Source	Geographic origin	Latitude (^{0}N)	Longitude (^{0}N)	Altitude (m)	Mean annual precipitation (mm)
West-central India (Semi-arid to arid region)	**S1**	Bhagya	University of Horticulture Science, Bagalkot, Karnataka	16.18	75.69	542	562
	S2	Konkan Ruchira	Dr. Balasaheb Sawant Konkan Krishi Vidypeeth, Dapoli, Maharastra	17.75	73.18	164	1915
	S3	Dantiwara	Sardar Krushinagar Dantiwada Agricultural University, Gujarat	25.24	73.32	311	578
	S10	Jodhpur	ICAR-CAZRI, Jodhpur, Rajasthan	26.25	72.99	236	323
Northern India (Subtropical region)	**S4**	PAU-1	PAU Ludhiana, Punjab	30.90	75.81	249	876
	S5	PAU-2	PAU Ludhiana, Punjab	30.90	75.81	250	876
	S6	PAU-3	PAU Ludhiana, Punjab	30.90	75.81	248	876
Southern India (Tropical region)	**S7**	PKM-1	Tamil Nadu Agricultural University, Tamil Nadu	11.31	76.93	310	952
	S8	PKM-2	Tamil Nadu Agricultural University, Tamil Nadu	11.31	76.93	310	952
	S9	ODC-3	Agri Farm, Tamil Nadu	8.15	77.59	31	1457
	S11	Mandya	Mandya, Karnataka	12.52	76.89	683	806
	S12	Mysore	Mysore, Karnataka	12.31	76.64	772	777

Sample collection and sample preparation

Samples were collected from each ecotype from two leaf phenophases, i.e., tender leaves [Early Leaf Expansion Stage (ELE)] with the 15-20 days of growth; mature leaves [Mature Dark Green Leaf stage (MDGL)] after the 60 days of growth (Singh et al., 2021a), and twigs containing tender, mature leaves and tender shoots in the month of August. The experiment was repeated for two consecutive years, i.e., 2020-2021. Plant samples were air-dried at room temperature in well-ventilated laboratory for 6-7 days and then dried in oven at 105^{o}C until the samples achieved a constant weight. Completely dried samples were then milled and sieved through a 2 mm sieve using a hammer mill before being assessed for the nutritional and fodder values.

Nutritional characteristics

Dry matter (DM) content was determined after drying samples in an oven at 105°C to constant dry weight (Singh, 2021). Macro-Kjeldahl technique was used to quantify the Nitrogen content (AOAC, 2005). The crude protein (%) content was then calculated by multiplying the nitrogen content with 6.25 factor. The standard protocols of AOAC were used to determine the crude fibre and crude fat content (AOAC 2005). Dubois et al. (1956) protocol was used to determine total soluble sugars. Total carbohydrate was calculated by using crude protein, crude fat and ash content as per the formulae given by Martin and Coolidge (1978). Similarly, the total energy value as food was estimated using Martin and Coolidge's (1978) equations and given in Kcal/100g of DM. The leaf samples for each phenophases in triplicates were used.

Proximate analysis and fodder quality estimation

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were quantified by the method given by Van-Soest et al. (1999). Acid detergent lignin was determined by using standard procedures of AOAC (2005). Hemicellulose and cellulose were calculated by the differences of NDF and ADF; ADF and acid detergent lignin (ADL), respectively (Goering & Van-Soest 1975). Relative feed value (RFV) was calculated from the estimates of digestible dry matter (DDM) and dry matter intake (DMI) (Horrocks & Vallentine, 1999); Dry matter digestibility (DMD) % = 88.9 - (0.779 × %ADF); DMI = 120/%NDF; Relative feed value (RFV) = (%DDM × %DMI)/1.29. DDM values were then used to estimate digestible energy (DE) (kcal/kg) using the regression equation reported by Fonnesbeck et al. (1984); DE (Mcal/kg) = 0.27 + 0.0428 (DDM %). Then, metabolized energy (ME) was calculated as per the formula: ME (Mcal/kg) = 0.821 × DE (Mcal/kg) (Khalil et al., 1986). *In vitro* dry matter digestibility (IVDMD) was assessed by using standard procedures of Tilley and Terry (1963).

Statistical analysis

The mean data recorded on various traits were analysed by using one-way analysis of variance as per the method laid down for complete randomized design (Panse & Sukhatme, 1989) using Proc GLM (SAS Software 9.3, SAS Institute Ltd. USA) function.

Results and Discussion

Effect of phenophases, leaf maturity and genotype on nutritional values

The mean nutritional values (g/100g DM) of fourteen ecotypes of *M. oleifera* collected from different geographical regions of India showed significant differences ($p \leq 0.01$) for studies characteristics (Figure 2 and 3; Table 2). The tender leaves of seed source S13 (PAU-4) registered maximum mean value (21.12 g/100g) for dry matter which was statistically at par with S14 (20.71 g/100g) (PAU- 5) and S12 (Mysore, Karnataka) (20.04 g/100g). For crude protein the maximum value was recorded in S12 (Mysore, Karnataka) (22.22 g/100g DM) which was followed by S9 (19.86 g/100g DM) and S10 (18.64 g/100g DM). Highest content for the crude fat was observed in local seed source S14 (5.73 g/100g DM) followed by S13 (5.63 g/100g DM), S12 (4.50 g/100g DM) and S8 (4.20 g/100g DM). The largest value for crude fibre was observed in S12 (20.95 g/100g DM) followed by S4 (19.10 g/100g DM), S11 (19.05 g/100g DM) and S2 (18.80 g/100g DM). Local variety S5 (PAU-2) had highest mean value for total carbohydrate (79.19 g/100g DM) and followed by S3 (78.56

g/100g DM), S4 (77.60 g/100g DM) and S2 (76.82 g/100g DM). The maximum content for total soluble sugar was registered in Gujarat seed source S3 (Dantiwara) (11.49 g/100g DM) trailed by S2 (11.33 g/100g DM), S1 (11.27 g/100g DM) and S4 (11.12 g/100g DM). The average value of total energy ranged in between 363.24 to 388.07 kcal/100g. The local seed source S14 (PAU-5) possessed the extreme value (388.07 kcal/100g) followed by S12 (385.14 kcal/100g), S10 (384.67 kcal/100g) and S9 (384.23 kcal/100g).

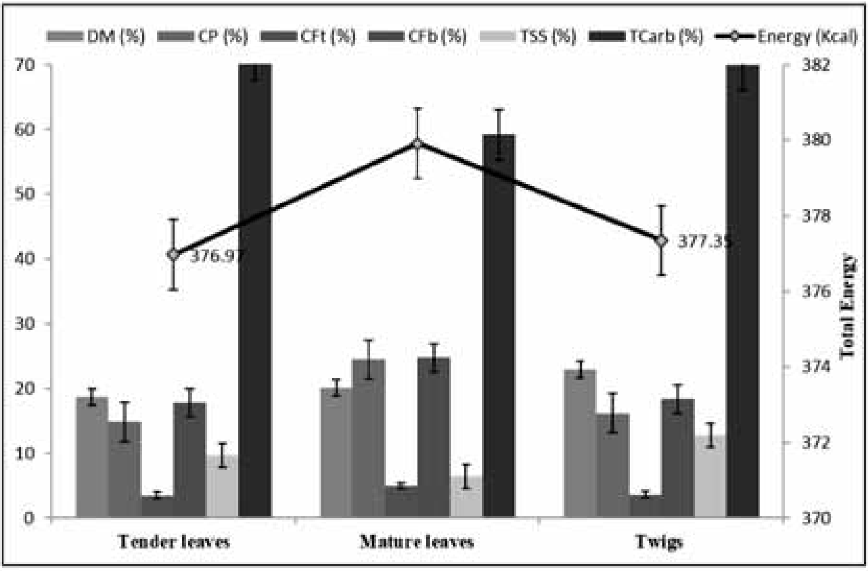
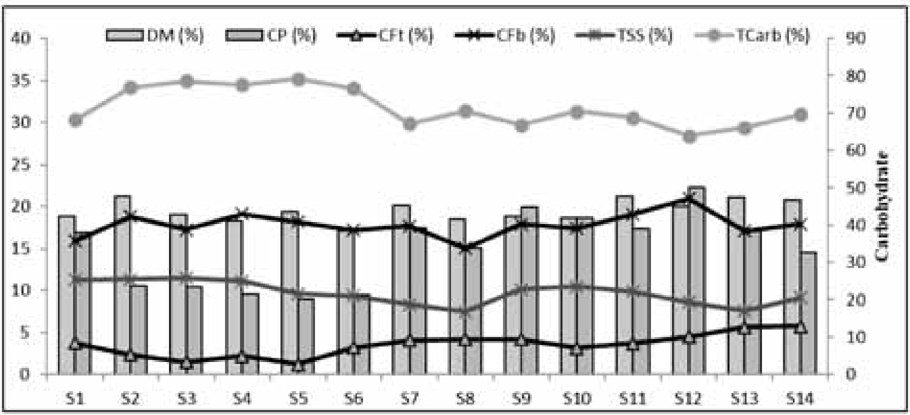


Figure 2: Effect of phenophases and maturity on nutritive values in *Moringa oleifera*



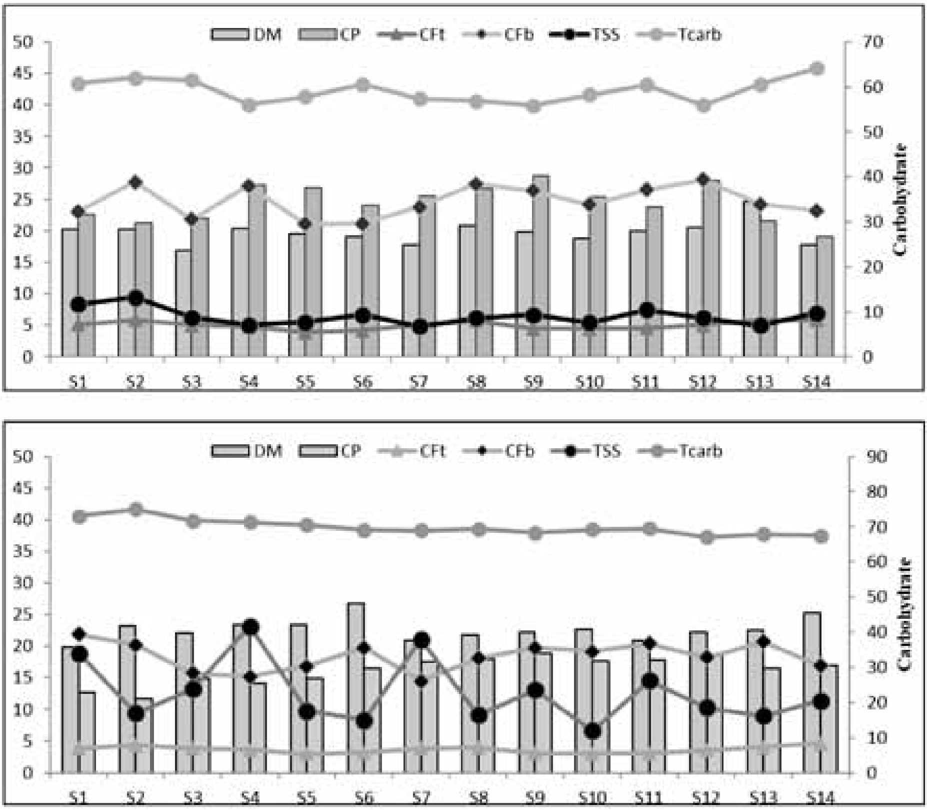


Figure 3: Genotypic variability for nutritive values of (a) tender leaves, (b) mature leaves, and (c) twigs of *Moringa oleifera*

The mature leaves of seed source S13 (PAU-4) registered maximum mean value (22.69 g/100g) for dry matter and it was followed by S14 (21.81 g/100g), S8 (20.84 g/100g) and S12 (20.49 g/100g). For crude protein the maximum value was observed in S9 (ODC-3, Tamil Nadu) (28.70 g/100g DM) which was followed by S12 (28.09 g/100g DM). Highest content for the crude fat was observed in two seed source S2 and S14 (5.87 g/100g DM) and they were followed by S13 (5.83 g/100g DM), S8 (5.70 g/100g DM) and S1 (5.17 g/100g DM). The highest value for crude fibre was recorded in S12 (28.15 g/100g DM) followed by S2 (27.80 g/100g DM), S8 (27.50 g/100g DM) and S4 (27.15 g/100g DM). Local variety S14 (PAU-5) registered highest mean value for total carbohydrate (64.22 g/100g DM) followed by S2 (62.00 g/100g DM), S3 (61.57 g/100g DM) and S1 (60.85

g/100g DM). The maximal content for total soluble sugar was registered in S2 (Konkan Ruchika, Dapoli) (9.48 g/100g DM) succeeded by S1 (8.38 g/100g DM), S11 (7.46 g/100g DM) and S14 (6.99 g/100g DM). The mean values of total energy ranged from 374.11-386.33 kcal/100g. The local seed source S14 (PAU-5) possessed the extreme value (386.33 kcal/100g) which was at par with S2 (386.05 kcal/100g) and S8 (385.54 kcal/100g).

The twigs of *M. oleifera* leaves of seed source S6 registered maximum mean value (26.80 g/100g) for dry matter followed by S14 (25.34 g/100g), S13 (24.53 g/100g) and S4 (23.41 g/100g).The maximum value for crude protein was observed in S9 ODC-3, Tamil Nadu (18.81 g/100g DM) which was followed by S12 (18.64 g/100g DM).The highest content for the crude fat was observed in local seed source S14 (4.69 g/100g DM) followed by S2 (4.40 g/100g DM), S13 (4.08 g/100g DM) and S8 (3.99 g/100g DM). The largest value for crude fibre was recorded in S12 (20.95 g/100g DM) followed by S1 (21.90 g/100g DM), followed by S13 (20.70 g/100g DM) and S11 (20.40 g/100g DM).The seed source S2 registered highest mean value for total carbohydrate (75.05 g/100g DM) succeeded by S1 (73.03 g/100g DM), S3 (71.75 g/100g DM) and S4 (71.37 g/100g DM).The maximal content for total soluble sugar was registered in local seed source S4 (PAU-1) (23.17 g/100g DM) followed S7 (21.06 g/100g DM), S1 (18.88 g/100g DM) and S11 (14.59 g/100g DM).The average total energy value ranged in between of 371.15 and 386.32 kcal/100g. The local seed source S2 possessed the extreme value (386.32 kcal/100g) which was at par with S8 (385.47 kcal/100g).

The four ecotypes had significant higher crude protein in tender leaves and five in mature leaves than nationally adopted variety, i.e.S7 (PKM-1), of which, S12 had about 27% more protein than S7 in tender, however, S9 (ODC-3) had maximum in mature leaves which was significantly at par with S12. Our results revealed that protein content was negatively correlated with the carbohydrate at both maturity levels, whereas, fat content and total energy value was not affected with advancing maturity stage. These data indicates that tender leaves of S12 for culinary proposes and mature leaves of S9, S12 and S4 for food and feed consumption are suggested due to high amount of protein and fibre content and likely to be excellent *M. oleifera* ecotype for producing leaves in this region. This makes the leaves of *Moringa* a good source of protein (Hassan & Umar, 2006). Besides, twigs only contained higher values of dry matter percentage and total soluble sugars. The nutritional composition of our cultivars was similar to those presented by several authors Mutayoba et al. (2011), Olugbemi et al. (2010) for fibre and fat content; Nweze and Nwafor (2014), Ogbe and Afiku (2011) for carbohydrates; Yameogo et al. (2011), Melesse et al. (2011) for energy value. Likewise, protein and fibre content observed in the tested cultivars revealed moringa leaves as a precious source of nutritional diets with potential application for the development of novel foods and feeds that could contribute to fulfil the nutritional requirements of humans and livestock, reducing the dependence on foreign sources of dietary proteins and thus, improving the competitiveness of the local agro-food sector (Mendieta-Araica et al., 2011).

Effect of phenophases, leaf maturity and genotype on fodder quality characteristics

The leaves of *M. oleifera* can be a good fodder substitute as it possesses good quality fodder characteristics and had significant differences (Figure 4 and 5; Table 2). The tender leaves possessed fair amount of fodder parameters. The seed source S5 registered maximum mean value of NDF content (47.65 g/100g DM) followed by S7 (44.95 g/100g DM), S4 (40.95 g/100g DM) and S6 (39.95 g/100g DM). The average value for ADF and ADL content ranged from 18.70-37.55 g/100g DM and 2.95-12.55 g/100g DM. The largest value of hemicelluloses was recorded in S8 (12.75 g/100g DM) which was at par with S9 (12.55 g/100g DM) and S13 (12.09 g/100g DM), whereas the lowest mean value was observed in S5 (10.10 g/100g DM). The seed source S5 registered maximum mean value (28.75 g/100g DM) for cellulose content followed by S6 (25.55 g/100g DM), S7 (21.60 g/100g DM) and S3 (20.55 g/100g DM). The maximum value for DMI, DDM and RFV was noticed in source S11 (Mandya, Karnataka) (3.96 g/100g DM), (74.33 g/100g DM) and (228.30 g/100g DM). The highest total digestible nutrients (TDN) was observed in S11 (68.33 %) and minimum was in S6 (60.17 %). The highest value for IVDMD content was registered in local source S14 (78.50 %) which was at par with two seed sources S2 (77.30 %) and S8 (77.30 %). The average metabolized energy ranged in between of 254 (S6) and 283 kcal/100g DM (S9 and S11 sources).

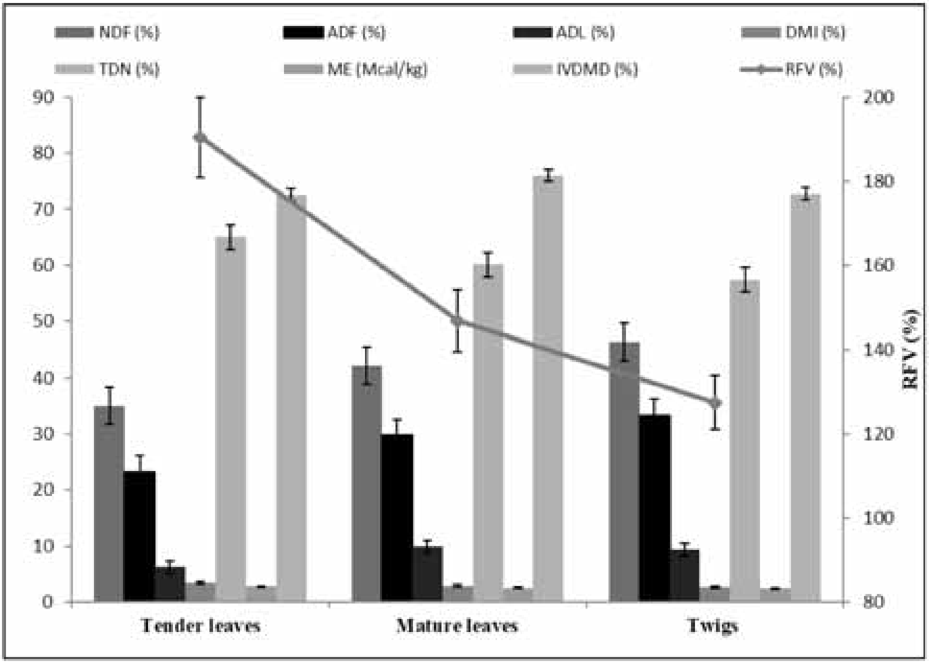


Figure 4: Effect of phenophases and maturity on fodder quality in *Moringa oleifera*

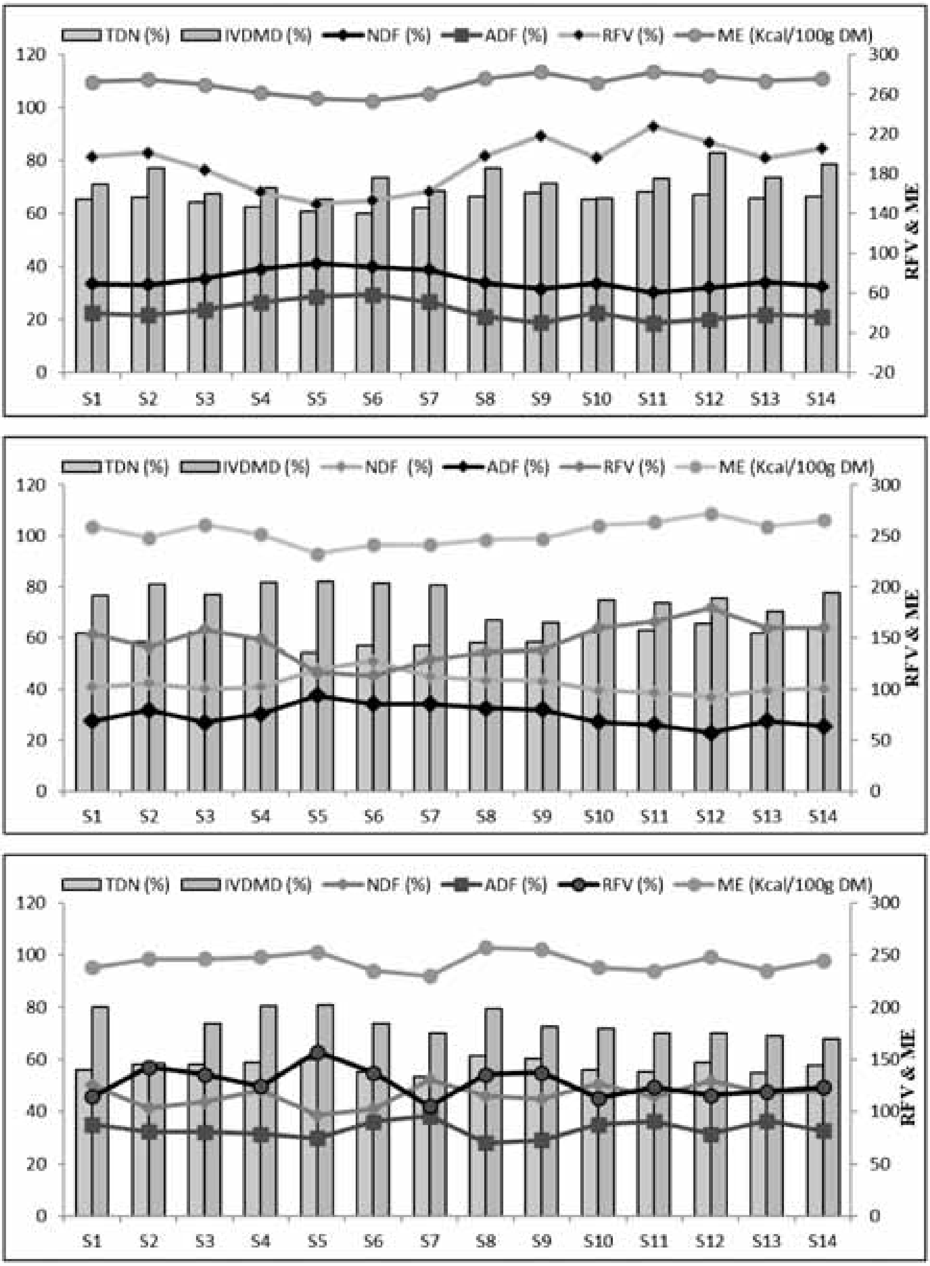


Figure 5: Genotypic variability for fodder values of (a) tender leaves, (b) mature leaves, and (c) twigs of *Moringa oleifera*

Table 2: Genotypic and phenophase variability for total energy value, acid detergent fibre and dry matter intake in *Moringa oleifera*

Geographical sources	Total Energy Value (Kcal/100g DM)			Acid Detergent Lignin (%)			Dry Matter Intake (% of BW)		
	Tender ± SE_m	Mature ± SE_m	Twigs ± SE_m	Tender ± SE	Mature ± SE	Twigs ± SE	Tender ± SE	Mature ± SE	Twigs ± SE
S1	373.89 ±0.72	380.55 ± 0.42	377.73 ± 0.43	2.95 ± 0.14	6.25 ± 0.26	10.00 ± 0.35	3.57 ± 0.01	2.94 ± 0.02	2.40 ± 0.02
S2	370.91 ± 0.49	386.05 ± 1.52	386.32 ± 0.51	3.40 ± 0.23	11.65 ± 0.61	8.25 ± 0.38	3.61 ± 0.07	2.83 ± 0.03	2.90 ± 0.04
S3	369.09 ± 0.39	380.02 ± 0.46	381.24 ± 0.48	3.20 ± 0.17	7.05 ± 0.20	9.00 ± 0.40	3.37 ± 0.06	3.00 ± 0.02	2.74 ± 0.01
S4	368.39 ± 0.61	376.96 ± 0.17	374.20 ± 0.07	11.00 ± 0.52	13.95 ± 0.49	9.55 ± 0.32	3.07 ± 0.02	2.93 ± 0.01	2.49 ± 0.03
S5	363.24 ± 0.21	374.11 ± 0.14	368.63 ± 0.22	8.80 ± 0.23	11.70 ± 0.46	6.25 ± 0.38	2.91 ± 0.01	2.52 ± 0.03	3.09 ± 0.04
S6	373.25 ± 0.42	376.65 ± 0.58	371.15 ± 0.06	4.00 ± 0.23	9.25 ± 0.32	9.30 ± 0.35	3.00 ± 0.02	2.35 ± 0.01	2.92 ± 0.03
S7	375.25 ± 0.01	377.78 ± 0.11	380.88 ± 0.04	10.85 ± 0.32	12.55 ± 0.38	13.55 ± 0.43	3.08 ± 0.01	2.67 ± 0.03	2.30 ± 0.02
S8	380.48 ± 0.01	385.54 ± 0.02	385.47 ± 0.15	4.45 ± 0.14	8.35 ± 0.32	6.05 ± 0.43	3.53 ± 0.05	2.76 ± 0.05	2.61 ± 0.02
S9	384.23 ± 0.01	378.73 ± 0.04	376.55 ± 0.10	5.25 ± 0.20	8.85 ± 0.26	6.15 ± 0.20	3.81 ± 0.06	2.78 ± 0.01	2.68 ± 0.03
S10	384.67 ± 0.11	375.22 ± 0.72	375.18 ± 0.04	7.15 ± 0.26	9.20 ± 0.35	11.15 ± 0.26	3.56 ± 0.02	3.04 ± 0.04	2.37 ± 0.03
S11	377.33 ± 0.68	377.99 ± 0.13	376.46 ± 0.69	5.85 ± 0.20	9.05 ± 0.14	11.70 ± 0.23	3.96 ± 0.05	3.11 ± 0.03	2.63 ± 0.04
S12	385.14 ± 0.20	381.97 ± 0.23	375.13 ± 0.24	7.05 ± 0.32	10.75 ± 0.20	10.80 ± 0.23	3.73 ± 0.09	3.24 ± 0.04	2.32 ± 0.02
S13	383.65 ± 0.37	380.85 ± 0.39	374.02 ± 0.79	7.70 ± 0.17	10.65 ± 0.43	10.15 ± 0.26	3.52 ± 0.03	3.03 ± 0.03	2.55 ± 0.03
S14	388.07 ± 0.04	386.33 ± 0.17	379.99 ± 0.06	5.95 ± 0.49	9.65 ± 0.32	9.20 ± 0.17	3.67 ± 0.06	2.98 ± 0.02	2.52 ± 0.03
Mean ± SE_m	**376.97 ± 0.39**	**379.91 ± 0.53**	**377.35 ± 0.37**	**6.26 ± 0.29**	**9.92 ± 0.36**	**9.36 ± 0.32**	**3.46 ± 0.05**	**2.87 ± 0.03**	**2.61 ± 0.03**
$LSD_{0.05}$	**1.14**	**1.52**	**1.08**	**0.84**	**1.03**	**0.94**	**0.14**	**0.08**	**0.08**

The mature leaves have good amount of fodder parameters. The local seed source S6 (PAU-3) registered maximum mean value of NDF content (51.05 g/100g DM) followed by S8 (43.55 g/100g DM), S9 (43.20 g/100g DM) and S2 (42.40 g/100g DM). The average value for ADF and ADL content ranged from 22.85-34.18 g/100g DM and 6.25-13.95 g/100g DM. The maximum value of hemicelluloses was recorded in S6 (16.88 g/100g DM) followed by S14 (14.70 g/100g DM), whereas the lowest mean value was observed in S2 (10.65 g/100g DM). The seed source S6 registered maximum mean value (24.93 g/100g DM) for cellulose content followed by S8 (24.10 g/100g DM) and S9 (23.15 g/100g DM). The highest value for DMI, DDM and RFV was noticed in variety S12 (Mysore, Karnataka) (3.24 g/100g DM), (71.10 g/100g DM) and (178.84 g/100g DM). The highest TDN was observed in S14 (63.18 %) and minimum was in S5 (54.16 %). The highest mean value for IVDMD content was registered in local source S5 (PAU-2) (81.90 %) followed by S4 (81.50 %), S6 (81.35 %), S2 (81.10 %) and S7 (80.40 %). The average metabolized energy ranged in between of 232 (S5) and 272 Kcal/100g DM (S12 source).

As far as twigs are concern, twigs didn't have any significance over leaves of *M. oleifera*. The seed source S7 registered maximum mean value of NDF content (52.20 g/100g DM) followed by S12 (51.80 g/100g DM). The average value for ADF and ADL content ranged from 28.15-38.35 g/100g DM and 6.05-13.55 g/100g DM. The highest value of hemicellulose was recorded in S6 (26.90 g/100g DM) succeeded by S8 (17.85 g/100g DM), S4 (16.75 g/100g DM) and S9 (15.60 g/100g DM), whereas the lowest mean value was observed in S6 (4.90 g/100g DM). The local seed source S6 registered maximum mean value (26.90 g/100g DM) and minimum content in S12 (20.70 g/100g DM) for cellulose content. The highest value for DMI, DDM and RFV was noticed in variety S5 (3.09 g/100g DM), S8 (66.97 g/100g DM) and S7 (105.21 g/100g DM). The highest TDN was observed in S8 (61.23 %) and minimum was in S7 (53.56 %). The highest mean value for IVDMD was registered in seed source S5 (81.00 %) which was followed by S4 (80.60 %), S1 (80.10 %) and S8 (79.60 %). The average metabolized energy ranged in between of 230 (S7) and 257 Kcal/100g DM (S8 source).

Conventional forage crops regulate the continuous fodder supply but non-conventional resources should be incorporated to regulate fodder availability during scarcity period especially in extreme summer and winter. *Moringa oleifera* can be used as low-cost non-conventional fodder resource that provides the adequate amount of green fodder in lean period. It is an established fact that *M. oleifera* had high concentration of nutrients and biochemical ingredients (Dhakad et al., 2019). The deterioration in crude protein (CP) and fibre with advance in plant growth has been reported (Contreras-Govea et al., 2009) but our finding contradicts with that of Sebola et al. (2019) who indicated that *Moringa* tender leaves contained more CP than mature. Data revealed that CP increases as with the decreasing DM content. The CP in *Moringa* was in excess than recommended for growth (11.3%) and lactation (12.0%) in ruminant animal (ARC, 1984) and more than conventional crops (Gupta et al., 2004). The CP content is above 16% for mature leaves and twigs which is the one required for growing higher ruminants making them ideal for use as a protein supplement (Moyo et al., 2011), while low protein in tender leaves have a greater potential as a feed additive to poultry animals (Sebola et al., 2019). However, the presence of fibre and secondary metabolites may reduce the bioavailability of protein. Detergent fibre is major indicator of digestibility and negatively affects feed quality (Han et al., 2003). Both, NDF and ADF increased with advancing maturity and increasing proportion of shoots in fodder, this indicates the reducing fodder quality.

The highest value of TDN found in tender leaves followed by mature leaves, and twigs which were due to the high concentration of dietary fibres or ADF concentration in twig part contained tender shoots which limits an animal ability to utilize the nutrients present in forage (Carmi et al., 2006). According to Horrocks and Vallentine (1999), forage with RFV value >151 is considered best. In the present study, leaves contained the excess amount of that limit, while twigs had values below the RFV standards. High RFV value for tender leaves suggests that harvest should be taken frequently to avoid the maturity of leaves and as a result decrease in RF value of moringa fodder similar to the Maize which is being harvested at early stage of maturity (Brar et al., 2019). Hence, it is assumed that leaves of moringa can be used as a valuable fodder and may consider as potential substitute to the conventional fodder crops. The relative more energy values indicated that *M. oleifera* could be used as concentrated sources of energy for livestock. Fibre content is affecting ME content due to the fact that digestibility of protein will decrease when fibre intake increases and that will decrease the ME content (Baer et al., 1997). IVDMD parameter is the most important parameter of forage quality. Hence, forage will be considered best for feeding the animals. The mature leaves of moringa possessed high IVDMD followed by twigs and tender leaves. The tender leaves showed significantly at par digestibility with twigs might be due to low ADF and proportionate distribution of tender and mature leaf content in twigs (Arif et al., 2020). The PAU ecotypes were observed best for the IVDMD which was statistically at par with S1 (Bhagya, University of Horticulture Science (UHS), Bhagalkot) and S8 (PKM-2, Tamil Nadu) ecotypes. Besides, *M. oleifera*, in general had high IVDMD than other tropical top feed tree species like *Anogeissus pendula*, *Ficus religiosa*, *F. recemosa* and *Eugenia jambolana* (Singh et al., 2021b).

Conclusions

The analyses revealed that *Moringa* leaves are promising sources of essential nutrients that can be exploited as ingredients for the preparation of food supplements and animal feed. It can also be used to obtain adequate amounts of nutrients with the overall benefits on body growth and development. The 60-days-old mature leaves showed high protein and fibre content required for human growth. However, the carbohydrate and sugar contents were observed to be high in 15-20-days-old tender leaves which reduced with advancing maturity. The variability in chemical ingredients with maturity stage signify the appropriate time for harvesting the leaves as a raw material for manufacturing of value-added products and thus, the intelligent combination of tender and mature leaves in specific proportions may contribute to provide health benefits due to their potential immune-boosting effect. On the other hand, significant difference among the ecotypes and phenophases for cell wall fractions, fodder quality traits, and *in vitro* dry matter digestibility was observed in twigs. Mean maximum crude protein (~28.70 g/100g DM) was recorded in S9 (ODC-3; tropical ecotype). Maximum *in vitro* dry matter digestibility (~81.90%) was recorded in S14 (PAU-5 subtropical ecotype), and metabolized energy (~2.83 Mcal/kg) were in S11 (Mandya, Karnataka) and S9 (ODC-3) equally. Based on the findings, north Indian ecotypes (subtropical region) were found at par for food and feed quality to the procured germplasm. The presence of adequate amount of fibre content reported in *Moringa* forage can be considered best for animal feed with high dry matter digestibility. High protein content and adequate metabolized energy were relatively equal to conventional forage plants.

Author Contributions

Conceptualization and design of the experiments - AKD; Resources availability - AKD, Data recording and curation – KS and AKD; Investigation - AKD, HKO; Methodology - HKO; Data analysis and validation – AKD and HKO; Writing-original draft – AKD and KS; Writing-review and editing – HKO.

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Production of Pectinase from *Bacillus licheniformis* Using Agriculture Wastes as Substrates

Haneef Ur Rehman[1]*, Fatima Aslam[1], Granaz Baloch[1], Muhammad Asif Nawaz[2] and Asad Karim[3]

[1]Department of Natural and Basic Sciences, University of Turbat, Kech, Balochistan

[2]Department of Biotechnology, Shaheed Benazir Bhutto University Sheringal, Der KPK

[3]International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

***Email: haneef.baloch@uot.edu.pk**

Abstract

Pakistan is agriculture-based country and produces large amount of agriculture waste material with limited economic use. In this work, different types of agriculture wastes including wheat bran, rice bran, orange peels, date fruit wastes, vegetable wastes and sugarcane bagasse were screened as substrate for pectinase production by *Bacillus licheniformis*. *B. licheniformis* produced higher concentration of pectinase using wheat bran as substrate as compared to other agricultural and vegetable wastes. Furthermore, the fermentation parameters were optimised for maximum pectinase production using wheat bran as substrate. *B. licheniformis* produced maximum pectinase using 1.5% wheat bran as substrate at pH 7.0 and 40°C after 48 hrs of incubation period. The overall results showed that agriculture wastes have great potential to be utilized as substrate for the production of commercially important enzymes.

Keywords: Enzyme, Plant pathological process, Polygalacturonase, Wheat Bran

Introduction

Pakistan is among the top agriculture economical based country and produces different varieties of crops and fruits including cotton, wheat, rice, sugarcane, mango, orange and other seasonal fruits. Being an agrarian economy, Pakistan generates significant amount of agricultural wastes from cultivated crops (Azam & Shafique, 2017). The new concept of bioeconomy and biorefinery has been developing among the scientific communities to emphasize the recycling of agro-industrial wastes and biomass into industrial important product. Most of the agricultural wastes composed of lignocellulosic material and contained cellulose, hemicellulose, lignin and pectin with some amounts of proteins, lipid and secondary metabolites (Thiviya et.al., 2022). The household food wastes are readily available wastes to be used due to the systematic disposal management. The rice bran, wheat bran, wheat straw, sugarcane bagasse, and corncob are cheapest available natural carbon sources that can be utilised as substrate for the production of industrially important enzymes (Ravindran et.al., 2018). The enzymes have been produced from different types of microorganisms from agricultural wastes. The enzymes have various applications in different industrial processes for food, drug, textile, and dye. The uses of agricultural wastes as carbon sources have exhibited great potential to reduce the enzymes production cost and increase its industrial capabilities (Bharathiraja et.al., 2017).

The enzymes are relatively expensive molecule and increase the operational cost of the process in which they are utilized (Tufvesson et. al., 2011). Therefore, different efforts have been made through conducting of various research to reduce the production cost of enzymes for better industrial applications (Sindhu et.al., 2016. The screening of the agricultural wastes (soybean meal, sunflower meal, maize bran, maize pericarp, olive oil cake and wheat bran) and bio-wastes as substrate for the production of enzymes is not only reduce the production cost enzymes but also support the recycling of wastes (Brandão et.al., 2021).

Pectinase is an enzyme that has significant importance in various industrial preparations including fruit juice extractions and clarifications, degumming plant fibres, plant oil extractions, wine productions and coffee, and tea processing (Shet et.al., 2018). The pectinase shared 25% of the total global of sales of food enzymes (Uzuner et.al., 2019). Pectinase play a very significant role in various biological processes across the entire field of living organism. The pectinase is widely distributed in nature and produced by different living organisms such as plants, microorganisms, insects, nematodes and protozoa (Rehman et.al., 2021). Pectinase is one of the important factors in the plant pathological process, plant-microbe symbiosis and in the decomposition of plant decay matters. The microbial pectinase is playing important role in nature by contributing of natural recycling of carbon in the environment (Rehman et.al., 2015).

Different microorganisms are known to produce pectinase with different molecular mass and catalytic properties. *Aspergillus niger* is mostly used for the industrial production of pectinase (Patil & Dayanand, 2006). The pectinases from fungus sources are usually acidic in nature and only can work in acid conditions. Production of alkaline pectinase remains under developed, as only few reports are available on the production alkaline pectinase by bacterial strains (Cao et al., 1992; Hoondal et al., 2002; Kavuthodi et al., 2018). Alkaline pectinase can be used for the treatment of pectineus substances containing wastewater from vegetable and food processing industries (Rehman et.al., 2015). *Bacillus licheniformis* is widely used for production of commercially important thermostable enzymes like protease and α-amylase which are stable at 105°C – 110°C for short period of times (Schallmey et al., 2004).

This study was carried out to screen the agricultural wastes as source of carbon for the production of pectinase by *B. licheniformis*. Furthermore, the concentration of agricultural wastes, pH, and temperature and fermentation period was optimised for maximum pectinase production.

Materials and Methods

Bacterial strain

B. licheniformis KIBGE-IB21 which was previously isolated for polygalacturonase production (Rehman et.al., 2012). The cells were harvested after 48 hours by centrifugation at 10,000 rpm for 15 minutes and the cell free supernatant (CFF) was used for enzymatic activities (Rehman et.al., 2015).

Production of Pectinase

One ml of overnight culture of *B. licheniformis* was transferred in the fermentation medium containing in the Erlenmeyer flask containing citrus pectin (1%), ammonium sulphate (0.14%), dipotassium hydrogen phosphate (0.6%), potassium dihydrogen phosphate (0.20%) and magnesium sulphate (0.01%), pH 7.0 and incubated at 37°c for 48 hours (Aslam et.al., 2021).

Screening of agricultural wastes for pectinase production

The agricultural wastes including rice bran, wheat bran, sugarcane bagasse, vegetable wastes, orange peels and date fruit wastes were screened as carbon source for the production of pectinase by *B. licheniformis*. 1.0% of above-mentioned agricultural wastes was individually used in replacement of citrus pectin in submerged fermentation technology.

Effect of agricultural wastes concentration on the production of pectinase

The effect of agricultural wastes concentration on the production of pectinase was analysed by using different concentration of wheat bran ranging from 0.1% to 3.0% in fermentation medium separately.

Effect of pH on pectinase production of pectinase

The effect of initial pH on the production of pectinase was screened by preparing fermentation medium with different pH ranging from pH 4.0 to pH 10 keeping the other parameters constant. The *B. licheniformis* was separately fermented in each medium for pectinase production.

Effect of temperature on pectinase production

The effect of temperature on pectinase production was investigated by incubating the *B. licheniformis*in different incubation temperature ranging from 20°C to 50°C keeping the other fermentation conditions constant.

Effect of fermentation period on pectinase production

The influence of fermentation period on the production of pectinase by *B. licheniformis* was assessed through incubating it for different incubation period ranging from 24 hours to 120 hours and keeping the other parameters constant.

Pectinase assay

The enzyme assay of pectinase is performed through colorimetric method using 0.5% citrus pectin as substrate. One ml of crude enzyme in cell free filtrate was added in 1.0 ml of pectin at pH 7.0 and incubated at 40°C for 15 minutes. After 15 minutes, 1.0 of reaction mixture was transferred to DNS (32 52 dinitrosalicylic acid) solution and boiled 10 minutes. Then absorbance of samples was recorded at 546 nm using UV-Visible spectrophotometer. One unit of pectinase is defined as "the amount of enzyme required to release 1 µmole of galacturonic acid per minute under standard assay conditions" (Rehman et.al., 2013).

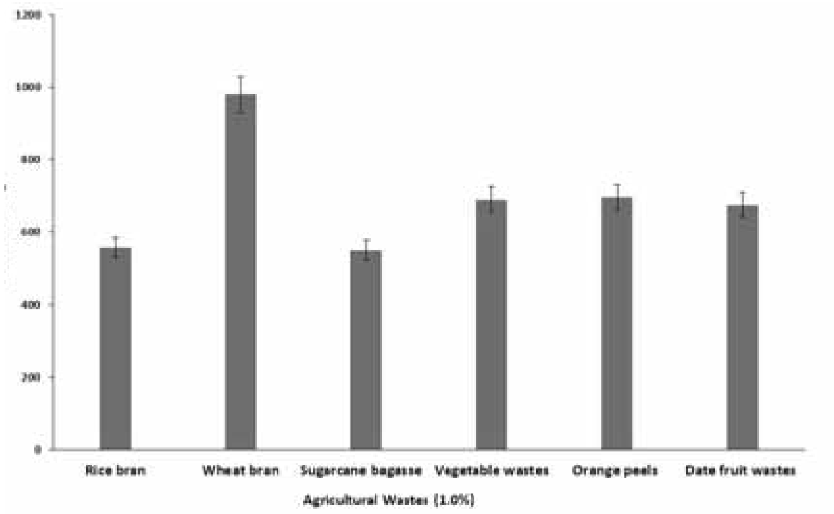
Estimation of total protein

The total protein of cell free filtrate was estimated using bovine serum albumin as a standard (Lowry et.al., 1951) for the calculation of specific activity of pectinase.

Results and Discussion

Screening of agricultural wastes for pectinase production

The agricultural wastes are considered the cheapest available natural carbon sources that can be utilised as substrate for the production of industrially important enzymes. Therefore, different agricultural wastes including rice bran, wheat bran, sugarcane bagasse, vegetable wastes, orange peels and date fruit wastes were screened as carbon source for the production of pectinase by *B. licheniformis* (Figure 1). It was observed that *B. licheniformis* produce significant amount of pectinase by utilization of agriculture wastes and high titre of pectinase production was observed in the medium containing wheat bran as carbon source as compared to others. The high nutritional value and surface area of wheat bran may be the reason to support the bacterial strains for high enzyme production. Furthermore, wheat bran has better air circulation and loose particle binding activities to facilitate the microbial penetration and enhance to extracellular enzyme production. Wheat milling industries in Pakistan produced large amount of wheat bran as by product and mostly available cheapest substrate for pectinase production using fermentation technology.



Figuree 1: The influence of various agricultural wastes on pectinase production by *B. licheniformis* (means ± S.E., n = 6)

Effect of wheat bran concentration on the production of pectinase

The optimising of substrate or carbon source concentration is very important to increase the enzyme production. The effect of wheat bran concentration on the production of pectinase by *B. licheniformis* was analysed by using different concentration of wheat bran (0.1-3%) in fermentation medium (Figure 2). The *B. licheniformis* increased the production of pectinase with the increased of wheat bran concentration and maximum production of pectinase was seen in the medium containing 1.5% wheat bran as a carbon source. Further increased of wheat bran concentration decreased the production of pectinase. The limitation of air and nutrients circulation in the microbial growth medium at higher concentration of wheat bran may negatively affect the microbial growth and enzyme production (Karim et al., 2015). The lower concentration of wheat bran seems to be not enough to provide chemical ecosystem to microbial strain for high titre production of pectinase.1.5% wheat bran is enough to provide physio-chemical environment to the *B. licheniformis* for the maximum production of pectinase. However, dual fermentation technology could be used to connect liquefaction with saccharification to improve the degradation of high concentration of agricultural wastes for microbial enzyme production (Betiku et al., 2013).

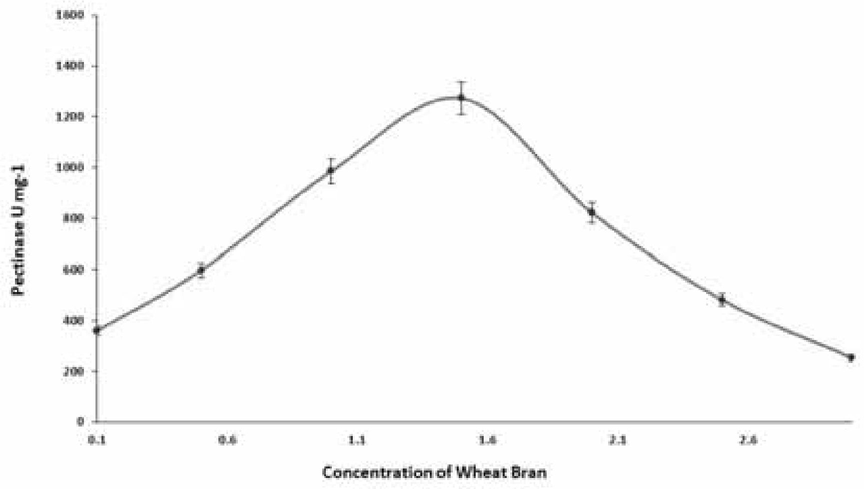


Figure 2: The influence of different concentration of wheat bran on pectinase production by *B. licheniformis* (means ± S.E., n = 6)

Effect of pH for pectinase production

The effect of pH on the production of pectinase by *B. licheniformis* was analysed by performing the fermentation under pH conditions. The initial pH of fermentation medium was adjusted from pH 4 to pH 10 keeping the other parameters of fermentation constant. The production of pectinase was gradually enhanced with increased of pH and maximum production was reached at pH 7.5 (Figure 3). The specific of pectinase was decreased when the pH was further increased and *B. licheniformis* declined more than 50% of its pectinase productivity at pH. The pH plays significant role to maintain the metabolic growth of microorganisms and it affects the environmental conditions that are relevant to microbial enzymes production. The pH refers to the reactivity of protons to control redox reactions, nutrients distribution, surface molecular association for signal transduction and extracellular activities of microorganisms (Ma et.al., 2016). Furthermore, the pH balances the salinity and composition of aqueous solutions need for biological system to perform different reactions as well as control the bioavailability of nutrients and trace elements (Hughes & Poole, 1991). pH 7.5 is optimum to provide environmental support to *B. licheniformis* to utilise wheat bran as substrate for maximum pectinase production.

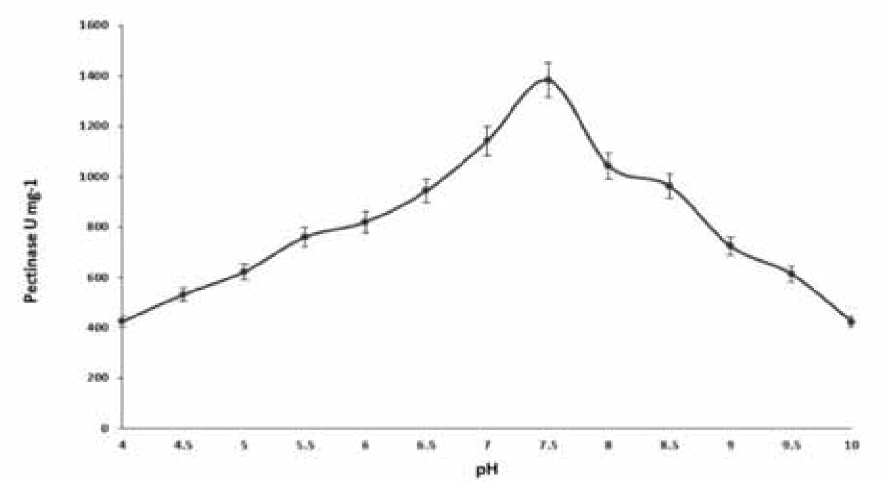


Figure 3: The influence of various pH on production of pectinase by *B. licheniformis* (means ± S.E., n = 6)

Effect of temperature for pectinase production

The effect of temperature on the pectinase production by *B. licheniformis* using wheat bran as substrate was analysed by incubating the bacterial strain in temperature ranging from 20°C to 50°C. The pectinase activity of *B. licheniformis* was increased with the increased of temperature and maximum pectinase production was obtained at 40°C (Figure 4). The pectinase production was decreased when the temperature was increased to 45°C and *B. licheniformis* lost more than 60% pectinase productivity at 50°C. The thermal denaturation of metabolic proteins of *B. licheniformis* at higher temperature may be the reason for reduction of pectinase production. The temperature is important factor to control the physiological of activities and growth of microorganisms through regulating their metabolic reactions (Turner et.al., 2007). The optimum temperature characterised the microbial strains into mesophilic or thermophilic. It has been concluded that the *B. licheniformis* is mesophilic in nature with reference of pectinase production using wheat bran as substrate.

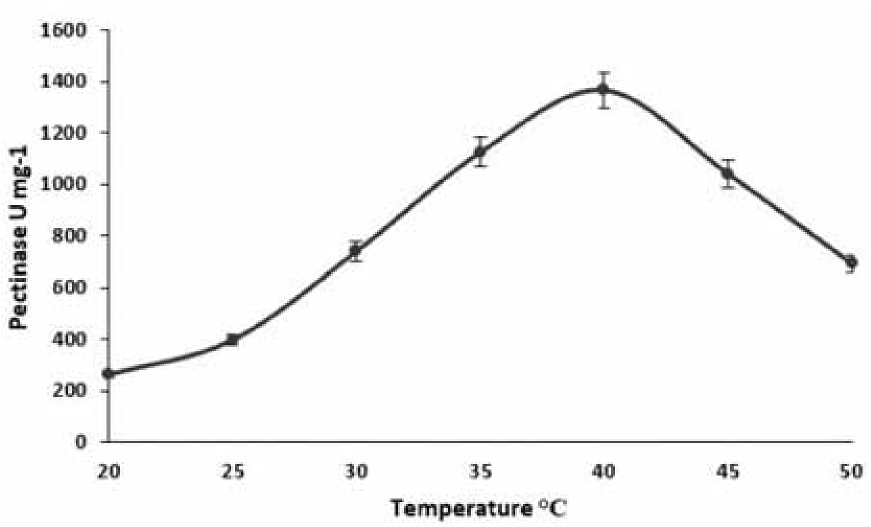


Figure 4: The influence of different temperature on the production of pectinase by *B. licheniformis* (means ± S.E., n = 6)

Effect of fermentation period for pectinase production

The effect of fermentation period on the production of pectinase was determined to calculate the total time needed by *B. licheniformis* to reach the exponential growth phase in term of pectinase production. The bacterial strain was incubated at 45°C for different time period ranging from 24 hours to 120 hours and after every 24 hours pectinase activity was measured to estimate pectinase production rate. *B. licheniformis* increased the production of pectinase with the increased of time and maximum production of pectinase was observed after 48 hours of fermentation time (Figure 5). The pectinase production was gradually reduced after 72 hours and the *B. licheniformis* almost lost its more than 90% pectinase productivity after 120 hours of incubations. In term of pectinase production, *B. licheniformis* reached its lag phase after 24 hours due to enough nutrient availability and the maximum multiplication of the strain is been seen after 48 hours with high titre pectinase production. The strain started the decline phase after 72 hours and almost lost its 90% growth after 120 hours because of declination of nutrients and environmental toxicities of metabolic by products.

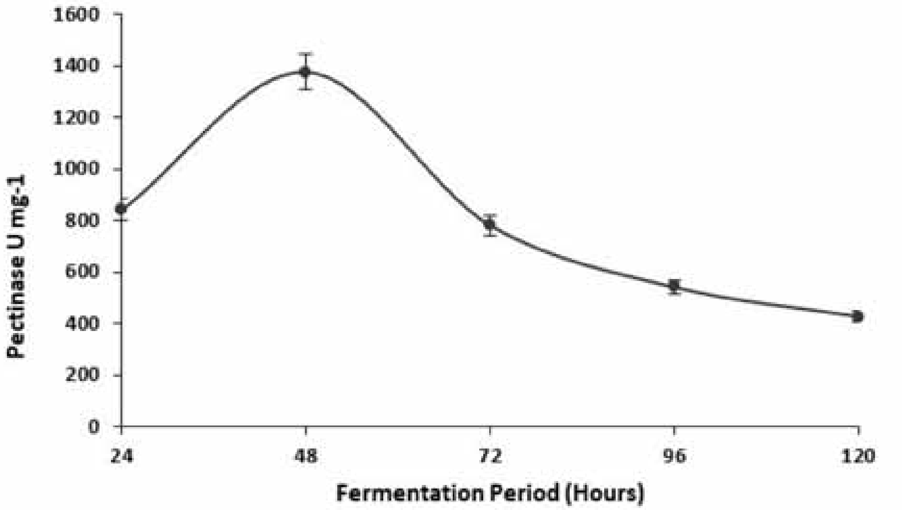


Figure 5: The influence of fermentation period on the production of pectinase by *B. licheniformis* (means ± S.E., n = 6)

Conclusion

In current study various agriculture wastes including wheat bran, rice bran, orange peels, date fruit wastes, vegetable wastes and sugarcane bagasse were tried as substrate for pectinase production by *B. licheniformis*. The wheat bran support higher production of pectinase as substrate by bacterial strain as compared to other agricultural and vegetable wastes. *B. licheniformis* produced maximum pectinase using 1.5% wheat bran as substrate at pH 7.0, 40°C temperatures and after 48 hours of incubation period. It has been concluded that the wheat branexhibited great nutritional potential as substrate for microbial strains for the production of commercially important enzymes.

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Macrofungi along Elevation Gradient in Palpa District, Lumbini Province, Nepal

Hari P. Aryal[1,2]* and Lal B. Thapa[1]
[1]Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2]Bio Resource and Bio Environmental Consultant Services Pvt. Ltd
***Email: hahariprasadaryal06@gmail.com**

Abstract

Macrofungi have diverse impacts on biology, ecology and economy. The floristic composition of Palpa district has been studied by several researchers, but the macrofungi which forms an important component of the ecosystem has been largely neglected in biodiversity studies. The present work is mainly focused on distribution of macrofungi in altogether 10 community forests along different elevation ranges (<500, 500-1000 and e" 1000 masl.) in Palpa district, Lumbini Province of Nepal. Total of 139 species of macrofungi were reported from the study area belonging to the Phyla Ascomycota and Basidiomycota. Less number of macrofungi (7.75%) were reported from the Phyla Ascomycota than Basidiomycota (92.8%). The Ascomycetous macrofungi were the members of the classes Leotiomycetes, Sordariomycetes and Pezizomycetes and orders Helotiales, Xylariales and Pezizales. Agaricomycetes, Dacrymycetes and Tremellomycetes were the classes of Basidiomycotous and among them, most of the members (127 species, 98.44%) represented the class Agaricomycetes. Agaricales was the richest having a total of 68 species (53.08%) followed by Polyporales (29 species, 22.31%) and Russulales (13 species, 10%). Comparing the three elevation zones, the richest elevation zone was > 1000 masl in the study area having 85 macrofungi species followed by 75 species from 500 to 1000 masl and 52 species from the elevation <500 masl. The diversity trend along elevation gradients show that it is the highest in the subtropical regions. Hence, this study creates an enthusiasm towards intensive exploration on the works.

Keywords: Ascomycota, Basidiomycota, Diversity, Habitats, Elevation range

Introduction

The fungi producing visible fruiting bodies mainly from the Phyla Ascomycota and Basidiomycota are referred as the macrofungi. They are important components of terrestrial ecosystem as they are directly or indirectly involved in the ecosystem processes (Dighton, 2018). Major processes in an ecosystem such as wood or organic matter decomposition, nutrient cycling and succession are run by the involvement of the macrofungi (Marcot, 2017). Economically, food and medicinal values of macrofungi have direct contribution to human livelihood at one hand and on the other hand their poisoning is another issue to be considered (McLellan & Brown, 2017; Devkota & Aryal, 2020; Bhambri et al., 2022).

Different bioclimatic zones in Nepal host large number of macrofungi. There are 1291 species of macrofungi belonging to 108 families and 357 genera with 165 species of Ascomycota and 1126 species of Basidiomycota (Devkota & Aryal, 2020). Along with, 34 endemic species, 159 edible, 74 medicinal and 100 poisonous species of macrofungi have been reported from the country (Devkota & Aryal, 2020). From different explorations by Devkota (2008); Aryal and Budhathoki (2015); Aryal et al. (2016); Acharya and Parmar (2016) have reported several macrofungi including many species of *Termitomyces* from tropical to temperate regions.

The scientific reporting of macrofungi in Nepal was started from 18th century by Lloyd (1808) and Berkeley (1838). Several areas across the country are yet virgin and need to be explored. Hence, this study was carried out in the Palpa district, Lumbini Province of Nepal with aim to document wild macrofungal species along elevation gradient.

Materials and Methods

Study area

This study was carried out in Palpa district, Lumbini Province, Nepal during 2016 to 2017 in rainy seasons. The district covers lower tropical to subtropical climates, the elevation ranging from 300 m to 2000 masl (Barnekow Lillesø et al., 2005). For the macrofungi survey, three elevation zones were selected (i) lower (< 500 masl) (ii) middle (500-1000 masl.) and (iii) upper (>1000 masl.). In each elevation zone, altogether three survey sites (Community Forests) were chosen as follows (Table 1, Figure 1).

Table 1: Survey sites selected for survey of macrofungi in Palpa district, Lumbini Province, Nepal

Elevation range	Survey sites	Location	Municipality or Rural Municipality
< 500 masl	1. Siddhababa Sacred Forest	27.34136N, 83.44643E	Tinau RM
	2. Dewrali Bhanjang CF and Kalika Mahila CF	27.36451N, 83.50969E	Mathagadhi RM
	3. Sirsire Danda CF	27.40499N, 83.51700E	Nisdi RM
500 to 1000 masl	4. Bayerdanda CF	27.39258N, 83.52119E	Purbakhola RM
	5. Chidipani CF	27.42415N, 83.54736E	Rainadevi Chhahara RM
	6. Rokaha CF	27.48835N, 83.56570E	
>1000 masl	7. Ratamata CF	27.51511N, 83.57220E	Rampur M
	8. Jogepani CF	27.52267N, 83.65399E	Mathagadhi RM
	9. Radhakrishna CF	27.5730N, 84.2200E	Rainadevi Chhahara RM

Note: CF: Community Forest, RM: Rural Municipality, M: Municipality

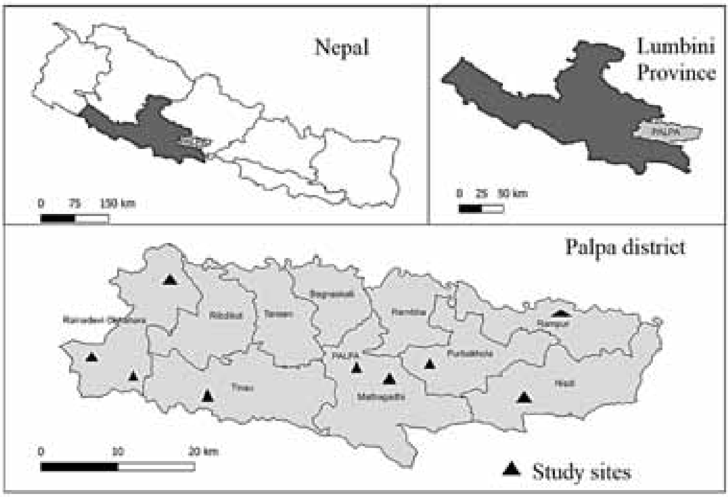

Figure 1: Study area showing survey sites in Palpa district, Lumbini Province, Nepal

In each survey site a total of nine plots of size 25 m × 25 m were sampled along horizontal transects (Elliott, 1971). All the macrofungi present in each plot were recorded. The voucher specimens were collected, well dried and packed in wax paper bags by wrapping with aluminum foil paper bags (Saini & Atri, 2000). The preserved specimens were thoroughly checked and dried at an interval of 1 to 2 months. The fleshy specimens were preserved in liquid medium (distilled water: alcohol: formalin ratio 70:25:5) (Hawksworth et al., 1995). Photographs and spore print were also taken in the field (Kuo, 2006).

The specimens were transported to laboratory of Central Department of Botany, Plant Pathology Unit, Tribhuvan University, Nepal for microscopic studies. The identification of the fungi was done based on diagnostic morphological characteristics and microscopic examinations. Standard and relevant literatures were consulted during identification (Pacioni, 1985; Purkayastha & Chandra, 1985; McKnight & McKnight, 1987). Online databases (Index Fungorum, and Tropicos.org) were also consulted. The voucher specimens are deposited in Tribhuvan University Central Herbarium (TUCH).

Results

Species composition

A total of 139 species of macrofungi belong to 46 families, seven (7) from Acomycota (Helvellaceae, Hypoxylaceae, Pezizaceae, Pezizellaceae, Pyronemataceae, Tricladiaceae and Xylariaceae) and 39 from Basidiomycota (Agaricaceae, Amanitaceae, Auriculariaceae, Bolbitiaceae, Boletaceae, Cerrenaceae, Cortinariaceae, Clavariaceae, Dacrymycetaceae, Entolomataceae, Fomitopsidaceae, Geastraceae, Gomphaceae, Hydnaceae, Hydnangiaceae, Hymenochaetaceae, Hymenogastraceae, Inocybaceae, Lycoperdaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Mythicomycetaceae, Omphalotaceae, Phallaceae, Phanerochaetaceae, Phyllotopsidaceae, Physalacriaceae, Polyporaceae, Psathyrellaceae, Rhizopogonaceae, Russulaceae, Schizophyllaceae, Sclerodermataceae, Sparassidaceae, Steccherinaceae, Stereopsidaceae, Strophariaceae, Tricholomataceae) were reported from the study area. Less number of macrofungi (7.75%) were reported from the Phyla Ascomycota (10 species) and the majority of macrofungi (92.8%) were reported from the Phyla Basidiomycota (129 species) (Table 6). The Ascomycetous macrofungi were the members of the classes Leotiomycetes, Sordariomycetes and Pezizomycetes and orders Helotiales, Xylariales and Pezizales (Table 2).

Table 2: Classes and orders in the Phylum Ascomycota

SN	Class	No. of species	SN	Orders	No. of species
1	Leotiomycetes	2	1	Helotiales	2
2	Sordariomycetes	4	2	Xylariales	4
3	Pezizomycetes	4	3	Pezizales	4
	Total	10		Total	10

Similarly, Agaricomycetes, Dacrymycetes and Tremellomycetes were the classes of Basidiomycota. Among them, most of the members (127 species, 98.44%) represented the class Agaricomycetes (Table 3). The Basidiomycetous macrofungi found in the study area belonged to 13 orders as given in Table 4. Among the orders, Agaricales was the richest having a total of 68 species (53.08%) followed by Polyporales (29 species, 22.31%) and Russulales (13 species, 10%). The orders Cantharellales, Boletales and Geastrales have 2-5% species and the orders Auriculariales, Dacrymycetales, Geastrales, Hymenochaetales, Phallales, Thelephorales and Tremellales were represented by single species (>1%) (Table 4).

Table 3: Classes in the Phylum Basidiomycota

SN	Class	No. of species	Percent
1	Agaricomycetes	127	98.44
2	Dacrymycetes	1	0.78
3	Tremellomycetes	1	0.78
Total		129	100

Table 4: Orders in the Phylum Basidiomycota

SN	Orders	No. of species	Percent
1	Agaricales	68	53.08
2	Polyporales	29	22.31
3	Russulales	13	10.08
4	Cantharellales	6	4.65
5	Boletales	4	3.10

6	Geastrales	3	2.33
7	Dacrymycetales	1	0.78
8	Auriculariales	1	0.78
9	Hymenochaetales	1	0.78
10	Phallales	1	0.78
11	Thelephorales	1	0.78
12	Tremellales	1	0.78
Total		129	100.00

Species distribution in three elevational ranges

Comparing the three elevation zones, the richest elevation zone was > 1000 masl in the study area having 85 macrofungi species i.e., 61% among 139 species reported. A total of 75 species (36%) was reported from 500 to 1000 masl and the lower elevation (<500 masl) was reported as less diverse region in terms of number of species (52 species i.e., 24%) (Figure 2).

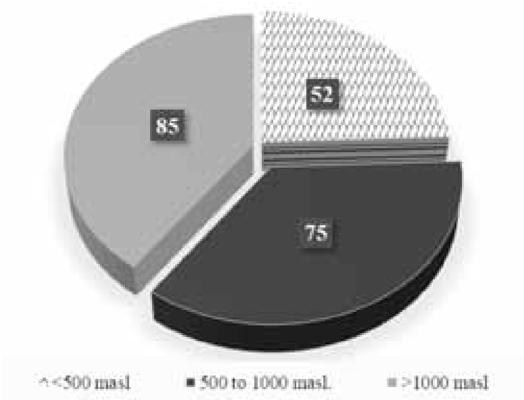

Figure 2: Number of macrofungi in three elevation ranges in Palpa district, Nepal

A total of 52 macrofungi were reported from the sampling sites below < 500 masl in the study area. Only the members of Basidiomycotina and class Agaricomycetes were reported. The largest order was Agaricales having 30 species (for example: *Agaricus sylvicola, Agaricus xanthodermis, Amanita caesarea, Auricularia auricula-judae, Coprinus sterquilinus, Leucopaxillus giganteus* etc.) followed by Polyporales (7 species such as *Coriolus versicolor, Fomitopsis vinosa, Lentinus tigrinus, Polyporus arcularius, Trametes hirsute* etc.) and Russulales (9 species such as *Lactarius acerrimus, Russula acetolens, R. foetens, R. japonica, R.*

mairei, R. rubra var. *sapida, R. sanguinaria, R. virescens, R. vitellina*). The order Cantharellales had only 3 species (*Craterellus cornucopioides, C. tubiformis* and *Clavulina amethystinoides*) and the orders Geastrales, Boletales, Gomphales, Hymenochaetales and Thelephorales were represented by single species each (Table 5 and 6).

Four species of Ascomycota were present in the elevation band 500 to 1000 masl belonging to the classes and orders Leotiomycetes (Helotiales), Pezizomycetes (Pezizales) and Sordariomycetes (Xylariales) (Table 5 and 6). Most of the macrofungal members (70 species) belonged to the Phylum Basidiomycota and classes Agaricomycetes in this elevation range while 6 species were of Ascomycota. The richest order in Basidiomycota was Agaricales having 41 species (example: *Agaricus sylvicola*, *Agaricus xanthodermis, Parasola plicatilis, Chlorophyllum rhacodes, Ramariopsis kunzei, Termitomyces arghkhachensis, T. palpensis, Tricholoma album* etc.) followed by Polyporales (12 species such as *Neoantrodia serialis, Daedalea quercina, Grifola frondose, Laetiporus sulphureus, Panus tigrinus, Parasola plicatilis* etc.) and Russulales (7 species). The Order Cantharellales had 3 species viz. *Cantharellus cibarius, Craterellus tubaeformis* and *Clavulina cinerea* and Boletales had 2 species. Rest, of the orders Dacrymycetales, Phallales, Thelephorales and Tremellales had only single species each (Table 5 and 6).

Similar to the mid elevation, three classes and orders viz. Pezizomycetes (Pezizales), Leotiomycetes (Helotiales) and Sordariomycetes (Xylariales) were represented in upper elevation i.e., >1000 masl with 8 macrofungi species (Table 5 and 6). In the same elevation, 39 species were reported from the orders Agaricales (*Bovista nigrescens, Candolleomyces candolleanus, Coprinus disseminates, C. comatus, Cortinarius caperatus, Entoloma albotomentosum, Hebeloma crustuliniforme, Laccaria ohiensis, Marasmius oreades* and several species of *Termitomyces* etc.). The members of Russulales included 11 species (*Russula nobilis, R. fragilis, R. adusta, R. rosacea, R. melliolens, R. sanguinaria* etc.), Polyporales (18 species), Boletales (4 species) and Geastrales (2 species). Rest of the three orders (Auriculariales, Hymenochaetales and Cantharellales) were represented by single species (Table 5 and 6).

Table 5: Number of species, orders and classes based on elevation gradient

Elevation band	SN	Class	Orders	No. of species
< 500 m	**Phylum: Basidiomycota**			
	1	**Agaricomycetes**	Agaricales	30
			Polyporales	7
			Russulales	9
			Cantharellales	3
			Geastrales	1
			Boletales	1
			Thelephorales	1
Total				**52**
500 - 1000 m	**Phylum: Ascomycota**			
	1	Leotiomycetes	Helotiales	1
	2	Pezizomycetes	Pezizales	3
	3	Sordariomycetes	Xylariales	2
	Phylum: Basidiomycota			
	1	Agaricomycetes	Agaricales	41
			Polyporales	12
			Russulales	7
			Cantharellales	3
			Boletales	2
			Dacrymycetales	1
			Phallales	1
			Thelephorales	1
	2	Tremellomycetes	Tremellales	1
Total				**75**
> 1000 m	**Phylum: Ascomycota**			
	1	Pezizomycetes	Pezizales	3
	2	Leotiomycetes	Helotiales	2
	3	Sordariomycetes	Xylariales	3
	Phylum: Basidiomycota			
	1	Agaricomycetes	Agaricales	39
			Russulales	11
			Polyporales	18
			Boletales	4
			Geastrales	2
			Auriculariales	1
			Hymenochaetales	1
			Cantharellales	1
Total				**85**

Among the macrofungi, 11 species were only confined to the lower elevation (<500 masl), 29 species to the mid elevation (500-1000 masl) and 43 species to the upper elevation (>1000 masl) (Figure 3, Table 5). The species found in the lower elevation were *Dissingia leucomelaena*, *Amanita caesarea, Auricularia cornea, Lepiota magnispora, Aspropaxillus giganteus* etc. (Table 6). Representatives of the mid elevation species were *Entoloma cetratum, Armillaria novae-zelandiae, Asterophora parasitica, Clavulinopsis fusiformis, Bovistella utriformis, Conocybe brunneola* etc. Similarly, *Daedaleopsis nipponica, Xylaria carpophila, Bovista nigrescens, Candolleomyces candolleanus, Rhodocollybia butyracea, Termitomyces robustus, Ganoderma* and *Russula* species in the upper elevation. List of species with elevation range is given in Table 6.

Interestingly, few numbers of macrofungi i.e., 9 species (*Cudoniella clavus, Coprinus sterquilinus, Limacella guttata, Ossicaulis lignatilis, Psathyrella* spp. *Lentinus tigrinus, Neofavolus alveolaris*) were not reported from the mid elevation whereas 22 species were reported from all elevation ranges (Figure 3, Table 6). Common members found in all elevation ranges were *Peziza domiciliana, Xylaria hypoxylon, Amanita fulva, Amanita pantherine, Amanita volvata, Lepiota spanista, Chlorophyllum rhacodes*, 5 species of *Termitomyces*, 7 species of *Russula* etc. (Table 6). Thirteen

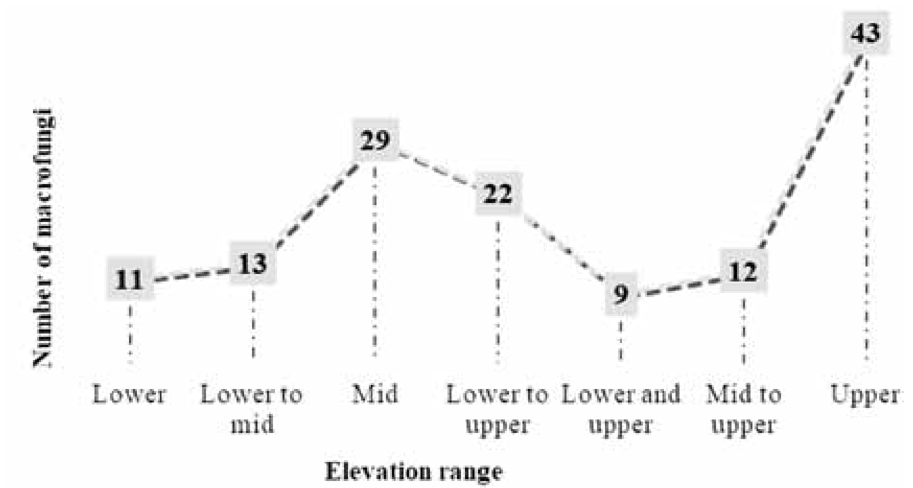

Figure 3: Distribution of macrofungi along elevation gradient in Palpa district, Nepal

species (*Agaricus sylvicola, Agaricus xanthodermus, Parasola plicatilis, Ramariopsis kunzei, Craterellus tubiformis, Ramaria flava,* 5 species of *Termitomyces* etc.) shared habitats of lower to mid elevation while 12 species (*Calycina citrina, Trichaleurina celebica, Geoscypha violacea, Bovista plumbea, Flammulina velutipes, Paragymnopus perforans* etc.) are distributed from mid to upper elevation. (Figure 3).

Table 6: List of macrofungi found in different elevation ranges in Palpa district, Lumbini Province, Nepal. Abbreviations: L = Lower elevation (<500 masl), M = Mid elevation (500-1000 masl), U = Upper elevation (> 1000 masl)

SN	Species Name	Elevation Range
Ascomycota, Pezizomycotina, Leotiomycetes, Helotiales		
1	*Calycina citrina* (Hedw.) Gray	M, U
2	*Cudoniella clavus* (Alb. & Schwein.) Dennis	L, U
Ascomycota, Pezizomycotina, Pezizomycetes, Pezizales		
3	*Dissingia leucomelaena* (Pers.) K. Hansen & X. H. Wang	L
4	*Geoscypha violacea* (Pers.) Lambotte	M, U
5	*Peziza domiciliana* Cooke	L, M, U
6	*Trichaleurina celebica* (Henn.) M. Carbone, Agnello & P. Alvarado	M, U
Ascomycota, Pezizomycotina, Sordariomycetes, Xylariales		
7	*Daldinia childiae* J. D. Rogers & Y. M. Ju	U
8	*Entonaema liquescens* Moller	M
9	*Xylaria carpophila* (Pers.) Fr.	U
10	*Xylaria hypoxylon* (L.) Grev.	L, M, U
Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricales		
11	*Agaricus sylvicola* (Vittad.) Peck	L, M
12	*Agaricus xanthodermus* Genev.	L, M
13	*Amanita caesarea* (Scop.) Pers.	L
14	*Amanita fulva* Fr.	L, M, U
15	*Amanita pantherina* (DC.) Krombh.	L, M, U
16	*Amanita volvata* (Peck) Lloyd	L, M, U
17	*Armillaria novae-zelandiae* (G. Stev.) Boesew	M
18	*Aspropaxillus giganteus* (Sowerby) Kuhner & Maire	L
19	*Asterophora parasitica* (Bull.) Singer	M
20	*Auricularia cornea* Ehrenb.	L
21	*Bovista nigrescens* Pers.	U
22	*Bovista plumbea* Pers.	M, U
23	*Bovistella utriformis* (Bull.) Demoulin & Rebriev	M
24	*Candolleomyces candolleanus* (Fr.) D. Wächt. & A. Melzer	U

25	*Chlorophyllum rhacodes* (Vittad.) Vellinga	L, M, U
26	*Clavulinopsis fusiformis* (Sowerby) Corner	M
27	*Condolleomyces candolleanus* (Fr.) D. Wacht. & A. Melzwr	L, U
28	*Conocybe brunneola* Kühner ex Kühner & Watling	M
29	*Coprinus comatus* (O.F. Müll.) Pers.	U
30	*Coprinus disseminatus* (Pers.) J. E. Lange	U
31	*Coprinus sterquilinus* (Fr.) Fr.	L, U
32	*Cortinarius caperatus* (Pers.) Fr.	U
33	*Deconica montana* (Pers.) P.D. Orton	M
34	*Entoloma albotomentosum* Noordel. & Hauskn.	U
35	*Entoloma cetratum* (Fr.) M.M. Moser	M
36	*Favolaschia calocera* R. Heim	M
37	*Flammulina velutipes* (Curtis) Singer	M, U
38	*Hebeloma crustuliniforme* (Bull.) Quél.	U
39	*Hymenopellis radicata* (Relhan) R.H. Petersen	M
40	*Inocybe geophylla* P. Kumm.	M
41	*Laccaria ohiensis* (Mont.) Singer	U
42	*Lacrymaria lacrymabundus* (Bull.) Pat.	L, U
43	*Lepiota magnispora* Murrill	L
44	*Lepiota spanista* Morgan	L, M, U
45	*Limacellopsis guttata* (Pers.) zhu L. Yang, Q. Cai & Y.Y. Cui	L, U
46	*Macrolepiota procera* (Scop.) Singer	M
47	*Marasmius oreades* (Bolton) Fr	U
48	*Marasmius rotula* (Scop.) Fr.	U
49	*Mycena vulgaris* (Pers.) P. Kumm.	U
50	*Mycetinis scorodonius* (Fr.) A.W. Wilson & Desjardin	M
51	*Omphalotus illudens* (Schwein.) Bresinsky & Besl	M, U
52	*Ossicaulis lignatilis* (Pers.) Redhead & Ginns	L, U
53	*Parasala plicatilis* (Curtis) Redhead, Vilgalys & Hopple	L, M
54	*Panaeolus papilionaceus* (Bull.) Quél.	U
55	*Paragymnopus perforans* (Hoffm.) J.S. Oliveira	M, U
56	*Phyllotopsis nidulans* (Pers.) Singer	U
57	*Psathyrella multissima* (S. Imai) Hongo	L, U
58	*Ramariopsis kunzei* (Fr.) Corner	L, M
59	*Rhodocollybia butyracea* (Bull.) Lennox	U
60	*Sagaranella gibberosa* (Jul. Schäff.) V. Hofst. Clemencon, Moncalvo & Redhead	M
61	*Schizophyllum commune* Fr.	L, M, U
62	*Termitomyces arghkhachensis* Aryal	M
63	*Termitomyces aurantiacus* (R. Heim) R. Heim	U

64	*Termitomyces eurrhizus* (Berk.) R. Heim	L, M
65	*Termitomyces badius* Otieno	M, U
66	*Termitomyces clypeatus* R. Heim	L, M
67	*Termitomyces fuliginosus* R. Heim	L, M, U
68	*Termitomyces globulus* R. Heim & Gooss.-Font.	L, M, U
69	*Termitomyces mammiformis* R. Heim	L, M
70	*Termitomyces microcarpus* (Berk. & Broome) R. Heim	L, M, U
71	*Termitomyces microcarpus f. santalensis* R. Heim	L, M
72	*Termitomyces palpensis* Aryal	M
73	*Termitomyces robustus* (Beeli) R. Heim	U
74	*Termitomyces schimperi* (Pat.) R. Heim	L, M, U
75	*Termitomyces striatus f. griseus* R. Heim	L, M, U
76	*Termitomyces striatus f. ochraceus* R. Heim	L, M
77	*Tricholoma album* (Schaeff.) P. Kumm.	M
78	*Tricholomopsis rutilans* (Schaeff.) Singer	U
Basidiomycota, Agaricomycotina, Agaricomycetes, Auriculariales		
79	*Dacryopinax spathularia* (Schwein.) G. W. Martin	U
Basidiomycota, Agaricomycotina, Agaricomycetes, Boletales		
80	*Buchwaldoboletus lignicola* (Kallenb.) Pilát	U
81	*Caloboletus radicans* (Pers.) Vizzini	M, U
82	*Rhizopogon roseolus* (Corda) Th. Fr.	U
83	*Scleroderma citrinum* Pers.	L, M, U
Basidiomycota, Agaricomycotina, Agaricomycetes, Catharellales		
84	*Cantharellus cibarius* Fr.	M
85	*Clavulina amethystinoides* (Peck) Corner	L
86	*Clavulina cinerea* (Bull.) J. Schröt.	M
87	*Clavulina coralloides* (L.) J. Schröt.	U
88	*Craterellus cornucopioides* (L.) Pers.	L
89	*Craterellus tubaeformis* (Fr.) Quél.	L, M
Basidiomycota, Agaricomycotina, Agaricomycetes, Geastrales		
90	*Geastrum fimbriatum* Fr.	U
91	*Ramaria aurea* (Schaeff.) Quél.	U
92	*Ramaria flava* (Schaeff.) Quél.	L, M
Basidiomycota, Agaricomycotina, Agaricomycetes, Hymenochaetales		
93	*Fomitiporia punctata* (P. Karst.) Murrill	U
Basidiomycota, Agaricomycotina, Agaricomycetes, Phallales		
94	*Phallus indusiatus* Vent.	M
Basidiomycota, Agaricomycotina, Agaricomycetes, Polyporales		
95	*Bjerkandera adusta* (Willd.) P. Karst.	U
96	*Cerrena unicolor* (Bull.) Murrill	U
97	*Coriolus hirsutus* (Wulfen) Lloyd	L

98	*Daedalea quercina* (L.) Pers.	M
99	*Daedaleopsis nipponica* Imazeki	M, U
100	*Ganoderma lucidum* (Curtis) P. Karst.	U
101	*Ganoderma neojaponicum* Imazeki	U
102	*Ganoderma valesiacum* Boud.	U
103	*Grifola frondosa* (Dicks.) Gray	M
104	*Laetiporus sulphureus* (Bull.) Murrill	M
105	*Lentinus arcularius* (Batsch) Zmitr.	L
106	*Lentinus tigrinus* (Bull.) Fr.	L, U
107	*Neoantrodia serialis* (Fr.) Audet	M
108	*Neolentinus lepideus* (Fr.) Redhead & Ginns	U
109	*Neofavolus alveolaris* (DC.) Sotome & T. Hatt.	L, U
110	*Nigroporus vinosa* (Berk.) Murrill	L, M
111	*Panus tigrinus* (Bull.) Singer	M
112	*Parasola plicatilis* (Curtis) Redhead, Vilgalys & Hopple	M
113	*Sparassis crispa* (Wulfen) Fr.	M, U
114	*Stereopsis burtiana* (Peck) D.A. Reid	U
115	*Steccherinum ochraceum* (Pers. Ex J. F. Gmel) Gray	U
116	*Trametes versicolor* (L.) Lioyd	L
117	*Trametes cinnabarina* (Jacq.) Fr.	M, U
118	*Trametes gibbosa* (Pers.) Fr.	U
119	*Trametes hirsuta* (Wulfen) Lloyd	L, M, U
120	*Trachyderma tsunodae* (Yasuda ex Lloyd) Imazeki	U
121	*Trachyderma* sp.	U
122	*Trichaptum abietinum* (Pers. ex J.F. Gmel.) Ryvarden	M
123	*Tyromyces sambuceus* (Lloyd) Imazeki	U
Basidiomycota, Agaricomycotina, Agaricomycetes, Russulales		
124	*Lactarius acerrimus* Britzelm.	L
125	*Russula adusta* (Pers.) Fr.	U
126	*Russula risigallina* (Batsch) Sacc	L
127	*Russula emetica* (Schaeff.) Pers.	U
128	*Russula foetens* Pers.	L, M, U
129	*Russula fragilis* Fr.	U
130	*Russula japonica* Hongo	L, M, U
131	*Russula melliolens* Quel.	L, M, U
132	*Russula nobilis* Velen	L, M, U
133	*Russula rosacea* (Pers.) Gray	U
134	*Russula sanguinaria* (Schumach.) Rauschert	L, M, U
135	*Russula virescens* (Schaeff.) Fr.	L, M, U
136	*Russula risigallina* (Batsch) Sacc.	L, M, U
Basidiomycota, Agaricomycotina, Agaricomycetes, Thelephorales		

137	*Thelephora palmata* (Scop.) Fr.	L, M
Basidiomycota, Agaricomycotina, Dacrymycetes, Dacrymycetales		
138	*Dacryopinax spathularia* (Schwein.) G.W. Martin	M
Basidiomycota, Agaricomycotina, Tremellomycetes, Tremellales		
139	*Tremella* sp.	M

Discussion

Among the largest orders reported in this study (Table 4), the order Agaricales includes the fungi that grow on wood or ground and composed of membranes which are found in different forms such as fleshy, woody, or gelatinous. The pore fungi Polyporales are involved in decaying wood which produce fruiting structure on standing dead wood, logs, and other woody debris (Money, 2016). The order Russulales includes russuloid agarics represent an independent evolutionary line of agarics such as Russula, Lactarius and their polyporoid and corticioid relatives. Majority of the members of these orders form ectomycorrhizal associations (Hongo, 1990; Hibbett, 2006; Likulunga et al., 2021). Similarly, the members of other genera such as Cantharellales, Boletales, Phallales, Tremellales, Dacrymycetales etc. are also very important genera for degrading organic materials to balance ecosystem (Floudas, 2021). Hence, the macrofungal diversity in the community forests of the Palpa district might have crucial role in balancing the forest ecosystems. Regarding the distribution of macrofungi along elevation range, the zone >1000m asl was found to be diverse than the lower elevations, thus showing the subtropical regions are favorable for macrofungal diversity. Because vegetation plays an important role in favoring species distribution. Besides these environmental factors such as precipitation, slope, aspects, canopy organic matters, soil types, soil pH, temperatures, humidity, altitude etc. also affect the growth and distribution of macrofungi.

The studies on distribution range of macrofungi, habitat analysis and documentation of macrofungi along elevation gradient are very scarce (Aryal, 2015). In this context, this study has analyzed diversity and distribution pattern of macrofungi in the Palpa district, Lumbini Province, Nepal. The study have identified the diversity trend along elevation gradients and the results indicated that the macrofungi diversity is high in the subtropical regions. Hence, this study has given a baseline idea for further studies on diversity and distribution pattern of macrofungi in other geographical regions along elevation or niche or habitat gradients in Nepal.

Conclusion

This study documents a total of 139 species of macrofungi from different community forests across different elevation zones of Palpa district, Lumbini Province, Nepal. This number of macrofungi indicates that the Palpa district is rich in macrofungi (mushrooms) diversity. The classes Leotiomycetes, Sordariomycetes and Pezizomycetes represented Ascomycota and the classes Agaricomycetes, Dacrymycetes and Tremellomycetes represented the Basidiomycota. Comparing to the Basidiomycota (129 species), the Phyla Ascomycota (10 species) has lesser number of species (Table 2 and 3). The fact indicates that the study habitats are suitable for diversification of mainly the Basidiomycetous macrofungi. This list of macrofungi is an important step towards producing a checklist of macrofungi in Palpa district. Hence the recent investigation creates an enthusiasm towards intensive exploration on the works and provides a background to appreciate the diversity and their relevance in ecosystem maintenance in general and human welfare in particular.

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Scientific Botanical Illustrations as a Taxonomic Tool for Flora writing

Neera Joshi Pradhan
Studio Petals, Khumaltar, Lalitpur, Nepal
Email: neera.joshi.pradhan@gmail.com

Abstract

Scientific botanical illustration is considered as an effective visual communication in plant science for the purpose of research, conservation and education of biodiversity. Visually appealing accurate scientific illustrations in the form of line drawings, sketches and different medium such as pen/ink line drawings in scale bar is widely acknowledged than hundreds scientific words. It is practiced and implemented worldwide as one of the valuable methods of taxonomic tool in modern botanical study. Scientific botanical illustrations require special skill of observation, description, analysis and depiction in sketching by hand and other instruments. This paper aims to draw an attention and stimulate an interest among botanists, taxonomists, researchers, its stakeholders and policy makers about the innovative scientific illustration in the present day. This technology is applied to describe the plant genus to species level with accurate line drawings of morphological structures and key characters of a plant. The comparative study of specimens thus aid to identify and find the possibilities of new species of a plant through illustrations. The illustrations are prepared after extensive study under the lenses directly from the live specimens from the field or dried specimens of the herbarium. There are rules and techniques to be followed while drawing diagrams. This paper accounts some examples of scientific illustrations of the flowering plants of Nepal, including *Sorbus himalaica, Sorbus arachnoidea* (Flora of Nepal, Vol III), *Ampelocissus rugosa, Pleione hookeriana* (CITES listed plants) and *Lilium nepalense* (Indigenous plant).

Keywords: Flowering plants, Line drawings, Herbarium specimens, Visual communication

Introduction

From the beginning of Paleolithic and Minoan period 1550 BC, botanical illustrations have been found on different media such as jars, coins and walls. The art of botanical illustrations was highly appreciated, popular and reached a peak on its achievement in 19th century. The time period from 1740 AD to 1840 AD was also known as the golden age of botanical art (Wilfrid, 1994). It was the great period of exploration, discovery and promotion of the botanical art. During that period, the plant scientists, explorers and collectors got together to record and study the preserved plant specimens in the form of accurate drawing.

In the beginning of 19th century, Francis Buchanan Hamilton, the first botanical collector of plants in Nepal (1802 to 1803 AD) discovered Nepalese flora and introduced new species to westerners. During his collection, Hamilton not only collected plants but also asked an Indian artist Haludar (Noltie & Watson, 2018) to draw some of the plants of Nepal.

After Buchanan, several British Botanists such as Nathaniel Wolff Wallich, Joseph Dalton Hooker and Isaac Henry Burkill extensively collected Himalayan plants from Nepal, Assam, Sikkim and China. Indian artists Vishnu Prasad and Gorachand have served Wallich (1786-1854) for the uncolored drawings of Nepalese plants when he was in mission to East India Company in 1813 and in charge of Calcutta Garden, India in 1824 (Noltie, 1999). When looking back, to Nepalese work of illustrative Botany, the "Chandra Nighantu" remains the classical documentation of Nepalese medicinal plants prepared during 1885-1901 AD, illustrated and authored by Pandit Ghana Nath Devkota. During the Prime Ministership of Bir Shumsher Rana, Pandit Ghana Nath Devkota started "Bir Nighantu" but did not complete due to death of Bir Shumsher. Later, it was revised and named into "Chandra Nighantu" according to reigning Prime Minister Chandra Shumsher Rana. Later in 1969 his son Kosh Nath Devkota had written "Nepali Nighantu" with some elaborated account and published by Royal Nepal Academy (IUCN, 2000), which has also included some illustrations of medicinal plants. Later Vaidya Mana Bajracharya (1928-2001) exhibited botanical paintings drawn and painted by himself on medicinal plants and published "Ayurvedic Medicinal Plants and General Treatment" in 1979 at Mahaboudha at his residence (Bajracharya, 1979). Likewise botanical illustrations were also reported, exhibited and published 37 botanical paintings of indigenous flowers from Kathmandu valley in which contemporary botanical art was introduced for the first time in Nepal in terms of presentation, composition with personal style in explaining the art of botany (Joshi Pradhan, 1999).

Today, a full comprehensive illustrative documentation of Nepalese flora is awaiting. Several botanical illustrators from different institutions and individuals have contributed scientific illustrations (line drawings) in Flora of Nepal Vol III (Magnoliaceae –Rosaceae) (2011). The inner front page of Flora of Nepal Vol III consists of color plate of *Sorbus himalaica* Gabrieljanin and some other illustrations of the species like *Sorbus arachnoidea* Koehne, were contributed by the author of this article (Watson et al., 2011).

There have been rarely noted implementation of scientific line drawings in Flora writing, however very few examples of scientific illustrations including *Ampelocissus rugosa* (Wall.) Planch. (Anonymous, 2073); *Ziziphus budhensis* Bhattarai & M.L.Pathak (previously treated as a new species) (Bhattarai, et al., 2015); CITES listed orchid species, *Pleione hookeriana* (Lindl.) Rollisson (Joshi Pradhan and Joshi, 2019) and *Lilium nepalense* D.Don, an indigenous plant was illustrated on the cover page of the book "Plants of National Botanical Garden" in 2016 (NBG, 2016). Likewise, the portraits of plants in water color medium by the author such as *Leucos ceptrumcanum* Sm., *Hedychium flavescens* have been displayed in "Flora of Nepal: a 200-year connection. A celebration of contemporary and historic plant portraits." and in collection of Florilegium of Royal Botanic Garden Edinburgh (2016) (https://stories.rbge.org.uk/archives/34621).

In modern botany, accurate visual sketches, illustrations, line drawings denoted in scale bar or different portraits of specimens drawn by hand in different mediums such as graphite, pen and ink and water color using scientific instruments are defined as scientific botanical illustrations. It can be applied by artists, scientists, biologists or anyone who have observational skill and talent to draw accurately from live or preserved dry specimens called herbariums. It is one of the challenging jobs to document the key characters of the plants in visual form as well as in scale by indicating them in scale bar.

Botanical illustrations play important role for the plant taxonomists to reach at a scientifically accurate illustration that will lead to identify plants by representing the salient features (Joshi Pradhan & Shakya, 2015). Therefore, one need to follow the strict rules and regulations while selecting the key characters of the specimen which will lead botanists and taxonomists to identify, and record research findings up-to genus and species level.

Accurate drawing is a challenging job. Drawn into reduced or enlarged form of characteristic structures in scale is the key method in describing the plants. The scale bar indicated in each characteristic structures are meant to reveal the real size of the specimen. The most important crucial fact is the observation skill, those when captured into visual language leads to analyze and identify the specimens by comparative studies in taxonomic botany. Therefore, the study of botanical illustrations is to unveil the real size of the plant specimen, to identify and describe key characters in visuals form of the genus and species in flora writing.

Materials and Methods

Tools for botanical illustration

- Plant Specimen's - i) live material ii) herbarium.
- Illustrator's divider, Microscope, Hand Lens, Hand loop and Scale.
- Dissecting Tools (different forceps, needles, brush and sharp blades.)
- Tracing Paper, Drafting Paper and Sketch book.
- Rotring pens 0.25,0.18 and 0.13.
- Lead pencil and special rubber.
- Archival paper/glass slides/cover slip for slides archival glue and detergent.

Illustration from Live specimens

For the illustration of fresh plant material i.e., branches with inflorescence for example here, *Leucos ceptrumcanum* (Figure 1) was collected in full bloom from the field and the material was studied under the microscope and then it was drawn. In case of *Pleione hookeriana* (Figure 2) the plant was observed carefully in the habitat and was drawn directly, and also a sketch was drawn in the green house by using pens, graphite pencils and water color, divider, hand lens and hand loop, while *Lilium nepalense* (Figure 3) with a full bloom was drawn in the field.

Figure 1: Live specimen: *Leucos ceptrumcanum* in the lab
Figure 2: *Pleione hookeriana* in the wild and field drawing
Figure 3: Live plant of *Lilium nepalense* & water color on paper

During drawing or sketching, measuring all parts are important, therefore, for less than 5mm structures, measuring devices such as hand loop should be used. The location of specimen using global positioning system (GPS), tag numbers and other important information should be noted. For the pen and ink medium scientific line drawings, necessary dissection, slide preparation of required materials should be carried out. The refined sketches and diagrams are verified from trained taxonomists or botanists after confirming the drafts of drawings; each drawing is then transferred to drafting paper by inking by different numbers of micron pens as required. For shadowing and showing different structures the conventional method like stippling was applied indicated by a scale bar. Scale bar should be drawn according to size of each drawing in reduced or enlarged format and maintained uniformity of the unit mentioned. All the illustrated morphological structures, habit sketches and other key characters are properly well labeled.

Here, live fresh specimens of *Schima wallichii* (DC.) Korth. (Figure 4), and *Aesculus indica* (Wall. ex Cambess.) Hook. (Figure 5) were also collected from the field and similarly as mentioned above, different morphological structures, important characters are drawn in professional art papers. Water color medium is applied for the painting the habit sketch as a portrait of a plant, and morphological characters, directly from the observation of the specimens either reduced or enlarged form by measuring devices.

Figure 4: Live fresh specimen of *Schima wallichii* and water color on paper
Figure 5: *Aesculus indica* with a blooming branch

Illustration from herbarium specimens

Different herbariums were studied for example, different species of *Sorbus*, and *Ampelocissus rugosa* (Figure 8). The characteristics features were noted with careful examination without damaging the specimen. Available live specimens of same herbarium specimen were also studied. For the species *Sorbus himalaica* (Figure 6), extra materials provided in the capsule of the herbarium sheet were used to reveal the detail morphological features, and key characters are drawn. The fresh material of the same herbarium was also examined for comparative observation simultaneously, if available. Similarly other herbarium specimens of different species *Sorbus arachnoidea,* and *S. hedlundii* C.K. Schneid., were studied thoroughly under the microscope. After the species are confirmed the sketches and diagrams are illustrated by pen and ink medium after several revisions in terms of size, shape and accuracy. The increased or reduced habit sketch, leaf, flower and their reproductive parts and diagrams are finalized and labeled (Figure 7 & 9).

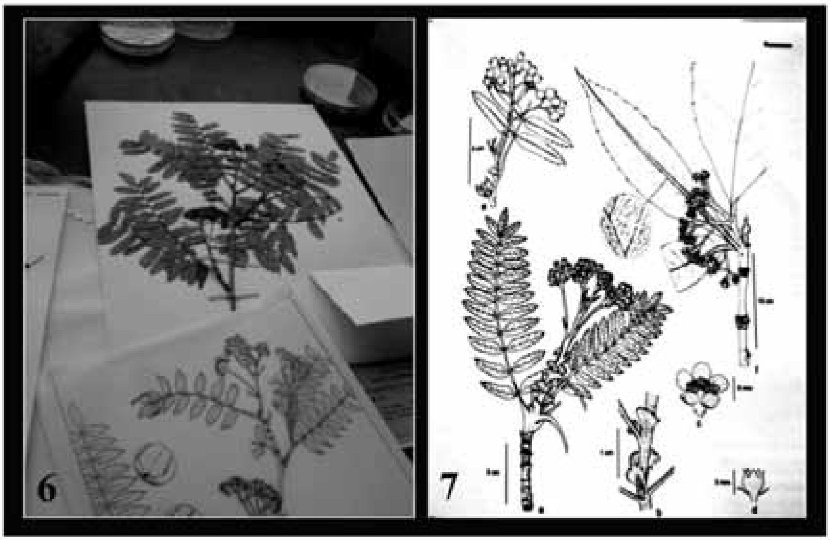

Figure 6: *Sorbus himalaica* Gabrieljan, Stanton,Sykes & Williams 815(31.5.1954), Tukucha of North west Nepal

Figure 7: *Sorbus arachnoidea* a) inflorescence and leaves, b) stipules, c) flower, d) hypanthium, e) infructescence and leaves of *Sorbus hedlundii,* f) inflorescence and leaves (llustrations by Neera Joshi Pradhan)

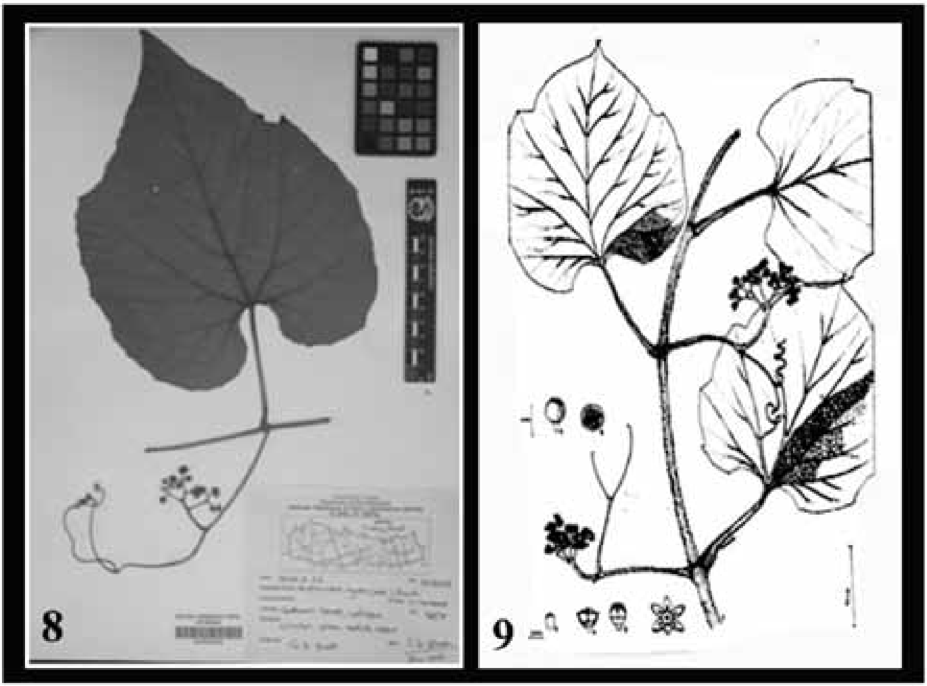

Figure 8: *Ampelocissus rugosa* (Wall.) Planch.KATH101974 Collection Number: 201103 Bhatta, G. D. Nepal ISO Standards: NP Godawari forest, Lalitpur Altitude :1650m
Figure 9: *Ampelocissus rugosa*. Illustration of Habit sketch and morphological structures

Results and Discussion

Line drawings and portrait with morphological structures of *Sorbus himalaica* (Figure 17), *Sorbus arachnoidea, Sorbus hedlundii* C.K. Schneid (Flora of Nepal VOL III) (Figure 7), *Ampelocissus rugosa* (Figure 9), and *Ziziphus budhensis* (Figure 11) were prepared from dried herbarium specimens as well as references available fresh specimens. Line drawings of *Leucos ceptrumcanum* (Figure 10), and *Pleione hookeriana* (Figure 13) were prepared from fresh specimens directly from the field or wild as well as in the lab. The comparative study of five species of *Rhododendron* (Figure 18) was also studied carefully several times through the processes mentioned above and was prepared and labeled accordingly. Botanical portraits with morphological key characters were prepared in watercolor medium of three live specimens, viz., *Lilium nepalense* (Figure 16), *Aesculus indica* (Figure 15) and *Schima wallichii* (Figure 14). *Schima wallichii* along with many other plants portraits by six botanical artists from Royal Botanic Garden, Edinburgh were exhibited on the occasion of exhibition "Flora of Nepal: a 200-year" connection. This was a celebration of contemporary and historic plant portraits." 2016 at Royal Botanic Garden, Edinburgh. (https://stories.rbge.org.uk/archives/34621;https://stories.rbge.org.uk/archives/21610.; http://plantdatabase.kath.gov.np/plants/search)

Illustrations from Live specimens

Figure 10: *Leucosceptrum canum.* Illustration of habit sketch and morphological structures

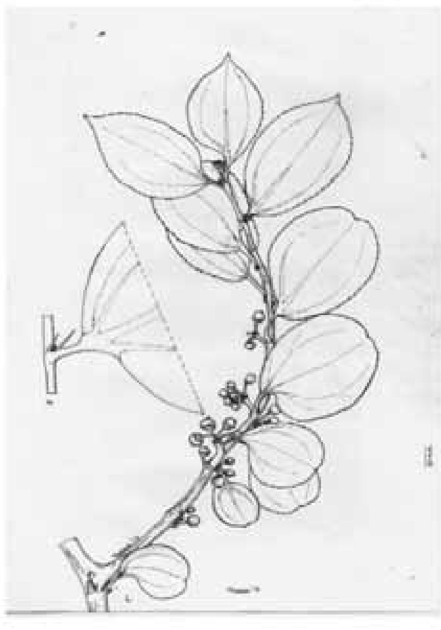

Figure 11: *Ziziphus budhensis.* Fruiting branch with leaves and portion of leaf attachment

Figure 12: *Pleione hookeriana* in the wild.

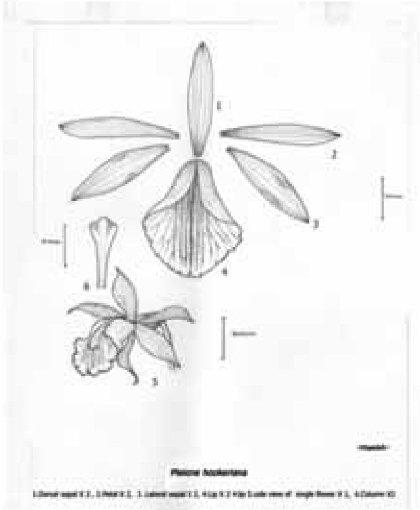

Figure 13: *Pleione hookeriana.* Illustration of morphological structures

Figure 14: *Schima wallichii*. Habit sketch and morphological structures, Water Color on paper, 15'' x 22'', Plant portrait by Neera Joshi Pradhan

Figure 15: *Aesculus indica.* Habit sketch and morphological structures, water Color on paper, 22''x30'', Plant portrait by Neera Joshi Pradhan

Figure 16: *Lilium nepalense.* Habit sketch and morphological structures, water color on paper, 15'' x 22''Plant portrait by Neera Joshi Pradhan

Illustrations from Herbarium specimens

Figure 17: *Sorbus himalaica* Gabrieljan STANTON, SYKES & WILLIAMS 815 (31.5.1954), Tukucha of North west Nepal

Figure 18: Comparative study of morphological structure and inflorescence of Rhododendron species 1. *Rhododendron pumilum* 2. *Rhododendron pendulum* 3. *Rhododendron anthopogan* 4. *Rhododendron barbatum* 5. *Rhododendron cowanianum* (Illustrations by Neera Joshi Pradhan)

Conclusion

Visual communication is fruitful in plant science for the purpose of research, conservation and education of biodiversity which aids to identify species in visuals as well as discovers the real size of specimens by measuring published images. Implementation of the scientific botanical illustration as a taxonomic tool can play a vital role in flora writing. Several species in plant diversity is unknown due to lack of comprehensive illustrative form. Plants have been described and identified with photographic images rather than scientific botanical illustrations, but the latter is very useful for revealing the exact size and detailed information.

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Medicinal Plant Species in Western Nepal: Potential for the Treatment of COVID-19 and Viral Diseases

Man D. Bhatt[1], Dirgha R. Joshi[2], Anil Thapa[3], Kanti P. Thapa[4], and Ripu M. Kunwar[4]*

[1] Department of Botany, Siddhanath Science Campus, Tribhuvan University, Nepal
[2] Department of Biochemistry, Tribhuvan University, Nepal
[3] District Ayurveda Office, Kaski
[4] Ethnobotanical Society of Nepal, Kathmandu
***Email: ripukunwar@gmail.com**

Abstract

Developing a supportive mechanism for antiviral efficacy and immune-modulatory attributes is highly pressing amid the Corona crisis and also the preference and recommendations of traditional medicines and medicinal plants nowadays. The present study focused to shortlist the potential antiviral medicinal plants in Nepal that could advance the therapies and save the life of many vulnerable communities. Total 100 medicinal plant species with 851 use reports were recorded for the treatment of 15 different viral diseases and boosting the human immunity. Of which the highest number of plant species were used to treat Asthma (46) followed by Cough (38 species), Hepatitis (37) and Common Cold (34). At species level, the highest use was recorded for *Curcuma longa* (58) followed by *Ocimum tenuiflorum* (40) and *Tinospora sinensis* (39). *Curcuma longa* was only one species with the highest values of Relative Importance (RI) and Use Value (UV). RI and UV, two indices were applied to test the cultural importance of plants in antiviral and immune-booster efficacies. Use reports > 2, UV ≥ 0.06 and RI > 0.4 shortlisted the first list into 100. The richness of useful medicinal plants showed that Kanchanpur district has 73 species out of 100 while Tanahu has the least 27 and the rest, Kaski, Kailali and Syangja, have over 50% (53-55 species). The finding of disproportionate results was attributed to the subjective sampling. An intensive comparison between the present and earlier findings (N=35) inferred that the about 60% medicinal plants species are potential for treatment for viral diseases including HIV-AIDs, COVID and asthma and for boosting the immunity. The highest Sorensens similarity index value, was found in *Zingiber officinale* (0.20) followed by *Terminalia chebula* (0.16) and *T. sinensis, C. longa, T. bellirica, O. tenuiflorum, Glycerhiza glabra, Phyllanthus emblica*, *Withania somnifera* and *Allium sativum* (0.13 each) had, revealing that these species were frequently cited for their anti viral efficacies. The recommended prospective list holds the potentiality for advancing the development of effective antiviral and immune-booster measures in Nepal.

Keywords: *Curcuma longa*, Ethnomedicinal plants, Hepatitis, Immuno-booster, Traditional healers

Introduction

Owing to diverse geography and bioclimates, Nepal has over 13,000 species of plants (Chaudhary et al., 2020), including about 7,000 species of flowering plants (Shrestha et al., 2018) and 2,500 species of medicinal and aromatic plants (Kunwar et al., 2021). Medicinal plants have long been used in Nepal for subsistence, household economy (Pyakurel et al., 2018), and traditional medicines (Adhikari et al., 2019). Ethnomedicine and its traditional knowledge are entrenched and they reveal how poor people in the distant locations utilize local plants for primary health care using the conventional procedures (Raut et al., 2012). Urbanization has expanded as a result of the population's exponential growth, which speeds up the spread of epidemics, as was the case with COVID-19 in Wuhan, China (Spernovasilis et al., 2021). The novel coronavirus (COVID-19) has been dubbed the global pandemic and is responsible for a concerning number of fatalities, particularly in vulnerable populations and nations worldwide. Nepal, a developing nation, is extremely vulnerable to the COVID-19 epidemic as it spreads. As of August 11th, 2022, the nation reported over 992,146 COVID-19 cases, resulting in 11,982 fatalities (MoHP, 2022). All known treatment options have been considered in the quest to stop this pandemic at all costs. In order to combat the COVID-19 epidemic, the WHO has advised employing traditional and indigenous medical techniques. Fortunately, medicinal herbs are the fundamental components of local medicines in isolated and high-altitude regions, and the traditional medical system is seen as a primary source of care, a primary lifeline, and frequently the first option (Dhar et al., 2002).

The preference locals have for traditional herbal medicines, the lack of alternatives available to them, poverty, and faith in the efficacy of folklore herbal cures all contribute to the higher percentage of ethnomedicinal applications of plants in rural areas (Bhattarai, 1992). Several medicinal plants, some of which have broad-spectrum antiviral action, have showed promise in the treatment of a number of viral illnesses (Taylor et al., 1996a). Treatments for the majority of viral illnesses only provide temporary relief from symptoms while you wait for your immune system to eradicate the virus. Recent research has investigated the immune-stimulatory effects of plant extracts with antiviral characteristics (Webster et al., 2006). The ability of plant extracts to strengthen the body's natural immune system defenses against viruses may use shared pathways.

The current study focused on cataloging and short listing potential antiviral medicinal plants in Nepal that could facilitate treatment and save the lives of many vulnerable communities who could not afford the allopathic medicines. This was done under the auspices of traditional medicines and medicinal plants as well as the essence of developing supportive mechanisms of antiviral efficacy and immune-modulatory attributes in the benefit to local and global human communities. Finding antiviral medicinal herbs that could benefit alternative and complementary treatments for COVID-19 (GoN, 2020; Gyawali et al., 2020; Khadka et al., 2021; Thapa Chhetri et al., 2021) is the main aim of this study. Finding the best immune-modulating and antiviral medicinal plants is one of the study's specific goals. Another is to inform communities and other interested parties about how to use these plants safely to treat COVID-19 and to persuade them to protect antiviral plants for the benefit of people and the environment.

Description of study area

Kailali-Kanchanpur is Far-western Tarai districts of Nepal having 57.6 percentage of literacy. These districts are expanded from the northern ridge of Chure to the southern boundary of lowland Tarai linked with India. Kailali-Kanchanpur districts, a lowland fertile Tarai plain area composed of alluvial soil are base of Chure, a young and fragile mountain range of the outer Himalayas. The districts are enriched with phyto-diversity and indigenous population (Singh et al., 2012). The altitude of districts ranges from 175 m in south to 1575 m in north and expanded from Mahakali River in west. The annual rainfall is recorded 1717 mm, temperature ranges from $3^{o}$to 42^{o} C, and the climate is tropical to mid temperate (DDC, Kanchanpur, 2008). The districts are gifted with a number of rivers, rivulets and gorgeous lakes like Betkot tal, Ranital, Pyara tal, Sundue tal, Jhilmila tal, etc. (Bhatt et al., 2023).

Similarly, Kaski-Syangja-Tanahun (KST) districts are the mid-western districts of Nepal located in Gandaki province having 97.1 percentage of literacy. The KST districts lie between 27°26'15" and 29°12'01" north latitude and 82°52'45" and 85 °12'01" east longitude and stretches between 1500m and 4500m altitude. The annual rainfall is recorded 146mm to 5284mm and temperature ranges from 9.67°C to 18.55°C and the climate is temperate to alpine. Peoples of the area are categorized in to Brahmin (21.6%), Magar (18.9%), Chhetri (13.4%), Gurung (11.4%), Kami (8.7%), Newar (4.1%) etc. Summer grazing, herding and collection of medicinal plants and their uses are still persistent with some adaptation (Messerschmidt, 1976) among Gurung communities in Panchase area of Kaski and Syangja districts (Pokharel et al., 2019). These traditional practices and ethnic community help hold and share the higher knowledge among the communities. Midhill is highly populated region of Nepal and characterized by rich plant biodiversity (MoFE, 2018) and medicinal plants (Kutal et al., 2021). Traditional practices and ethnic community help hold and share the higher knowledge among the communities.

Materials and Methods

The fieldwork for this study was carried out in five districts from western Nepal (Figure 1). Altogether 36 traditional healers (N=36), 12 from Kailali-Kanchanpur complex and 24 from Kaski-Syangja-Tanahun complex were interviewed.

Traditional healers (Vaidhyas) were subjectively selected following village level references and earlier publications. Snowball sampling followed up the sampling process once a couple of Vaidhyas and their peers were identified. Vaidhyas are traditional medicinal practitioners particularly of the western Nepal and adjoining areas of India (Kunwar et al., 2010). Three district Ayurvedic offices were collaborated for identifying the Vaidhyas of the district and facilitating the interview processes. Oral prior informed consent was obtained before executing the interview process. In order to assess plant use knowledge, a total of 36 healers (N = 36) including 25 men and 11 women age ranged between 45 years and 75 years were interviewed following Kunwar et al. (2019). While interviewing, traditional medicinal practitioners was encouraged to explain the locally available medicinal plants that are useful to treat the following 15 viral diseases (smallpox, the

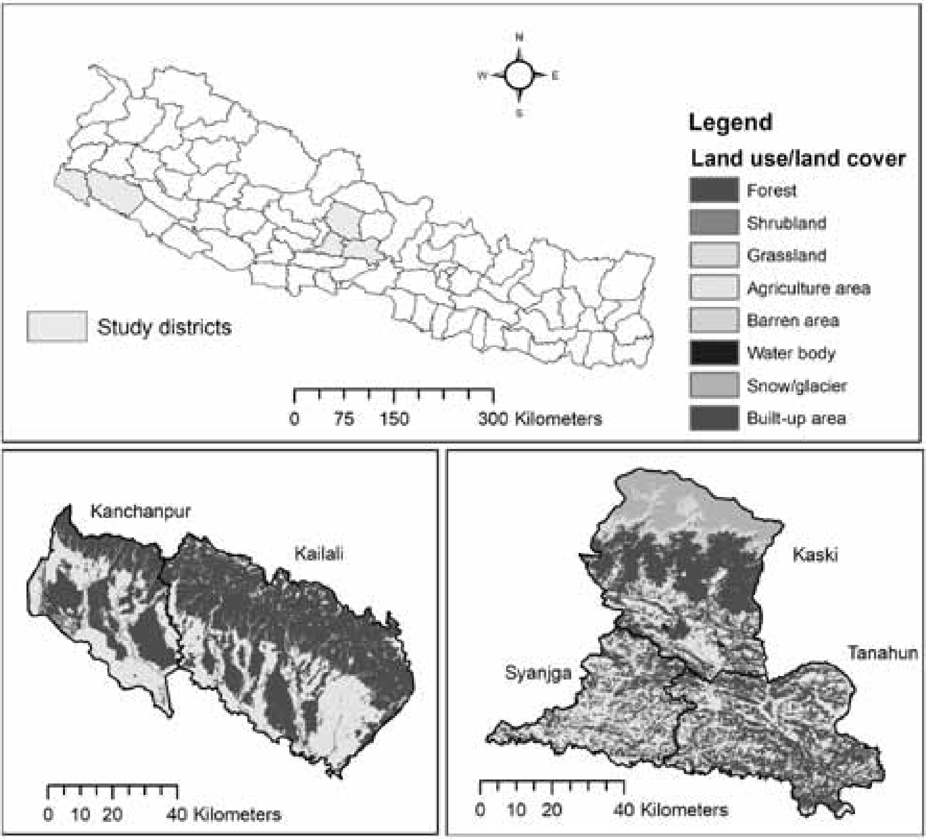

Figure 1: Study area map showing five study districts and their land use pattern

common cold (Rugha), cough (Khoki), asthma (Dum), different types of flu (respiratory problem), measles (Dadura), mumps (Galfule, Hade, Soth), small pox (Bifar, Sitala), chicken pox (Theula), rubella (Karu), hepatitis (Kamal pitta, Janundice), cold sores (Veduwa), polio (Pakshyaghat), and rabies, COVID, and HIV-AIDS), and to boost the immunity. In order to structure and facilitate the interview, semi-structured Nepali script checklist along the Nepali names of viral diseases was used with the help of a local assistant. The assistant was helpful in collecting the voucher specimens of plants described by the Vaidhyas and transcribing the vernacular dialects. Supplementary information was collected through informal meetings while staying in the village with communities, walking in the trails, and having morning tea at tea-vendors (Putnam, 1975). The local importance of each medicinal plant species cited was calculated using two different techniques: Use-Value (UV) and Relative Importance (RI). The UV was calculated using the formula UV = “Ui/n (Albuquerque et al., 2006; modified from Phillips & Gentry, 1993), where: Ui = the number of uses mentioned by each informant for a given species, n = the total number of informants. As such, the UV of a given plant is determined by the number of uses locally attributed to it in relation to the number of informants.

The relative importance index (RI), indicative of the level of popularity of medical applications in the country, was computed for each reported medicinal plant by using the formula RI = NP + ND + NCS (adapted from Bennet & Prance, 2000), where NP is obtained by the number of citations for a species divided by the total number of citations attributed to the most versatile species (the number of highest citations). ND is obtained by number of districts the species was referred to for a particular disease divided by the total number of districts referred to the most versatile species. NCS is the number of diseases treated by a given species divided by the total number of diseases treated by the most versatile species. Species with RI value 3.0 (the highest possible value) are those with the highest popularity in the country for treatment of those diseases studied. We performed binomial logistic regression of UV and RI in order to assess their association and to find the species of greater importance. We used Shapiro-Wilk normality test to check the data normality. Once the ethnomedicinal plants with higher UV and RI were identified, the plant species with use reports > 2, UV ≥ 0.06 and RI > 0.4 were qualified for further analysis to develop the potential list of antiviral plants.

To verify and validate the usage of species for further proceeding, the collected information was compared with the earlier studies (Kunwar, 2006; Dhami, 2008; Burlakoti & Kunwar, 2008; Singh, 2014; Bhatt & Shakya, 2015; Bhatt et al., 2021; Kutal et al., 2021) carried out in the same study area. Later a comparative assessment was carried out to short-list the most potential species. The following 35 literature pertinent to the antiviral efficacy and immune-stimulatory of medicinal plants: Zhang et al. (1995); Hamidi et al. (1996); Taylor et al. (1996b); Smit et al. (2000); Rajbhandary et al. (2001); Jassim et al. (2003); Cinatl et al. (2003); Wu et al. (2004); Watanabe et al. (2005); Wang et al. (2006); Rajbhandary et al. (2007); Balasubramanian et al. (2007); Fani et al. (2007); Zhang et al. (2007); Gewali and Awale (2008); Moyo et al. (2011); Nikomtat et al. (2011); Kala (2012); Singh et al. (2012); Ahmad et al. (2013); Sawale et al. (2013); Eidi et al. (2013); Mukharjee et al. (2013); Wu et al. (2015); Patel et al. (2016); Amber et al. (2017); Shen et al. (2017); Priya et al. (2017); Tiwari et al. (2018); Sahoo and Banik (2019); Joshi et al. (2020); GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Koshak et al. (2021); Pathak et al. (2021); were used for comparing the antiviral activities of plants. While comparing the findings of this and earlier studies, we calculated Sorensen similarity index. Sørensen index take values from 0 to 1. Using the index, a value of 0 means that the studies don't share any species for a treatment of a particular disease, and a value of 1 means have exactly the same species used.

The plant species referred to be useful by respondents were collected, pressed, dried, mounted and preserved based on standard methods as given by Forman and Bridson (1989). Before preservation, all the collected vouchers were examined and identified with the help of literature (Grierson & Long, 1983; Press et al., 2000; Bista et al., 2001). The voucher specimens were curated and identified with the peer copies deposited at Department of Botany, Siddhanath Science Campus, Mahendranagar. All voucher specimens were deposited at Department of Botany, Siddhanath Science Campus, Mahendranagar. Scientific name of plants and their families were verified with referring to the plant list (http://www.theplantlist.org/). The recommendations of the Code of Ethics of the International Society of Ethnobiology was followed during the data collection, recording and housing (https://www.ethnobiology.net).

Results and discussion

Disease and their treatment

Total 100 medicinal plants with 851 use reports were referred to the treatment of 15 different antiviral diseases and immunity booster. Of which the highest number of plant species were used to treat Asthma (46) followed by Cough (38), Hepatitis (37) and Common Cold (34) (Figure 2). The least number of medicinal plants were found to be used for HIV/AIDS as the HIV/AIDS cases were parsimoniously spelled out and consulted with the healers as the HIV/AIDS disease and victims were considered as socially disparaged. Total 29 medicinal plants were used for the support of treatment of COVID-19 including all 12 species except *Centella asiatica*, *Cuscuta reflexa* and *Citrus limon* (Table 1).

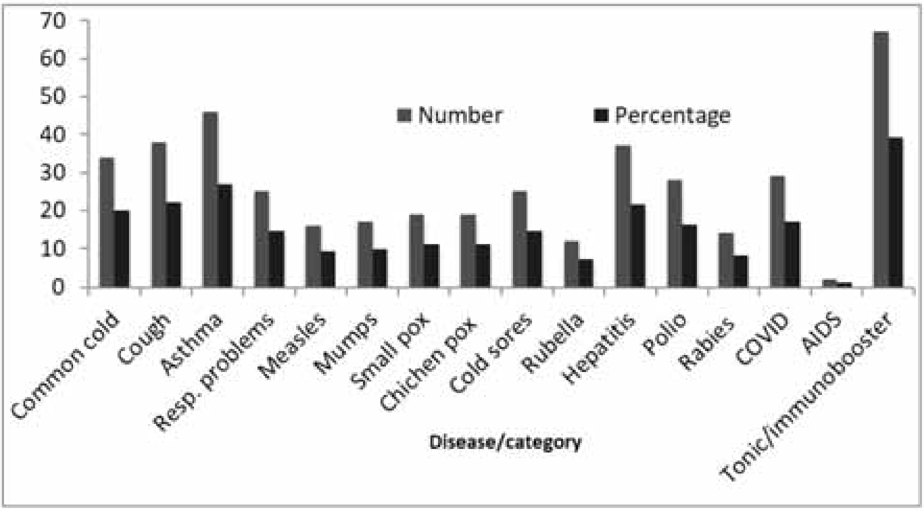

Figure 2: Number of plant species used against different diseases

At species level, the highest use was reported for *Curcuma longa* (58) followed by *Ocimum tenuiflorum* (40) and *Tinospora sinensis* (39). These three species consecutively hold the first, second and third order for both UV and RI indices. Twelve species, including the former three species, managed the top 10 position of both UV and RI (Table 1). The species UV ranged between 0.03 and 1.93 and RI between 0.3 and 3.0. *Curcuma longa* was only one species with the highest values of RI and UV.

Table 1. Species with the highest UV and RI values and their rank

Species	Use reports	Use Value (Order)		Relative Importance (Order)	
Curcuma longa	58	1.933	I	3	I
Ocimum tenuiflorum	40	1.333	II	2.272	II
Tinospora cordifolia	39	1.333	III	2.222	III
Centella asiatica	27	0.900	VI	2.132	IV
Zingiber offcinale	31	1.033	IV	2.117	V
Terminalia chebula	21	0.700	VII	2.112	VI
Piper nigrum	16	0.533	-	2.025	VII
Cuscuta reflexa	29	0.966	V	1.96	VIII
Phyllanthus emblica	19	0.633	VIII	1.910	IX
Artemisia vulgaris	15	0.533	-	1.841	X
Justicia adhatoda	18	0.600	IX	0.6	-
Citrus limon	17	0.566	X	0.56	-

Table 1 lists the ordination of the 10 most important species with respect to the values of RI and UV indices. Both considered three species (*C. longa, O. tenuiflorum* and *T. sinensis*) as highly important. The seven species with the highest values for both indices maintained the top seven positions while the order was different. However, some differences were observed when comparing all the values of two indices. For example, *Justicia adhatoda* and *Citrus limon* only appeared on the Use-Value list, probably because these two plants were used for a few subject diseases (five and four only) with the higher use reports. Byg and Balslev (2001) encountered a positive correlation between the Use-Value, and the number of use reports. We encountered the correlation coefficient of UV and use reports (r = 1) whereas that of RI and use reports was (r = 0.90). The absence of *C. limon* and *J. adhatoda* in the RI list was probably related to the fact that this technique only valorizes species with a significant number of uses, independent of the number of informants that may cite them. Likewise, *Piper nigrum* and *Artemisia vulgaris* appeared on the RI list but not the UV list (Table 1).

The reason for their presence on the RI list lies in the fact that these two species had used for the treatment of larger number of diseases, nine and seven respectively. RI evaluates the usefulness of species how it is being used in different categories (here in the sample diseases and sample districts). The Relative Importance (RI) emphasizes those plants that have the greatest absolute number of uses (Albuquerque et al., 2006). It depends less on use reports and more on popularity. The importance of a plant may not be solely determined by the number of uses it has, but also by how well known it is (Byg & Balslev, 2001). It can be used with small numbers of interviewees with heterogeneous background and diverse cultural knowledge. Ethnobotany of a landscape can be assessed by RI and that of a small site or a cultural group is well versed in UV. The UV places more emphasis on species that have many uses, even if these uses are reported by a few people (Silva et al., 2006). If a single person cites many uses for a given plant, the UV would seem to be idiosyncratic. Thus, UV is based on idea of consensus whereas the RI does in fact seem to be influenced by a combination of consensus, use categories (diseases in this study) and the species popularity. *Acorus calamus*, a species popular for treatment of hoarseness of sound, sore throat, cough and respiratory problem had the highest RI value in one study (Kunwar et al., 2016).

The Shapiro-Wilk normality test showed that the sample data precisely deviates from normality ($W = 0.63733$, $p < 2.2e\text{-}16$). The plot ($p = < 0.001$) of UV and RI regression shows that the values are significantly positively associated but R^2 value was 91 (Figure 3). It supports that RI does not reflect directly on the consensus.

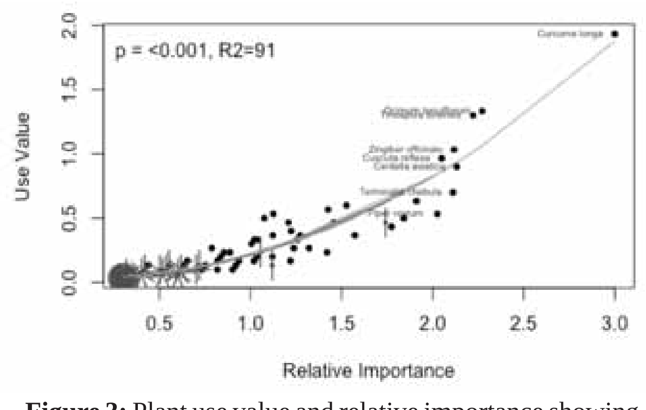

Figure 3: Plant use value and relative importance showing the highly convergent species

Comparative assessment of useful species

The use reports > 2, UV ≥ 0.06 and RI > 0.4 were considered for further analysis as the study has scope of finding out the most potential 10-15 antiviral plants in Nepal. Information from at least three respondents was counted as a common response and qualified for further quantitative analysis (Reyes-Garcia et al., 2006). This screening sorted out the 100 species (Annex 1).

The richness of useful medicinal plants showed that Kanchanpur district has 73 species out of 100 while Tanahu has the least 27 and the rest, Kaski, Kailali and Syangja, have over 50% (53-55 species). There are over 2,500 medicinal plants in Nepal (Pyakurel et al., 2018) and many are yet to be explored (Dhami, 2008; Kunwar et al., 2021). Among the species, there are over 500 antiviral medicinal plants and many are found in lowland Tarai (Bhatt & Kunwar, 2020). Kanchanpur and Kaski districts are enriched with the tropical and temperate medicinal plant species that are frequently being used in traditional medicines (Dhami 2008; Bhatt & Shakya 2015; Bhatt et al., 2021; Kutal et al., 2021). Out of 100 ethnomedicinal plants, the use reports for 41 species were matched with Dhami (2008) and 32 species with Singh (2014). The highest number of common useful species, 62 between Bhatt and Shakya (2015) and present study was attributed to the fact that both studies were carried out from the central part of Kanchanpur district. There were 23 common medicinal plant species between this study and Bhatt et al. (2021). An account of total 58 plant species was reported as useful in ethno-medicine of Kaski and Baitadi districts. Among them 28 species were common to our study and the high use values were reported to *Asparagus racemosus, Zanthoxylum armatum, Centella asiatica, Cyanodon dactylon, Terminalia chebula,* etc. (Kutal et al., 2021). Rajbhandari et al. (2001) reported 23 medicinal species widely used in the traditional medicine of Nepal for the treatment of infectious and other diseases on their in vitro antiviral activity against influenza virus and Herpes Simplex-Virus (HSV). Out of 23 medicinal species, five were similar to our study, which were *Asparagus racemosus, Bombax ceiba, Butea monosperma, Nyctanthes arbortritis* and *Zanthoxylum armatum. Bergenia ciliata*, *Urtica dioica* and *Zanthoxylum* *armatum* showed *in-vitro* antiviral activity against Herpes Simplex Virus type 1(HSV-1) (Rajbhandari et al., 2007).

Common antiviral plants

The ethnomedicinal uses of 100 species were compared with the earlier literature (Annex 1). An intensive comparison between this study findings and earlier reports (N=30) found the 59% medicinal plants species, potential for treatment for viral diseases including HIV-AIDs, COVID and boosting the immunity. The highest Sorensens similarity index value (0.20) was reported for *Z. officinale* followed by *T. chebula* (0.16) and *T. sinensis, C. longa, T. bellirica, O. tenuiflorum, G. glabra, P. emblica, W. somnifera* and *A. sativum* (0.13 each). This gave us idea that these species were commonly used for the treatment of viral diseases and immune boosting in western Tarai and midhill districts of Nepal. Account of such antiviral properties and the pharmacological actions of the medicinal plants from midhills and southern part of Nepal was also described in earlier studies (Taylor et al., 1996b; Rajbhandari et al., 2001, 2007; Gewali & Awale, 2008; Dhami, 2008). By plant family, Fabaceae and Lamiaceae were found dominant in possessing the antiviral plants, consistent to the findings of Pathak et al. (2021).

To sum up, described 59 medicinal plants, the first potential list holds the strong potential for advancing the pharmacology for antiviral and immune-booster plants in Nepal. Intensive review of the species' pharmacological action and consultation of traditional healers, and studies on phytochemical analysis and isolation of active compounds of these plants seem quite rational for further proceeding. The cumulative knowledge held by traditional healers regarding the use of medicinal plants is being eroded and could be lost. Considering the richness and the fact that their profession is not being perpetuated, this study urges strong measures to save both traditional healers and their knowledge and medicinal plants before they get threatened.

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Annex 1: Plant species, their uses for common viral diseases, use methods and references antiviral and immune-booster uses

Scientific name	Local name	Family	Diseases	Parts used	Similarity index	Antiviral and or immune-booster uses
Abrus precatorius L.*	Ratgedi	Fabaceae	Chicken pox, Measles, Polio	Leaves and stem		
Acacia catechu (L.F.) P.J.H. Hurter and Mabb*	Khair	Fabaceae	Cold sores, Cough, Tonic	Stem		
Achyranthes aspera L.*	Apamarga	Amaranthaceae	Common cold, Cold sore, Mumps, tonic	Leaves and stem	0.03	Mukharjee et al. (2013)
Acorus calamus L.*	Bojo	Acoraceae	Common cold, cough, asthma, respiratory problems	Seed	0.06	Singh et al. (2012); Khadka et al. (2021)
Aegle marmelos (L.) Correa*	Bel	Rutaceae	Asthma, covid, mumps, small pox, polio, rabies, tonic	Leaves and bark	0.03	Khadka et al. (2021)
Aesculus indica (Wall. ex Cambess.) Hook.*	Pangar	Sapindaceae	Mumps, polio, tonic	Fruit		
Ageratina adenophora (Spreng.) King and H. Rob.*	Banmara	Asteraceae	Chicken pox	Leaves		
Allium hypsistum Stearn**	Jimmu	Amaryllidaceae	Common cold, COVID	Leaves	0.06	Khadka et al. (2021); Rajbhandary et al. (2007)
Allium sativum L.*	Lasun	Amaryllidaceae	Common cold, cold sore, covid, polio	Stem	0.13	Fani et al. (2007); GoN (2020); Khadka et al. (2021); Pathak et al. (2021)
Allium wallichii D.Don **	Ban Lasun	Liliaceae	Common cold, rubella	Fruit		
Aloe vera (L.) Burm. F.*	Ghyu Kumari	Asphodelaceae	Cold sore, hepatitis, tonic	Leaves	0.06	Sahu et al. (2013); Khadka et al. (2021)
Alstonia scholaris (L.) R.Br.*	Chhatiwan	Apocynaceae	Hepatitis, polio, tonic	Latex		
Areca catechu L.*	Supari	Arecaceae	Asthma, small pox	Seed		
Argyreia hookeri Lour.**	Sikari lahara	Convolvulaceae	Cold sores, tonic	Leaves		
Artemisia vulgaris L.*	Titepati	Asteraceae	Asthma, comon cold, cough, resporratory problems, chicken pox, polio, COVID, AIDS	Leaves	0.06	Singh et al. (2012); Khadka et al. (2021)
Asparagus racemosus Willd. *	Satavari	Asparagaceae	Measles, tonic	Root	0.06	GoN (2020); Rajbhandary et al. (2007)
Azadirachta indica A.Juss*	Neem	Meliaceae	Asthma, comon cold, cough, resporratory problems, chicken pox, cold sore, hepatitis, COVID, tonic	Leaves	0.06	Khadka et al. (2021); Pathak et al. (2021)
Bauhinia vahlii (Wight and Am., Benth) *	Malu	Fabaceae	Chicken pox, polio	Leaves	0.03	Taylor et al. (1996a)

Bergenia ciliata (Haw.) Sternb. Revis. Saxifrag. Suppl. *	Silfode	Saxifragaceae	Respiratory problems, tonic	Stem	0.06	Rajbhandary et al. (2001); Rajbhandary et al. (2007)
Bidens pilosa L.*	Kalo kuro	Asteraceae	Cough	Leaves		Bhatt et al. (2021)
Blumea lacera (Burm.fil.) **	Sahastrabuti	Asteraceae	Asthma, cough, hepatitis, tonic	Leaves		
Bombax ceiba L.*	Simal	Malvaceae	Chicken pox, measles	Bark	0.06	Rajbhandary et al. (2001); Singh et al. (2012)
Callicarpa macrophylla vahl. **	Dahijalo	Lamiaceae	Asthma, cough, hepatitis	Bark		
Calotropis gigantea (L.) Dryand.*	Aank	Apocynaceae	Rabies, AIDS	Fruit		
Capsicum annuum L.*	Khursani	Solanaceae	Asthma, rabies	Fruit	0.03	Khadka et al. (2021)
Carica papaya L.*	Mewa	Caricaceae	Chicken pox, hepatitis	Fruit	0.10	Kala (2012), Khadka et al. (2021); Pathak et al. (2021)
Cassia fistula L.*	Raj briksha	Fabaceae	Cough, chicken pox, hepatitis, tonic	Fruit	0.03	Jassim et al. (2003)
Ceastrum noctumum L.*	Rato rani	Solanaceae	Cold sore, rubella	Flower		
Centella asiatica (L.) Urban*	Ghodtapre	Apiaceae	Asthma, cough, common cold, cold sore, hepatitis, mumps, respiratory problems, tonic	Leaves and stem	0.03	Khadka et al. (2021)
Cinnamomum camphora J. Presl**	Kapoor	Lauraceae	Asthma, respiratory problems	Fruits		
Cinnamomum tamala (Buch.-Ham.) T.Nees and C.H. Eberm.*	Dalchini	Lauraceae	Asthma, cough, common cold, cold sore, tonic	Bark	0.06	Gyawali et al. (2020); Khadka et al. (2021)
Cissampelos pareira Rhodes**	Musebelo	Menispermaceae	Mumps, small pox, COVID, tonic	Leaves	0.03	GoN (2020)
Citrus limon (L.) Osbeck*	Kagati	Rutaceae	Common cold, mumps, small pox, chicken pox, tonic	Leaves	0.10	Shen et al. (2017); Khadka et al. (2021); Pathak et al. (2021)
Cleistocalyx operculatum(Roxb.) Merr. Et L.M. Perry, 1937**	Kyamun	Myrtaceae	Common cold, respiratory			
*Cuminum cyminum*L.*	Jeera	Apiaceae	Common cold, mumps, COVID	Fruit	0.03	Khadka et al. (2021)
Curcuma longa L.*	Besar	Zingiberaceae	Asthma, cough, common cold, cold sore, chicken pox, respiratory problem, measles, mumps, rabies, rubella, COVID, AIDS, tonic	Stem	0.13	GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Pathak et al. (2021)
Cuscuta reflexa L*	Aakashbeli	Lauraceae	Asthma, cough, small pox,	Stem		

			hepatitis, measles, mumps, rubella, polio, tonic			
Cynodon dactylon (L.) Pers.*	Dubo	Poaceae	Rabies, common cold	Leaves		(Bhatt et al. (2021); Bhatt& Kunwar (2020); Dhami (2008)
Dactylorhiza hatagirea (D.Don) Soo*	Panchaule	Orchidaceae	Respiratory problems, tonic	Root	0.03	Khadka et al. (2021)
Datura metel L.*	Kalo dhaturo	Solanaceae	Asthma, polio, rabies	Seed		
*Dioscorea bulbifera*L.*	Geetho	Dioscoreaceae	Measles, mumps, tonic	Seed		
Drymaria cordata(L.) Willd. ex Schult.**	Abijalo	Caryophyllaceae	Common cold			Taylor et al. (1996b); Nikomtat et al. (2011)
Eclipta prostrat(L.)L.*	Bhiringi jhar	Asteraceae	Cold sore, hepatitis, rabies, tonic	Whole plant		
*Ficus auriculata*Lour.*	Timla	Moraceae	Asthma, tonic	Fruit		
*Ficus palmata*Frossk.*	Bedu	Moraceae	Rabies	Latex		
Ficus religiosa L.*	Pipal	Moraceae	Cough, respiratory problems, hepatitis	Fruit	0.03	Khadka et al. (2021)
Glycerrhiza glabra L.**	Jethimadhu	Fabaceae	Respiratory, COVID, tonic	Root	0.13	GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Cinatl et al. (2003)
Hordeum vulgare L.*	Jaun	Poaceae	Hepatitis, rabies	Leaves		
Jasminum amabile H.Hara**	Jai	Oleaceae	Chicken pox, cold sore	Leaves		
Juglans regia L.**	Okhar	Juglandaceae	Tonic		0.03	Eidi et al. (2013)
Justicia adhatoda L.*	Basingo	Acanthaceae	Asthma, cough, common cold, respiratory problems, COVID	Stem	0.06	Amber et al. (2017); Khadka et al. (2021)
Kalanchoe pinnata (Lam.) Pers.*	Kan karal, Pathhar chatta	Crassulaceae	Rabies, tonic	Fruit	0.03	Joshi et al. (2020)
Lepidium sativum L.**	Chamsur	Brassicaceae	Asthma, tonic	Leaves		
Mentha spicata L.*	Mentha	Lamiaceae	Respiratory problems, rubella	Leaves	0.03	Khadka et al. (2021)
*Moringa oleifera*Lam.**		Fabaceae	Cold sore, rubella		0.10	GoN (2020); Gyawali et al. (2020); Moyo et al. (2011)
Myrica esculenta Buch.-Ham. ex D.Don*	Kaphal	Myricaceae	Cough, hepatitis, tonic	Bark		
Neopicrorhiza kurroa Royle ex Benth.*	Kutki	Plantaginaceae	Asthma, respiratory problems, small pox, Hepatitis, polio, COVID, tonic	Stem	0.06	Smit et al. (2000); Wang et al. (2006)
*Nigella sativa*L.*	Kalo Jeera	Ranunculaceae	Asthma, cough, common cold, small pox, COVID, tonic	Leaves	0.06	Ahmad et al. (2013); Koshak et al. (2021); Khadka et al. (2021)

Nyctanthes arbor-tristis L.*	Parijat	Oleaceae	Smallpox, hepatitis	Leaves	0.06	Rajbhandary et al. (2001); Khadka et al. (2021)
Ocimum Sanctum L.*	Tulsi	Lamiaceae	Asthma, cough, common cold, small pox, hepatitis, COVID, tonic	Leaves	0.13	GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Pathak et al. (2021)
Ophiocordyceps sinensis (Berk.) G.H.Sung, Hywel-Jones and Spatafora*	Yarsa Gumba	Ophiocordycipitaceae	Tonic, asthma	Root	0.03	Gewali & Awalie (2008)
Oroxylum indicum (L.) Benth. Ex Kurtz*	Faltate	Bignoniaceae	Asthma, cough, hepatitis, polio, rubella	Fruit		
Paris polyphylla Sm.*	Satuwa	Melanthiaceae	Polio, respiratory problems, tonic	Whole plant	0.06	Zhang et al. (2007); Joshi et al. (2020); Khadka et al. (2021)
Paspalum scrobiculatum L.**	Kodo	Poaceae	Cold sore, chicken pox, measles, rubella	Seed		
*Peuraria tuberosa*Roxb. ex Willd.) DC.**	Bidari	Fabaceae	Tonic			Sawale et al. (2013); Patel et al. (2016)
Phyllanthus emblica L.*	Amala	Phyllanthaceae	Asthma, cough, common cold, cold sore, polio, COVID, tonic	Fruit	0.13	GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Pathak et al. (2021)
*Phyllanthus urinaria*L.*	Bhuiamlaki	Phyllanthaceae	Hepatitis			Wu et al. (2015)
Piper longum L.*	Pipala	Piperaceae	Asthma, cough, common cold, COVID, tonic, respiratory problems	Fruit	0.10	Hamidi et al. (1996); GoN (2020); Priya et al. (2017)
Piper nigrum L.*	Kalo mirch	Piperaceae	Asthma, cough, common cold, respiratory problems, small pox, hepatitis, COVID, tonic, rabies	Seed	0.03	Khadka et al. (2021)
Pogostemon benghalemsis (Burn.filo.). Kuntze**	Rudilo	Lamiaceae	Common cold	Root		
Psidium guajava L.*	Amba	Myrtaceae	Cold sore, mumps, hepatitis, tonic	Leaves	0.06	Balasubramanian et al. (2007); Khadka et al. (2021)
Punica granatum L.*	Anar	Lythraceae	Cough, tonic	Bark	0.10	Zhang et al. (1995); GoN (2020); Pathak et al. (2021)
Raphanus sativus L.*	Mula	Brassicaceae	Hepatitis	Root		
Rauvolfia serpentina (L.) Benth. ex Kurz*	Sarpagandha	Apocynaceae	Asthma, polio	Whole plant	0.03	Wu et al. (2004)
Reinwardtia indica Dumort*	Pyauli	Linaceae	Common cold, tonic	Leaves		
Rhododendron arboreum Sm. *	Guras	Ericaceae	Measles, small pox, hepatitis, rabies	Flower		
Rubus ellipticus Sm.*	Aiselu	Rosaceae	Asthma, common cold,	Fruit		

			resporatory problems, small pox, hepatitis, COVID			
Saccharum officinarum L.*	Ukhu	Poaceae	Hepatitis	Stem		
Sapindus mukorossi Gaertn.*	Reetha	Sapindaceae	Mumps	Stem		
Solanum xanthocarpum L.**	Kantakari	Solanaceae	Respiratory problems, tonic	Seed	0.03	GoN (2020)
Spilanthes calva Jacq.**	Marethi	Asteraceae	Hepatitis, tonic	Fruit		
Swertia chirayita L. (Roxb.ex Fleming) Karsten*	Chiraito	Gentianaceae	Common cold, cold sore, measles, chicken pox, small pox, rubella, hepatitis, polio, tonic	Leaves	0.06	Gyawali et al. (2020); Khadka et al. (2021)
Syzygium aromaticum (L.) Merr. and L.M. Perry*	Lwang	Myrtaceae	Common cold, cough, tonic	Fruit	0.03	Khadka et al. (2021)
Syzygium cumini (L.) Skeels.*	Jaamun	Myrtaceae	Asthma, tonic	Leaves	0.03	Khadka et al. (2021)
Terminalia alata Willd.**	Asna	Combretaceae	Cold sore, chicken pox, polio	Bark		
Terminalia bellirica (Gaertn.) Roxb.*	Barro	Combretaceae	Asthma, cough, cold sore, small pox, polio, COVID, tonic	Fruit	0.13	Taylor et al. (1996a); GoN (2020); Khadka et al. (2021); Pathak et al. (2021)
Terminalia chebula Retz.*	Harro	Combretaceae	Asthma, common cold, cough, cold sore, chicken pox, small pox, polio, COVID, tonic	Fruit	0.16	Taylor et al. (1996a); GoN (2020); Joshi et al. (2020); Khadka et al. (2021); Pathak et al. (2021)
Tinospora sinensis (Lour.) Merr.**	Gurjo bela	Menispermaceae	Asthma, cough, cold sore, chicken pox, respiratory problems, hepatitis, polio, COVID, tonic	Stem, leaves	0.13	Tiwari et al. (2018); GoN (2020); Gyawali et al. (2020); Khadka et al. (2021)
Toona sinensis(A.Juss.) M.Roem.**	Dallo	Meliaceae	Measles, rabies	Bark, latex		
Trachyspermum ammi (L.) Sprague ex Turrill*	Ajwain	Apiaceae	Cough, cold, respiratory problems, COVID, tonic	Fruit	0.03	Khadka et al. (2021)
*Trichosanthes cucumerina*L.**	Kal Indreni	Cucurbitaceae	Cold sore, polio		0.03	GoN (2020)
Trigonella foenum-gracecum L.**	Methi	Fabaceae	Asthma, respiratory problems	Seed	0.03	Khadka et al. (2021)
Urtica dioica L.*	Sisno	Urticaceae	Asthma, polio, rubella	Leaves	0.03	Rajbhandary et al. (2001)
*Vitex negundo*L.**	Simali	Lamiaceae	COVID, tonic	Leaves		
Vitis vinifera L.*	Kismis	Vitaceae	Asthma, tonic	Fruit	0.03	Khadka et al. (2021)
Withania somnifera (L.) Dunal*	Ashwagandha	Solanaceae	Respiratory problems, tonic	Whole plant	0.13	GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Pathak et al. (2021)

Woodfordia fruticosa (L.) Kurz**	Dhuniya	Lythraceae	Tonic	Flower		
Zanthoxylum armatum DC.*	Timur	Rutaceae	Cough, common cold, cold sore, respiratory problems, tonic	Fruit	0.10	Rajbhandary et al. (2001); Gyawali et al. (2020); Khadka et al. (2021)
Zingiber officinale Roscoe*	Aduwa	Zingiberaceae	Asthma, cough, common cold, cold sore, mumps, polio, COVID, tonic	Stem	0.20	Amber et al. (2017); Sahoo & Banik (2019); GoN (2020); Gyawali et al. (2020); Khadka et al. (2021); Pathak et al. (2021)
Ziziphus mauritiana Lam.*	Bayar	Rhamnaceae	Measles, mumps, chicken pox, small pox	Fruit		

Note: * indicate the plant species reported from Kailali-Kanchanpur district and ** indicate the plant species reported fromKaski-Syangja-Tanahun district

Summary of the International Conference

Biodiversity – the diversity of life on Earth – underpins human wellbeing, livelihood and sustainable development. Plants are recognized as a vital component of biodiversity and healthy ecosystems. They provide food, fiber, fuel, shelter, medicine and a range of ecosystem services. Yet, biodiversity is declining at an unprecedented rate. Globally, land-use change, resource use and exploitation, climate change, pollution, and invasive alien species are the direct drivers contributing to deterioration and loss of biodiversity. The potentiality of biodiversity can be tapped through bioprospecting – an exploration of biodiversity for new resources of social and commercial values, has multiple goals including conservation and sustainable use of biodiversity for commercial purposes like medicinal drugs, biochemical, cosmetics. Bioprospecting has huge potential for conservation, sustainable use and the welfare of the society and humankind. Hence, we must find better ways to manage biodiversity for the current and future generations. Sharing the existing research findings, innovations and experiences and exploring new opportunities on biodiversity and bioprospecting is important for a prosperous future.

In this backdrop, an "International Conference on Biodiversity and Bioprospecting" was organized on June, 22-24, 2022 at Hotel Crowne Imperial, Ravi Bhawan, Kathmandu by Department of Plant Resources (DPR), the Ministry of Forests and Environment (MoFE), Government of Nepal in collaboration with Universities (Tribhuvan University, Agriculture and Forestry University, Mid-West University) and prominent national (NAST, NTNC) and international (ICIMOD, IUCN Nepal, WWF Nepal, Zoological Society of London, Nepal) organizations.

Aim and Objectives

The main aim of the conference was to bring academicians, scientists, researchers, experts, managers and policy makers together to share and discuss existing problems, latest innovations, emerging opportunities and future priorities in the field of biodiversity and bioprospecting. The specific objectives of the conference were as follows:

- Share and exchange research findings, knowledge and experiences on biodiversity and bioprospecting among wider stakeholders;
- Encourage scientists and researchers by providing platform to present research findings to strengthen capacity in plant and allied science;
- Develop strategic direction for future research and development in the field of biodiversity and bioprospecting.

In the three-day program, the first day sessions were divided into (a) Inaugural session, (b) Plenary session, and (c) Technical (oral) session. In the second day there was two sessions (a) Plenary session, and (b) Technical (oral) session. On the last day also there was two sessions (a) Plenary session, and (b) Technical (poster) session followed by declaration and valedictory session. The overview of program schedule is as follows:

	DAY 1 Wednesday (June 23 2022)		
07:30 – 08:30	**Registration and Breakfast**		
09.00-11.00	**Inaugural session: Imperial Hall**		
11:15 – 12:15	**Plenary Session 1: Biodiversity and Bioprospecting (Imperial Hall)**		
12:15 – 13:15	**Lunch**		
	Technical Session 1		
13:15 – 15:15	**Bioprospecting and Innovation (Imperial Hall)**	**MAPs and Natural Product (Drishya Dawn Hall)**	**Plant Diversity Conservation (Drishya Dusk Hall)**
15:15 – 15:30	**Tea/coffee break**		
	Technical Session 2		
15:30 – 17:30	**Ethnobotany and TK (Imperial Hall)**	**Bioprospecting and Innovation (Drishya Dawn Hall)**	**Plant Diversity Conservation (Drishya Dusk Hall)**
18:30 – 8:30	**Reception Dinner**		
	DAY 2 Thursday (June 23, 2022)		
07:30 – 08:30	**Breakfast**		
9:00 – 10:30	**Plenary Session 2: Biodiversity and Bioprospecting (Imperial Hall)**		
	Technical Session 3		
10:40 – 12:00	**Biodiversity and Invasion (Imperial Hall)**	**Climate Change (Drishya Dawn Hall)**	**Plant Systematics (Drishya Dusk Hall)**
12:00 – 13:00	**Lunch**		
13:00 – 14:30	**Plenary Session 3: Ethnobotany and Bioprospecting (Imperial Hall)**		
	Technical Session 4		
14:25 – 16:05	**Ethnobotany and TK (Imperial Hall)**	**Bioprospecting and Innovation (Drishya Dawn Hall)**	**Diversity Conservation (Drishya Dusk Hall)**

16:05 – 16:20	**Tea/coffee break**		
	Technical Session 5		
16:20 – 17:45	**Plant Systematics (Imperial Hall)**	**Bioprospecting and Innovation (Drishya Dawn Hall)**	**Plant Diversity Conservation (Drishya Dusk Hall)**
	DAY 3 Friday (June 24, 2022)		
07:30 – 08:30	**Breakfast**		
9:00 – 10:30	**Plenary Session 4: Plant Diversity Conservation (Imperial Hall)**		
10:15 - 11:15	**Poster Session (Imperial Hall)**		
11:15 – 12:15	**Valedictory Session (Imperial Hall)**		
12:00 – 13:00	**Lunch**		
13:15 – onwards	**National Botanical Garden Tour (For Registered persons)**		

Inaugural Session

The inaugural session of the conference was chaired by Dr. Buddi Sagar Poudel, Director General, Department of Plant Resources (DPR). The conference was inaugurated by Chief Guest, Honorable Minister, Ramsahay Prasad Yadav, Minister, Forests and Environment. The session was also graced by the Special Guests, Ms. Saloni Pradhan Sigh, Honorable Member, National Planning Commission and Dr. Pem Narayan Kandel, Secretary, Ministry of Forests and Environment.

Mr. Saroj Kumar Chaudhary, Deputy Director General of DPR, delivered the introduction and welcome speech with the highlight of the objectives and relevance of conducting ICBB. The program was conducted by Dr. Samjhana Pradhan, Scientific Officer and Mr. Pramesh Bahadur Lakhey, Assistant Scientific Officer of DPR.

In the three-day program, the first day sessions were divided into (a) Inaugural session, (b) Plenary session, and (c) Technical (oral) session. In the second day there was two sessions (a) Plenary session, and (b) Technical (oral) session. On the last day also there was two sessions (a) Plenary session, and (b) Technical (poster) session followed by declaration and valedictory session. The overview of program schedule is as follows:

During the inaugural ceremony, two keynote addresses were delivered by Professor Emeritus Dr. Ram Prasad Chaudhary on "*Biodiversity* *conservation and bioprospecting: values, challenges and opportunities*" and Professor Emeritus Dr. Pramod Kumar Jha on "*SWOT observations on biodiversity research in Nepal*".

Key note address was followed by special remarks from Special Guests, Ms. Saloni Pradhan Singh, Honorable Member, National Planning Commission and Dr. Pem Narayan Kandel, Secretary, Ministry of Forests and Environment. Dr. Robbie Hart, Director of Missouri Botanical Garden, USA, Dr. Sanjay Kumar, Senior Principal Scientist of CSIR-Central Institute of Medicinal and Aromatic Plants and Dr. Sunil Babu Shrestha, Vice Chancellor of NAST gave short remarks and emphasized the relevance and importance of the conference in current global context. The inaugural address was delivered Chief Guest, Honorable Minister, Ramsahay Prasad Yadav, Minister, Forests and Environment assuring to implement the declaration of the conference.

On the occasion, a book entitled "Lichens of Nepal" published by National Herbarium and Plant Laboratories, DPR was launched by the Chief Guest, Honorable Minister. The vote of thanks and closing remarks was delivered by the Chair of the inauguration session, Dr. Buddi Sagar Poudel.

Plenary Session

During the three-day conference there were four plenary sessions, on three thematic areas 1. **Biodiversity and Bioprospecting**; 2. **Ethnobotany and Bioprospecting** and 3. **Plant Diversity Conservation**. Under the thematic area **Biodiversity and Bioprospecting**, papers were presented by Prof. Krishna Kumar Shrestha, Prof. Hari Datta Bhattarai, Dr. Robbie Hart, Dr. Sanjay Kumar, Dr. Bharat Babu Shrestha, and Dr. Uttam Babu Shrestha. In the second thematic area **Ethnobotany and Bioprospecting**, papers were presented by Dr. Rajendra Gyawali, Dr. Achyut Adhikari and Dr, Ripu Kunwar. In the third thematic area **Plant Diversity Conservation,** papers were delivered by Dr. Keshab Raj Rajbhandari and Prof. Dr. Sangeeta Rajbhandary.

Technical Session

Each plenary session was followed by technical sessions. There were altogether 5 technical sessions covering nine different thematic areas (72 oral and 17 poster sessions) were conducted. The thematic areas in the technical sessions were Bioprospecting and Innovation; MAPs and Natural Products; Plant Diversity and Conservation; Ethnobotany and Traditional Knowledge; Biodiversity and Invasions; Climate Change; Plant Systematics; and Diversity Conservation. Three parallel oral sessions were conducted at the same time in three different interactive halls. Each session was chaired by senior professors and eminent personalities of concerning fields and was accompanied by moderators to conduct the sessions.

Poster session

In the poster session on the third day 17 posters were displayed. As a motivation for the young researcher's, best oral presenter and best poster were awarded with certificate and Token of Appreciation in the valedictory session.

Space was made available for the exhibition. Total six organizations were invited for the exhibition (Table 1). They displayed and explained different herbal products to the guests and participants of ICBB.

Table 1: List of Exhibitors in ICBB 2022.

SN	Organization
1	Herbs Production and Processing Co. Ltd. (HPPCL)
2	Evans Life Sciences Pvt. Ltd.
3	Nature Nest Pvt. Ltd.
4	Bhaskar Herbaceuticals Pvt. Ltd.
5	Himalayan Bio Trade Pvt. Ltd.
6	Kanaya Herbs Store Pvt. Ltd.

Declaration

On the third day of the conference, opinion about the expectation, experience, and output of the conference was collected from the experts and chairs of the technical sessions. On the basis of the comments from the experts', a declaration was drafted and presented in the valedictory session. A Declaration Drafting Committee was formed including Prof. Dr. Krishna Kumar Shrestha, Prof. Dr. Sangeeta Rajbhandary, Dr. Bharat Babu Shrestha, Dr. Buddi Sagar Poudel, Mr. Saroj Kumar Chaudhary, Ms. Sangeeta Swar, and Mr. Subhash Khatri for the preparation of Kathmandu Declaration 2022. The declaration was endorsed in valedictory session.

Valedictory Session

The valedictory session was chaired by Dr. Buddi Sagar Poudel, Chairman of the conference organizing committee. The chief guest for the closing session was Dr. Pem Narayan Kandel, Secretary, Ministry of Forests and Environment. On behalf of the international and national participants, Dr. Rakesh E. Mutha and Dr. Nirmala Joshi represented and gave away their remarks regarding the conference. After the remarks from the participants, Chief Guest, Dr. Pem Narayan Kandel, gave away the award and Token of Appreciation to the best oral presenter to national presenter Yogendra Bikram Poudel from Central Department of Botany, Tribhuvan University and to Ms. Astha Chauhan from Himalayan Forest Research Institute (HFRI), Shimla, India an international participants. Best poster was awarded to Sabina Adhikari from Nepal and Waseem Raja from India. Chief Guest also handed over Token of Appreciation to the representative from the generous co-organizers and respected reviewers of the International Conference.

Prof. Dr. Sangeeta Rajbhandary, as a representative of Declaration Drafting Committee, presented the Kathmandu Declaration on Biodiversity and Bioprospecting in Nepal 2022, which was endorsed from the floor. Last but not the least, Chief Guest, Dr. Pem Narayan Kandel, Secretary, Ministry of Forests and Environment, gave away his special remarks. Finally, Chairperson Dr. Buddhi Sagar Poudel, Director General, Department of Plant Resources gave away his valedictory remarks thanking all participants, supporting organizations and individuals for making the conference a grand success and concluded the conference. Valedictory session was conducted by Mr. Pramesh Bahadur Lakhey, Assistant Scientific Officer of DPR.

Conference Committees

A strong conference committee leads to a successful and well-managed conference. It gives the event greater influence among the scientific community, and provides a wider reach for identifying quality topics, speakers and proper management for best outcome from the conference. There was an advisory committee, organizing committee, scientific sub-committees, management sub-committee, Technical committee and conference secretariat which are as follows:

Advisory Committee

Dr. Pem Narayan Kandel, Secretary, Ministry of Forests and Environment, GoN, Nepal

Dr. Sunil Babu Shrestha, Vice Chancellor, Nepal Academy of Science and Technology

Prof. Dr. Bir Bahadur Khanal Chhetri Dean, Institute of Forestry, Tribhuvan University

Prof. Dr. Balram Bhatta, Dean, Faculty of Forestry, Agriculture and Forestry University

Dr. Sudeep Thakuri, Dean, Faculty of Science and Technology, Mid-West University

Dr. Pema Gyamtsho, Director General, International Centre for Integrated Mountain Development

Dr. Prahlad Kumar Thapa, Country Representative, IUCN Nepal

Mr. Sharad Chandra Adhikary, Member Secretary, National Trust for Nature Conservation

Dr. Ghanshyam Gurung, Country Representative, WWF Nepal

Dr. Hem Sagar Baral, Country Representative, ZSL Nepal

Organizing Committee

Dr. Buddi Sagar Poudel, Director General, Department of Plant Resources Representative, Ministry of Forests and Environment, GoN

Prof. Dr. Santosh Rayamajhi, TU/ IOF

Mr. Shreehari Bhattarai, Assistant Professor, Faculty of Forestry, AFU
Ms. Savita Dhungana, Assistant Professor, Central Campus of Science and Technology, Mid-West University
Dr. Kanti Shrestha, Chief Scientific Officer, NAST
Dr. Sunita Chaudhary, Ecosystem Services Specialist, ICIMOD
Ms. Racchya Shah, Program Manager, IUCN Nepal
Dr. Chiranjibi Prasad Pokheral, Program Manager, NTNC
Dr. Ananta Bhandari, Head of Forests and Landscape Program, WWF Nepal
Dr. Bhagwan Raj Dahal, Deputy Country Manager, ZSL Nepal
Ms. Sangeeta Swar, Under Secretary (Tech.), DPR

Scientific Sub-Committee	**Management Sub-Committee**
Coordinator: Mr. Saroj Kumar Chaudhary Member: Mr. Dipak Lamichhane Member: Mr. Madhu Shudan Thapa Magar Member: Mr. Devi Prasad Bhandari Member: Dr. Gaurav Parmar Member: Dr. Tirtha Raj Pandey Member Secretary: Dr. Seerjana Maharjan	Coordinator: Ms. Sangeeta Swar Member: Ms. Jwala Shrestha Member: Mr. Subhash Khatri Member: Mr. Sishir Panthi Member: Mr. Anjani Kumar Adhikari Member Secretary: Mr. Dinesh Baral

Technical Committee (Computer, recording, Information and Documentation Section)
Mr. Jagya Prasad Regmi
Ms. Pradipika Acharya
Mr. Krishna K. Shah
Ms. Nishant Shrestha
Ms. Pratikshya Shrestha
Mr. Sujit Kumar Chaudhary
Mr. Krishna Prasad Poudel
Mr. Arjun Parajuli
Mr. Arjun Neupane
Mr. Sudip Pudasaini

Conference Secretariat
Dr. Seerjana Maharjan
Mr. Dinesh Baral

KATHMANDU DECLARATION

International Conference on Biodiversity and Bioprospecting (ICBB) June 22-24, 2022, Kathmandu Nepal

The International Conference on Biodiversity and Bioprospecting (ICBB), organized in Kathmandu, Nepal from June 22-24, 2022 (2079/8/10), Kathmandu, Nepal.

We the participants of the above conference, from the countries of India, Nepal, Pakistan, and USA

- *Noting* that although Nepal occupies only 0.1% of the global area, it is exceptionally rich in biodiversity due to its extreme topographic and climatic variability and characterized by a high number of endemic and endangered species of flora, is a centre of high genetic diversity of food and agricultural crops, livestock, and their wild relatives, and *that* local communities play a key role in the conservation and sustainable use of biological diversity;
- *Acknowledging* that the culturally diverse peoples in Himalayan region maintain time-tested indigenous knowledge and traditional practices pertinent to natural resources for their livelihood;
- *Recognizing with concern* however, that the region with its fragile ecosystems faces a variety of anthropogenic threats to biodiversity, in particular land-use changes, global climate change, deforestation, forest fires, illegal logging, and habitat fragmentation, and massive encroachments of invasive species have resulted into biodiversity loss and the loss of associated nature's contribution to people that has serious negative impact on the livelihoods particularly of the indigenous people and local communities;
- *Further recognizing* that there are important gaps in knowledge and information on biodiversity, nexus among drivers of biodiversity losses, and nature's contribution to people;
- *Further noting* that inadequate data and funds, and ambiguous governance constitute additional challenges to attain the goals of biodiversity conservation and sustainable development;

- *Recognizing further* the economic, ecological and cultural values of biodiversity and the need for its management through the participation of indigenous people and local communities, governments of different levels (local, province and national), and private sectors;

Expressing gratitude to the Honorable Minister and Secretary of the Forests and Environment; the organizers and co-organizers of the Conference, as well as the people for hosting the Conference and offering their warm hospitality;

Therefore, the declaration of the Conference has been passed, and it is here followed by Activities to Accomplish the Recommendations and Background of the Conference.

KATHMANDU DECLARATION

1. *Endorses* the decisions adopted by the CBD on the Nagoya Protocol on Access and Benefit Sharing (ABS), and by COP 26 of the IPCC.

2. *Recommends strongly* that Governments of Nepal to develop their national and provincial targets for conservation and sustainable use of biodiversity in accordance with their national and provincial priorities, and develop related indicators for assessing progress; the indicators as far as possible being measurable, time-bound and achievable, and building on the successes achieved towards the Post 2020 Global Biodiversity Framework Targets*[draft]*.

   Recommends scientific and research organizations to endeavor to develop and carry out research that respond to the needs of governments as identified:
   - Government's commitment to accomplish online and printed version of remaining volumes of Flora of Nepal
   - Lead for the Flora of Nepal should be taken by DPR
   - Initiation of Provincial and Protected Areas flora
   - Conservation Assessments for Red List Categorization of Floral diversity for National Red Data List
   - Conservation Strategies for Endemic and Threatened plants and their habitats
   - Prioritization of Biodiversity Exploration in Less Explored Areas

- Integrated approach to effectively address the problems of global environmental changes including land use and land cover change, over-exploitation of biological resources, climate change, pollution and biological invasions
- Conservation of biological resources, sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources
- An exploration of biodiversity for new resources of social and commercial value
- *in-situ* and *ex-situ* conservation of prioritized species
- Endorse the developed 'National Bio-prospecting strategy and Action Plan'
- Prioritize capacity building on bio-prospecting
- Value-addition, research and development for innovation
- Documentation of ethno-botanical and traditional knowledge in National depository system.

4. *Urges* scientific and research organizations to assist governments in assessing the recognized issues mentioned above along with the present and future impacts of global environmental change on biodiversity, nature's contribution to people and good quality of life.

5. *Calls upon* Governments and other stakeholders to work to reduce the impacts of global environmental changes on biodiversity and to promote good quality of life through ecosystem-based participatory approaches and conservation and sustainable management strategies. *Also call upon* for collaboration between national and international investors for the investment and technology transfer towards the establishment of industries based on plant raw materials supplied in a sustainable way.

6. *Encourages* Governments of all levels to promote synergy in the implementation of measures addressing biodiversity loss, land degradation, biological invasions and climate change through wider public awareness and participation and also state to pursue a policy aimed at identifying and protecting traditional knowledge, skills, and practices for bioprospecting.

Department of Plant Resources

Thapathali, Kathmandu

The Department of Plant Resources (DPR) (Then Department of Medicinal Plants) is one of the government institutions under the Ministry of Forests and Environment which was established in 1960 A.D. This organization is conducting researches and providing services on the plant resources in Nepal. It is a multidisciplinary organization comprising scientists mainly the botanists, the chemists and the pharmacists.

Objectives and Activities:

- Resource survey and collection of plant materials and preservation of the specimens in the National Herbarium and Plant Laboratories (KATH).
- Identification and certification of plant and its products (KATH).
- Establishment and maintenance of Botanical Gardens in different physiographic regions of the country for *ex-situ* and *in-situ* conservation of rare, endangered, threatened and medicinal plants.
- Technical support for sustainable utilization of plant resources through cultivation and processing; floriculture and landscaping.
- Chemical and Biological researches for the utilization of medicinal, aromatic and other valuable plants.
- Biotechnology research, improvement and propagation of plants of economic value.
- Agro-technology development of important and medicinal plants to provide services to the farmers on techniques of commercial cultivation.
- Conduction of trainings on plant resources conservation, management and provide garden services.
- Information dissemination through publications on various aspects of Nepalese plant resources.
- Bio-prospecting of plants of economic value.
- Library service for public, researcher, policy maker and all concerned.

DPR also functions as the following

- National Scientific Authority of plant resources for the implementation of the Convention on International Trade in Endangered Species of Wild Fauna & Flora (CITES).
- National focal point of Global Taxonomic Initiative (GTI)
- National focal point of Bio-safety
- Secretariat of Herbs & NTFPs Coordination Committee (HNCC): 33 species of medicinal plants have been prioritized for economic development of Nepal, among these 13 species have been prioritized for agro-technology development.

The Department has two divisions, three central level offices, seven district level plant research centers and twelve botanical gardens.

1. **Research and Planning Division :** This division is mainly responsible to formulate research and management plan for research and development of diverse plant resources of the country. Under this division there are Planning and Monitoring section, Herbs promotion and development section, Biotechnology section, Biodiversity and CITES section and Urban garden development section.

2. **Management and Development Division :** This division is responsible for application of research for sustainable utilization, herbs promotion and development, development of plant resources of the country. Under this division there are Instrument section, Quality determination section, Biological section, Herbs utilization and pilot section and Documentation section.

Central Level Offices

Natural Products Research Laboratory, Thapathali

This laboratory provides services to the public for industrial product development of plant origin and certification. Currently its main focus is on plant chemistry aiming for R&D activities. This lab also certifies plant products and essential oil as per ISO 17025/2017.

Natural Products Research Laboratory
Thapathali, Kathmandu, PO Box 2270
Tel: 977-01-4268247, 4266856
E-mail: nprl@dpr.gov.np

National Herbarium and Plant Laboratories

Established in 1962 as a full government undertaking, the Botanical Survey and Herbarium-now called as National Herbarium and Plant Laboratories (KATH) aims to support the nation building through R&D on the diverse plant resources of the country. This is the only institution having the mandate of conducting country-wise exploration, collection, identification, preservation and housing of dried plant specimens called as Herbarium.

National Herbarium and Plant Laboratories
Godawari-5, Lalitpur, PO Box No. 3708
Tel: 977-1-5174277
E-mail: nhpl@dpr.gov.np, Website: www.kath.gov.np

National Botanical Garden

Established in 1962, for the purpose of Collection, Conservation, Education and Scientific Research of living plants. This botanical garden is located at the foothill of Phulchoki hill, spreads over an area of 82 ha. More than 1000 different species of plants are conserved here. There are different landscapes and thematic gardens like Physic garden, Biodiversity Education Garden (BEG), Rock garden, Fern garden, Orchid house, Japanese style garden, Lily garden, Tropical garden, Taxonomic family garden, VVIP plantation area, Ethno-botanical garden, etc.

National Botanical Garden
Godawari, Lalitpur, PO Box No.3708
Tel: 977-01-5174279, 5174246, Fax: 977-01-5174279
E-mail: nbg@dpr.gov.np

Plant Research Center

There are seven plant research center under DPR.

- Plant Research Center, Ilam
- Plant Research Center, Dhanusa
- Plant Research Center, Makwanpur
- Plant Research Center, Banke
- Plant Research Center, Salyan
- Plant Research Center, Jumla
- Plant Research Center, Kailali

Botanical Gardens

Besides National Botanical Garden there are other botanical gardens which are managed by respective plant research offices

1. Maipokhari Botanical Garden, Ilam,2100 m (Estd.1992)
2. Dhanusha Botanical Garden, Dhanushadham, Dhanusha, 106.6m
3. Vrindaban Botanical Garden, Hetauda, Makwanpur, 500 m, (Estd. 1962)
4. Mountain Botanical Garden, Daman, Makwanpur, 2320 m, (Estd. 1962)
5. Tistung Botanical Garden, Tistung, Makwanpur, 1900 m, (Estd. 1962)
6. Dhakeri Botanical Garden, Banke, 160 m(Estd. 1980)
7. Mulpani Botanical Garden, Kapurkot, Salyan, 2000 m, (Estd. 1990)
8. Dhitachaur Botanical Garden, Jumla, 2498 m, (Estd. 1980)
9. Godavari Botanical Garden, Godavari, Kailali, (Estd. 1998)
10. Devoriya Botanical Garden, Dhangadi, Kailali, 170 m, (Estd. 1998)
11. World Peace Biodiversity Garden, Raniban, Pokhara, Kaski. Established in 2013 as per Government of Nepal (Secretary Level) decision dated 2070/8/18

Major Programs and Activities

Major programs and activities implemented by Government of Nepal in these botanical gardens are Medicinal plants (Jadibuti) Development Program and Research, Conservation and Garden Development.

The major activities under these programs are:

- Production of quality planting material of medicinal plants and distribution to farmers for commercial farming
- In-situ and ex-situ conservation of threatened and endemic plant species
- Research and study for agro-technology development of medicinal plants
- Documentation of indigenous knowledge related to plant resources
- Domestication and germ-plasm conservation of Medicinal and Aromatic Plants (MAPs)
- Variety development program of *Mentha* and *Chamomile*
- Friends of Botanical Garden (FoBG)
- Beautification of plant landscapes and thematic gardens
- Production of seasonal and perennial ornamental plants
- Research on indigenous ornamental plants
- Awareness program

Photo Plates


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International Conference on
Biodiversity and Bioprospecting
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Kathmandu, Nepal


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Bioprospecting


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FLORISTIC STUDY OF REMNANT TROPICAL
FOREST OF EASTERN NEPAL


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NATIONAL BOTANICAL GARDEN


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