Impact of Climate Change on Medicinal Plants of Selected Districts in Nepal-2020

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Abbreviations

AAM	= Ayurveda and Alternative Medicines
CAM	= Complementary and Alternative Medicine
CHAL	= Chitwan-Annapurna Landscape
CFU	= Colony formation per unit
GLOF	= glacial lake outburst flood
IPCC	= Intergovernmental Panel on Climate Change
I/NGO	= International/National non-governmental organization
LTDL	= Less than detectable limit
LVI	= Livelihood Vulnerability Index
MAPs	= Medicinal and Aromatic plants
NT	= Not tested
NCVST	= Nepal Climate Vulnerability Study Team
NTFPs	= Non-timber forest products
NTFP	= Non Timber Forest Products
OECD	= Organization for Economic Cooperation and Development
TAL	= Terai Arc Landscape
UCL	= University College London
WHO	= World Health Organization

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Executive Summary

The earth is undergoing rapid environmental changes because of human actions and ecological processes causing gradual slow increment of earth's temperature. In recent years, global climate change has been recognized as a significant driver of ecological change. The Himalayas are no exception of this environmental change worldwide. Various assessments show that the Himalayan Mountains are highly vulnerable to global climate change which is going to affect habitat of many living animals on this region including valuable plants. Human-caused environmental changes such as climate change, rise in extreme weather conditions, desertification, pollution, etc. are modifying the environmental conditions and affecting to the many of the medicinal plants globally. Although a good understanding of the extent and specific consequent changes to biodiversity is still unclear, shifts in vegetation, species extinctions, and changes to ecosystem service delivery are expected, with consequential cascading, downstream impacts on human livelihoods and lives such as animals and plants.

The most important is to loosing valuable herbaceous medicinal plants in this region. Nepal is one of the most vulnerable countries to climate change. Nepal's mountainous and challenging topography, socio-economic conditions, annual changes in the pattern of precipitation or rainfall, glacier lakes outbursts floods and extreme weather events adversely influence the distribution of floral and faunal diversity in different geographic regions.

We have been provided Ethical approval of this study from Nepal Health Research Council.

Furthermore, local uses, parts used, trades and livelihood of the plants have also been reviewed all around this study. Department of Plant Resources and Department of Food Technology and Quality Control have been officially required for conducting pharmacognostical study and parameters of standardization of the collected samples.

This house hold survey has been carried out in Madhuwan mathwal rural municipality of Parsa, Dhunebashi village of Dhading and Hunde of Manang districts. This study was both qualitative and quantitative type conducting among at least 100 house holds

from every rural municipality as mentioned above using semi-structured questionnaires, focal group discussion and in-depth interview with focal persons of the villages. This survey was used to determine livelihood vulnerability index by using LVI tools which have already been used by various previous studies.

Furthermore, this study was focused to study medicinal plants of selected districts on pharmacological and phytochemical study whether environmental changes have affected the quality of medicinal plants. This data was supposed to be baseline for further evaluation of the environmental changes on the same plants in the same locality after 10 years.

Parsa district has been noted the most natural vulnerability and climatic variation. The vegetations are getting affected by many anthropogenic pollutants near by the urban or metropolitan city. Microbial pesticides residue and heavy metals contamination are the major pollutant for vegetations which are consumed by people for food and medicines which is similar in Dhading district, the vulnerable one among the study sites. Manang is the remote among the study districts in the view of human created pollutant. However, heavy metal contamination was found higher among the study samples. The sample collected from Parsa district was found higher microbial contamination among the study samples. Other parameters such as foreign matter, loss of drying, total ash value, extractive values were all within the limit.

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CHAPTER-I: INTRODUCTION

Background

The earth is undergoing rapid environmental changes because of human actions and ecological processes. In recent years, global climate change has been recognized as a significant driver of ecological change(IPCC, 2007a, Parmesan, 2006). The Himalayas are no exception; assessments show that the Himalayan Mountains are highly vulnerable to global climate change (Beaumont et al., 2011, Li et al., 2013, Bawa et al., 2012). The IPCC projects that the average annual temperature in South Asia will increase by 3-4°C by 2080-2099 under an A1B (medium-high emissions) scenario, and is likely to be higher under an A2A scenario based on comparisons with historical averages from 1980-1999, while annual precipitation is expected to increase throughout this region(Meehl et al., 2007).

Temperature data collected between 1977 and 1994 indicate an average increase in temperature of 0.06°C per year nationally, and from 1996-2005 an average increase in the maximum temperature of 0.04°C per year. The increasing trends are, however, variable across the country. Precipitation data collected from 166 stations across Nepal from 1976 to 2005 shows an increasing trend in annual precipitation, but with considerable local variation, including in pre- and post-monsoon precipitation and winter precipitation(MoE, 2010). Himalayan glacier melt and retreat have also been documented, with 18 glacial lake outburst flood (GLOF) events recorded in Nepal between 1936 and 2000 (Callot et al., 2009).

Organization for Economic Cooperation and Development (OECD) (Agrawala et al., 2003) and the Nepal Climate Vulnerability Study Team(NCVST, 2009) jointly reported that there will be annual temperature increases of 1.2°C by 2030, 1.7°C by 2050, and 3°C by 2100 relative to a pre-2000 baseline. The OECD projections indicate a 5-10% increase in winter precipitation in eastern Nepal, but no change in western Nepal. But monsoon (summer) precipitation is projected to increase by about 15-20% across the country. The NCVST projects an increase in monsoon rainfall, especially in eastern and central Nepal. Although a good understanding of the extent and specific consequent changes to biodiversity is still unclear, shifts in vegetation, species extinctions, and

changes to ecosystem service delivery are expected, with consequential cascading, downstream impacts on human livelihoods and lives(Xu et al., 2009).

Human-caused environmental changes such as climate change, rise in extreme weather conditions, desertification, pollution, etc. are modifying the environmental conditions and affecting to the many of the medicinal plants globally. These environmental modifications might become a greater cause of species extinction than direct habitat destruction. Due to over-exploitation and un-scientific destructive harvesting, many important medicinal plants have almost become extinct viz. *Nardostachys jatamansi, Aconitum ferox, Picrorhiza scrophulriiflora, Dactylorhiza hatagirea, Podophyllum hexandrum, Rheum nobile, Maharanga emodi,* and *Aconitum spicatum*(Joshi et al., 2017). Besides, The fragile landscapes of the Himalayan region are highly susceptible to natural hazards, and there is an ongoing concern about current and potential climate change impacts which may include abnormal floods, droughts and landslides (Barnett et al., 2005, Cruz, 2007). Climate change has a great impact on the species dispersal. Minimum, maximum, and average temperatures affect plant growth and distribution. Similarly, rainfall impacts the balance of plant types in a specific area. Climate change exerts a tremendous adverse effect on medicinal and aromatic plants (MAPs), and MAPs are not immune to the effects. The noticeable impacts of climate change on MAPs are found on the life cycles and distributions of vegetation. Thus, climate change brings the risks to some endemic MAPs in the geographic regions because of their vulnerability to the changing climate conditions. Such serious issues and challenges are a continuous concern regarding to the survival and genetic integrity(Das et al., 2016). Furthermore, rapid climate change associated with increasing greenhouse gas emissions and that influences current and future vegetation patterns(Sala et al., 2000).

Nepal is one of the most vulnerable countries to climate change. Nepal's mountainous and challenging topography, socio-economic conditions, annual changes in the pattern of precipitation or rainfall, glacier lakes outbursts floods and extreme weather events adversely influence the distribution of floral and faunal diversity in different geographic regions. These adverse events have also impacts on the growth and distribution of MAPs(NHTC, 2018). The Terai Arc Landscape (TAL) is a stretch of

lowlands in the southernmost part of Nepal from east to west. This is the area with the highest biodiversity in the country.

The Chitwan-Annapurna Landscape (CHAL) falls partly within the Sacred Himalayan Landscape (SHL) that stretches from Bhutan in the east to Nepal's Kali Gandaki River in the west. CHAL itself is bounded by the Gandaki river basin. It exhibits rain shadow of the trans-Himalayan area and the snowcapped mountains of Annapurna, Manaslu and Langtang in the north, descending southwards through diverse topography to the mid-hills, Churia range and the flat lowlands of the Terai. It contains seven major sub-river basins: Trishuli, Marsyandi, Seti, Kali Gandaki, Budi Gandaki, Rapti and Narayani. CHAL has a human population of over four million people, and who are very dependent on forest resources for their livelihoods and wellbeing. Both landscapes overlap in the south, in Nawalparasi, Chitwan, Makwanpur and Palpa districts(Thapa et al., 2015).

There are several threatened and endemic plant species in CHAL. Several sites along the Marsyangdi, Madi, Seti, and Narayani river valleys have been identified as high species richness areas(Basnet et al., 2000). There was also listed 47 species of threatened plant species and 88 species that are endemic to Nepal from the CHAL region(Shrestha, 1996). The forests in these areas are already fragmented, and the climate projections indicate that the remaining subtropical broadleaf and hill and lowland *sal* forest areas will become extensively converted into several patches by 2050.

Therefore, there is an urgent need to assess and review the current understanding about the impacts of climate change on their morphological characters, life cycle, undulating primary and secondary metabolites formation and effect of soil contents and pollution in traditionally used Medicinal plants of Ayurveda healthcare system.

Soil

Climate change will also influence global precipitation patterns, altering both the amount of precipitation received and the distribution of precipitation over the course of an average year in many locations(IPCC, 2007c). With this change in climate there will be effects on the environment, including the soil(Brevik, 2012). Soils are also important to food security(Pimentel, 2006, Lal, 2010, Blum and Nortcliff, 2012, Brevik, 2013a) and climate change has the potential to threaten food security through its

effects on soil properties and processes(Brevik, 2013a). Understanding these effects, and what we may do to adapt to them, requires an understanding of how climate and soils interact and how changes in climate will lead to corresponding changes in soil.

Livelihood vulnerability index

The LVI uses data from household surveys to assess households across eight major vulnerability components: socio-demographic profile, livelihood strategies, social networks, health, food, water, natural disasters, and climate variability. Each component is associated with one of the three dimensions of vulnerability (exposure, sensitivity, and adaptive capacity), and is represented by a number of indicators.

Climatic factor

Climate Change (CC) models predict that the air temperature will rise by between 1 and 4°C by the end of the 21st century, because of increased greenhouse gases in the atmosphere(IPCC, 2007b). The temperature of the earth increased by an average of 0.7°C during the past century, and the rate of temperature increase rate is increasing twice as rapidly in Korea since 1980(IPCC, 2013). For this reason, the Korean Government has initiated research on mitigation and adaptation of key agricultural and horticultural crops to CC scenarios and to examine impacts of moderate and extreme CC scenarios by examining Representative Concentration Pathways (RCP) of 4.5 and 8.5 (Song et al., 2015). Air temperature is predicted to increase by approximately 6.0°C by 2100, the CO_2 concentration is expected to increase to 940 µmol/mol, and the precipitation to increase by 20.4% from existing conditions under the RCP 8.5 CC scenario.

The average annual temperature at different bioclimatic zones clearly indicates that temperature at higher elevations is increasing significantly more than at lower elevations. In lower tropical bioclimatic zone (LTBZ), upper tropical bioclimatic zone (UTBZ), lower subtropical bioclimatic zone (LSBZ), upper subtropical bioclimatic zone (USBZ), and temperate bioclimatic zone (TBZ), the average temperature increased by 0.022, 0.030, 0.036, 0.042 and 0.051 ◦C/year, respectively. The decade level temperature scenario revealed that the hottest decade was from 1999–2009 and average decade level increases of temperature at different bioclimatic zones ranges

from 0.2 to 0.27 ◦C /decade. The average temperature and precipitation was found clearly different from one bioclimatic zone to other(Luitel et al., 2020).

Morphological characters

Previous studies in South Korea have suggested that under high precipitation conditions, hot pepper is susceptible to outbreaks of anthracnose or phytophthora diseases which can significantly impact on yield(Hwang and Tae, 2001). Abnormal/extreme weather conditions in terms of extremely high temperature, heavy rainfall, and drought in summer due to climate change are known to be the main causes of a reduction in hot pepper production(Seungmi et al., 2010, Smittle et al., 1994). Previous research revealed that increases in air temperature(Park et al., 2014, Lee et al., 2001), CO_2 concentration(Hansen et al., 2000), and precipitation(Hwang and Tae, 2001) significantly retarded growth and reduced yield of hot pepper. Extreme hot summers lead to a decrease in the production of hot pepper by reducing both growth and increasing the occurrence of abnormal fruits (Park et al., 2014, Lee et al., 2018). A study conducted by Sin and Yun(Joo et al., 2010) founded that elevated CO_2 and temperature increased the incidence of hot pepper diseases. Under intense rainfall conditions, the rhizosphere can become anaerobic, affecting root penetration and impacting on growth and plant photosynthesis(Lee et al., 2017). In addition, the amount of irrigation has been found to be closely correlated with size and weight of hot pepper fruits(Smittle et al., 1994, Seungmi et al., 2010, Sezen et al., 2014). Physiological response studies with increasing air temperature and CO_2 concentrations for cucumber(Taub et al., 2000), radish, and Kimchi cabbage(Choi et al., 2011, Lee et al., 2016, Lee et al., 2009, Oh et al., 2015) have shown retarded growth and deducted yield, as well as increased physiological disorders under extreme weather conditions. Studies have been carried out to develop prediction models of the effect of various environmental factors such as temperature and precipitation on crop growth and yield(Hadley et al., 1995, Porter and Semenov, 2005).

Standardization

Evidenced-based traditional plants are believed to be a prospective and alternative source of safe and effective drug development both in developed and developing countries for fruitful remedy against acute and chronic diseases. Because mainline

allopathic medicines demonstrate versatile unwanted effect and become ineffective in the treatment of chronic therapy.

In order to fulfil this purpose, large number of traditional plants needs to be cultivated in homogeneous environmental condition, favorable geographical and climatic condition for optimize the yield and increase the production rate of the plant metabolites. Satisfactory climate, good quality of soil, and advantageous geographical condition yield huge number of cultivated medicinal plants with suitable growth. By maintaining such parameters of cultivation protocol, the farmers and cultivators may be capable to give homogeneous and optimized phytoconstituents containing crude extract for herbal drug discovery.

However, global changing of climate is modulating the weather condition of the same geographical condition, which in turns drastically jeopardize the secondary metabolite production rate, their composition in the organized medicinal plant extracts. Vis-a-vis, weather condition, rainfall, and humidity are versatile in different geographical locations, which could change the phyto-metabolites composition and yield when the same species medicinal plant is growing in different geographical locations. These variations are augmented due to difference in the soil quality, collection time, and dispensation methods of cultivated medicinal plants for secondary metabolites extraction. These consequences are resulting in the huge discrepancy in the reproducibility of bioactive phyto-metabolites extractive yield and composition from the same species of medicinal plant. Therefore, patient may not obtain uniform and optimized bioactivity from the same herbal formulations from different batch. In order to attenuate this challenges and hurdles, appropriate strategies should be taken by policy maker and related stakeholders to mitigate the climatic effects on medicinal plants to maintain the quality of herbal materials throughout the batches of the year.

Purpose of the study

Globally, there are several scientific evidences about the impacts of environmental changes such as climate change on the medicinal plants. The climate change has brought the several complexities in plants for examples; changes in the timing of seasonal life cycle events, range shift, food web disruption, support to the spread of pathogens, parasites and diseases, habitat destruction and species extinction(Settele

et al., 2015). Active agents of medicinal plants may alter with variation in altitude of habitat. A study in india reported that active agents of *Rauvolfia serpentina* was decreased with various higher altitudes. However, active agents of *Hyoscyamus niger* was found to be increased with different higher altitudes(Chatterjee, 2001). Studies on impact of climate change on traditionally used medicinal plants is seriously lacking except some sporadic works under controlled conditions in few species.

The climate change in Northern Hemisphere has declined the quality and trade of medicinal plants (UCL, 2019). As temperatures increase, movement of plants occurred to higher elevations at a median rate of 36 feet per decade, and to higher latitudes at a median rate of 10.5 miles per decade in North America. The expansion increased the competition with local factors and lead to extinction in their local habitat(Groffman et al., 2014). In India, the population of MAPs has also affected because of impact of climate change particularly in high altitude of central Himalayan and mountain regions of Uttarkhand. The impacts of climate change were observed more on indigenous medicinal plant species and their dependent communities(Maikhuri et al., 2018).

There are few available research findings in Nepal that reflect how climate change impacts on MAPs. For examples; decline in yields of eight indigenous medicinal plants and appearance of new sub-tropical species at the altitude of 3,000m and above were reported in lower Mustang area (Gyawali et al., 2016). In another study of (Rana et al., 2017) predicted the impact of future climate change on the distribution of two focal species (used in folklore medicine; *F. cirrhosa* and Ban Lasuna; *L. nepalense)* i.e., their shift towards the north-west region of Nepal, losing their potential habitat in hilly and lower mountainous regions by 2050.

The similar studies have enhanced our understanding of climate change impacts on traditionally used medicinal plants in Nepal. However, there is a serious lack of synthesized and systematic study showing the views of stakeholders and indigenous people from different ecological regions regarding impacts of environmental changes on the medicinal plants of Nepal. The evidence collected from indigenous people can support in future climate change risk reduction to medicinal plants through logical government planning for sustainable conservation and harvesting from their natural local habitat for quality production of Ayurvedic medicines. In this analysis, we

conduct species envelope projections to assess the impacts of climate change trajectories on forest vegetation communities in Nepal.

Rationale / Justification

Globally, there are several scientific evidences about the impacts of environmental changes such as climate change on the medicinal plants. The climate change has brought the several complexities in plants for examples; changes in the timing of seasonal life cycle events, range shift, food web disruption, support to the spread of pathogens, parasites and diseases, habitat destruction and species extinction (Settele et al., 2015). Active agents of medicinal plants may alter with variation in altitude of habitat. A study in india reported that active agents of Rauvolfia serpentina was decreased with different higher altitudes. However, active agents of Hyoscyamus niger was found to be increased with different higher altitudes (Chatterjee, 2001). Hence, quality of medicinal plants is also reported to alter by environmental changes. Studies on impact of climate change on traditionally used medicinal plants is seriously lacking except some sporadic works under controlled conditions in few species. There are plenty of evidences that changes in various factors of environment cause depletion of flora and fauna worldwide. Mountainous and Himalayan people significantly rely on medicinal plants for income generation or livelihood. Depletion of natural resources affects habitant in the the regions and ultimately threatens other resources. In this regards, environmental factors have less been assessed to associate these with one of the cause of rare vegetation of medicinal plants in Himalayan region of Nepal. This study therefore has been planned to carry out environmental factors evaluation and its impact on medicinal plants; and this impact has also been assessed to find any association with livelihood of the habitant of the region in small population of Parsa, Dhading and Manang districts of Nepal.

Objectives

General objectives: This study will assess and document the impacts of various environmental changes on the distribution, availability and threat to medicinal plants in different ecological regions of Nepal.

Specific objectives:

a. To collect information about impact of environmental changes on endemic medicinal plants used in Ayurveda medicine.

b. To identify impact of environmental change on traditionally used Ayurvedic herbal resources/availability and threat in high altitude, Middle Mountain and low land of Nepal.
c. To synthesize the information on threat to Ayurveda practices, herbal based profession and livelihood in study areas.
d. Collection of soil sample from study sites and analysis soil parameters.
e. To investigate the pharmacognostic parameters of the dry samples collected from the different geographic locations of study sites.
f. To prepare the phytochemical fingerprinting of volatile oil extracted from Ayurvedic medicinal plant samples from different geographic locations of study sites.

CHAPTER-II: LITERATURE REVIEW

Nepal is ranked as 9th among the Asian countries for its floral wealth with an estimated 9,000 species of flowering plants(Bhattarai et al., 2011) and 6,653 species of flowering plants have been reported (Kunwar RM, 2010). Among these, 25%–50% are enthnomedicinal(NP, 2002, Kunwar et al., 2006). Catalogues have recorded 1,792(Rokaya et al., 2010) to 2,331 (Baral and Kurmi, 2006) useful medicinal and aromatic plants in Nepal, reporting their importance in alleviating human suffering because they have long been used for subsistence, home remedies, and traditional therapies(NP, 2002, Rokaya et al., 2010, Kunwar RM, 2010). These plants are also important for local livelihoods(Manandhar, 1995) and income generation(Malla et al., 2015), and they do fetch higher market prices(Tiwari S, 2004).

A study was conducted in Rasuwa district of Central Nepal which recorded a total of 60 medicinal formulations from 56 plant species. Medicinal plants were used to treat various diseases and disorders, with the highest number of species being used for gastro-intestinal problems, followed by fever and headache. Herbs were the primary source of medicinal plants (57% of the species), followed by trees (23%). The average FIC value for all ailment categories was 0.82, indicating a high level of informant agreement compared to similar studies conducted elsewhere. High FIC values were obtained for ophthalmological problems, tooth ache, kidney problems, and menstrual disorders, indicating that the species traditionally used to treat these ailments are worth searching for bioactive compounds: Astil berivularis, Berberis asiatica, Hippophaes alicifolia, Juniperus recurva, and Swertia multicaulis. Sixteen medicinal plants were ranked as priority species, 13 of which having also been prioritized in a country-wide governmental classification (Uprety et al., 2010).

Similarly, this study documented the uses of plants in traditional herbal medicine for treatment of human and veterinary ailments in four village development committees in the Humla district of western Nepal. It also determined the homogeneity of informant's knowledge on medicinal plants suitable for different ailment categories and the most preferred plant species used to treat each ailment category in the study areas. A total 161 plant species belonging to 61 families and 106 genera used for

treating 73 humans and 7 veterinary ailments. It was also documented culinary uses and additional uses for 67 and 33 of medicinal plant species respectively. Most medicines were prepared in the form of powder and used orally. Roots were most frequently used plant parts. The uses of 93 medicinal plants were not mentioned in any previous studies(Rokaya et al., 2010).

In a study, a total of 132 ethnomedicinal plant species belonging to 99 genera and 67 families have been documented from two ethnic communities Magar and Majhi of Parbat district in Western Nepal. These plants are used to treat various diseases and disorders grouped under 12 disease categories, with the highest number of species(Saikia et al., 2006) being used for gastro-intestinal, parasitic and hepatobiliary disorders, followed by blood and lymphatic system category. The highest fidelity level (HFL) values recorded in Paris polyphylla (FL=96.0%) followed by Bergenia ciliata (FL=95.0%) confirms that these plants are the best plant species with medicinal properties(Kunwar et al., 2006).

Rokaya MB et, al. documented a total of 947 species belonging to 158 families and 586 genera used to treat gastrointestinal disorders in Nepal. Diarrhea was the disorder treated by the highest number of species (348), followed by stomachache (340) and dysentery (307). Among the reported species, five were endemic to Nepal, whereas 16 orchid species were protected under CITES. The randomization test showed that species belonging to 14 families were used less often than expected, whereas plants belonging to 25 families were used more often than expected(Rokaya et al., 2014).

A study was conducted to document a total of 161 plant species belonging to 86 families and 144 genera to cure 89 human ailments in a Tamang community dwelling in the Makawanpur district of central Nepal. Although 68 plant species were cited as medicinal in previous studies, 55 different uses described by the Tamang people were not found in any of the compared studies(Luitel et al., 2014).

A study showed that medicinal herbs were the main ingredients of traditional therapies, and they were considered a main lifeline and frequently were the first choice in the Baitadi, Darchula and Dadeldhura districts, far western region of Nepal. Use of *Cordyceps sinensis* as an aphrodisiac, *Berberis asiatica* for eye problems, *Bergenia ciliata* for disintegration of calculi, *Sapindus mukorossi* for dandruff, and

Zanthoxylum armatum for toothache were the most frequently mentioned. Medicinal plants are inseparable from local livelihoods because they have long been collected, consumed, and managed through local customs and knowledge(Kunwar et al., 2013).

A study conducted during 2010-2011 in the terai forest of western Nepal recorded 66 medicinal plant species belonging to 37 families and 60 genera. These plants were used to treat various diseases and ailments grouped under 11 disease categories, with the highest number of species(Subrat, 2002) being used for gastro-intestinal disorders, followed by dermatological disorders(Panthi and Chaudhary, 2003). Herbs (53%) were the primary source of medicine, followed by trees (23%). Curcuma longa (84%) and Azadirachta indica (76%) are the most frequently and popularly used medicinal plant species in the study area. Acacia catechu, Bacopa monnieri, Bombax ceiba, Drymaria diandra, Rauvolfia serpentina, and Tribulus terrestris are threatened species which needs to be conserved for future use (Singh et al., 2012).

A study has been carried out in Dunuwar community of Sindhuli found that the village folks have a wide range of herbal remedies for diseases like indigestion, diarrhea, dysentery, cough and cold, fever etc. Total 74 medicinal plant species has been recorded from local traditional healers and tribal chiefs' aged 40-60 years. That has been used by the local people for remedies of 24 ailments. Among these medicinal plants, 23 species have been used for the external application, 45 species for internal use and 6 species for veterinary medicine(Manandhar, 1990).

Ethno botanical study has been conducted in 23 villages of Padampur VDC and the surrounding north-east forest of Chitwan National Park has documented the indigenous knowledge of people inhabiting in the area regarding plants for medicine, fuel wood, fodder, handicrafts, oil extracts and ceremonial and cultural use, etc. This study has been recorded 185 plant species having medicinal value that have been used to treat 126 different human diseases whereas 3 species have been used for cattle diseases. The result revealed that the fixed numbers of pieces of plant's parts in garland made of either the root or steams were also worn to cure diseases like fever, headache, jaundice, cough, etc. (Rijal, 1994). This practice is also common in other region of the country.

A survey has been carried out in 10 villages of Jajarkot district reported 60 species of medicinal plants used by the local people for treating 25 types of diseases. The

information was collected through verbal interview with 10 herbalists from each village and 50 knowledgeable adults that include shepherds, wood-cutters, fodder collector, medicinal plants collector and others. The study showed that the common people used fresh medicinal herbs whereas the healers use both dried and fresh herbs(Manandhar, 1995).

A survey has been conducted in Sindhuli district among Brahmin chhetri, Tamang, Magar, Danuwar, Newar, Sunwar communities of 101 household documented 102 medicinal plant species belonging to 59 families and 92 genera used in traditional medicines for curing different diseases like ENT problem, respiratory disease, Trauma, Jaundice, skin disease, etc. The study revealed that faith healing system (Tantra/Mantra) was more common in lower caste whereas practice of herbal treatment was more common in the upper cast people. The study had also showed the poverty relation with traditional medicine that is related to utilization of medicinal plants(Basnet, 1998).

A survey has been documented a total of 47 species of plants used for the treatment of 17 types of diseases in the Raute community of Aampani and Rajaura villages of Dadeldhura district. The study reported that medicinal uses of 15 species were unrecorded from other parts of the country (Manandhar, 1998).

A study has been conducted in Kali Gandaki watershed area and documented 48 medicinal plants belonging to 31 families used for remedies. The study indicates that the inhabitant of the Kali Gandaki Watershed area rely on traditional medicine for their primary health care needs (Joshi and Joshi, 2000).

A study has been conducted in Tribhuvan University in 2000, has been reported altogether 50 medicinal plants species belonging to 50 genera and 37 families used for remedies of 24 different diseases like malaria, diarrhea, jaundice, toothache, internal fever etc. in the Lama community of Ichangue VDC of Kathmandu. The detail information on the usage and local name of medicinal plants, plant parts used in medicine, mode of preparation of drugs, dose and route of administration has been documented (RANJITKAR, 2005).

A database of medicinal and aromatic plants of Nepal has been published including 1624 species in wild or cultivated state, belonging to 938 genera and 218 families

along with their ethno botanical information, pharmacological action, mode of application and its chemical constituents (Shrestha et al., 2000).

It has been investigated the indigenous knowledge on the use of medicinal plants among the Tharu community of Bachhauli VDC of Chitwan District. In this study, socio-economic and demographic feature, traditional medical practices and local environment has been documented including183 species of medicinal plants used in the study area along with their mode of preparation(Chaudhary, 2001).

In 2001, Karki et.al gave an account of detail description of 75 species of medicinal plants belonging to 45 families and 68 genera used for 51 diseases at Ugrachandi and Tukucha VDCs of Kavrepalanchok district. It includes family name, English name, local name, local status, parts used etc. This study revealed that such knowledge has been handed down from generation to generation, and besides local healers, other people too have sound knowledge on it(Karki, 2001).

In 2001, Lama et, al. has compiled on 407 plants from Shey-phoksundo National Park and its buffer zone including 100 medicinal plants. This work was also emphasized on Sowa-Rigpa's knowledge and conservation. In addition, the indigenous use of these medicinal plants for curing different aliments has been reported that is relevant to the present research work(Lama et al., 2001).

A study has been recorded 529 species of medicinal plant species from Shey-Poksundo National Park of Dolpa. The study reported that about 94.3% of the total medicinal plants recorded have been used in traditional medicine by the Sow-Rigpa for remedies of more than 50 ailments like cough and cold, dysentery, typhoid, rheumatism etc [83].

A survey has been conducted in different ethnic groups like Gurung, Kami, Sarki, Pariyar, Chhetri, Bhujel of Chitre VDC (Parbat) and Bhadaure/Tamagi VDC (Kaski) to record indigenous knowledge of the communities. The study has been reported that the local communities have been practicing traditional medicine since time immemorial with 83 medicinal plant species belonging to 51 families and 77 genera used for remedies of 52 different ailments like fever, constipation, menstrual disorder, sore throat, heart diseases, typhoid, infertility etc. The study has further been documented their mode of preparation, parts used, quantity and route of administration, etc(Gurung, 2002).

A survey has been recorded a total of 274 species and 5 varieties of medicinal plant belonging to 63 families and 172 genera along with their use, availability and distribution from upper Dolpo region of Nepal. These medicinal plants were used to 64 different types of diseases like infection, intestinal disorder, cough and cold, cuts, wounds and injuries, fever, blood disorder etc. Out of 274 plant species, 79 species were reported as new medicinal plant species(Rokaya, 2002).

A study has been reported that traditional healing practices were very much prevalent among the local people of Langtang National Park of Rasuwa district, Central region of Nepal. In this study 200 species of medicinal plants have been recorded for curing more than 35 types of diseases; maximum numbers of the plants were recorded for remedies of wound and fever. The drugs were in the form of juice, paste, decoction, oil and powder(Shrestha I).

Balami reported 119 species of medicinal plant from Kharpa community forest of Pharping, Kathmandu which was used by the communities for the treatment of 35 types of disease like Epilepsy, Fever, Diabetics, Jaundice, Anthelmintic, Aphrodisiac, Constipation, Hemorrhage, etc. He studied the ecological parameters of medicinal and aromatic plants to Kharpa community forest of Pharping and reported their utilization along with parts used and form of drug's preparation for different diseases(Balami, 2003).

Gurung A carried out the study to document indigenous knowledge on plant resources for different purposes like medicine, fodder, food and other miscellaneous uses from three VDCs of Tinjure area of Tehrathum district. A total of 32 species of medicinal plants belonging to 25 families were documented with their local name, part used and purpose and mode of preparation(Gurung, 2003).

Oli BR has investigated the indigenous knowledge on the use of medicinal plants among the Limbu community of Tapethok VDC, Taplejung. He documented information on 40 widely used medicinal plants species belonging to 38 genera and 33 families along with their parts used, indication procedure of medication, route of administration and dosage, etc. The study revealed that most of the elderly people and traditional healers were known as Phedangba, Samba, Yabbaand, Bijuwa and had much knowledge on medicinal plants. He recorded more than 30 traditional health

practitioners. Out of them, 50 % traditional healers were young learners of age below 30 years(Oli, 2003).

A study has been carried out the ethno medicinal uses of wild plant species among 9 rural communities managing local forest resources in the Bonch VDC of Dolakkha district. The study revealed that 113 medicinal remedies derived from 58 plant species belonging to 40 families to treat a wide range of ailments like cardiovascular, urino-genital, ophthalmological disorders and injuries. The apparent reliance on the local herbs for the health care was associated with the lack of modern medication, poverty and their traditional beliefs on its effectiveness. The information was collected through interviewing 62 informants from different communities(Shrestha and Dhillion, 2003).

A field survey has been documented the indigenous knowledge on the use of plants and animals for different purpose like food, medicine, ceremonies, manure, broom, timber, music etc. among the major people of Tnimure VDC of Palpa district. The study recorded 43 medicinal plant species that had been used to treat 10 different diseases like fracture, bronchitis, burn, peptic ulcer, fever, asthma, and headache, toothache, cut wounds and eye problem by traditional healers, Lamas and elder members of Magar community(Dhakal, 2004).

Pant SR and Pant IR documented the indigenous knowledge of uses of medicinal plants by the ethnic groups comprising Dhami, Lohar, Tamata, Rawat, Damai and Bohara of Bhageati VDC of Darchula by the local traditional healers, traders, elder men and women and teachers. The local people have used 78 species of medicinal plants belonging to 50 families to cure for the remedy of 39 different types of human disorder(Saikia et al., 2006).

Rai SK, Subedi S, Mishra S carried out the study in Thumpathar VDC of Sindhupalchok district on the indigenous utilization pattern of plants as medicine by the local people. They investigated ethno-medicinal practices of plants on 6 communities namely Brahmin, Chhetri, Damai, Kami, Tamang and Sarki. The results revealed that their perception on medicinal plants did not vary significantly and documented 42 species of plants belonging to 34 families for curing 45 different types of ailments along with the doses and route of administration(Rai SK, 2004).

In 2004, a study was conducted in the ethnic Kumal community of Chirtungdhara VDC of Palpa and documented the information about traditional medicinal uses of plants. A total of 50 different species of plants belonging to 45 families were found in the practices for the remedy of diseases like measles, anthelminthic, sinusitis, bone fracture, ear infection, etc(Shrestha A, 2004).

Shrestha MP documented the indigenous knowledge of local medical practices by use of various plants to cure different diseases in the local Newar community of Tokha area. His study was reported 50 medicinal plant species belonging to 49 species belonging to 49 genera and 34 families. The most commons part of plants used for treatment was roots and leaf in the form of paste or juice(Pant SR, 2004).

A book published by Kunwar RM in 2006 on non-timber forest products of Nepal which aims at providing detailed description, inventory, harvest and management approach of 25 selected NTFPs representing important medicinal plants of Nepal along with their scientific name, family name, vernacular name, distribution and habitat, store and value addition, part used, indigenous use, chemical constituent and marketing information (Kunwar and Bussmann, 2008).

Pokhrel BM has carried out study on ethno botany of Bankariya of Makwanpur district and has documented the indigenous knowledge on the use of plants and animals for different purposes like food, ceremonies, medicine, fiber, broom, music etc. He documented 82 species of medicinal plants belonging to 45families and 74 genera used for the treatment of various ailments varying from simple cuts and burns to internal bodily disorder like urinary problem, diabetics, etc(Pokhrel, 2006).

Rajbhandary S and Ranjitkar S mentioned monographs on 30 commercially important and highly traded herbal drugs of Nepal that may be listed in the present study(Rajbhandary and Ranjitkar, 2006).

Plantation and protection, Conservation and collection of all types vegetation is regulated by department of forest and environment, and custom duties have been charged. Nepal exports 80 % of crude herbs to India, and 15% to China and rest to the other countries. Herbs are exported from Nepal of cost one billion in 2017-18 AD.

Complementary and alternative medicine (AAM) includes a wide range of practices that do not fit within the dominant biochemical model of health care and are not commonly provided within conventional medicine settings.

KUTAKI

Neopicrorhiza scrophulariiflora (Pennell) Hong

(Basonym: *Picrorhiza scrophulariiflora* Pennell)

It is perennial rhizomatous herb belonging to the tribe Veroniceae of the family Plantaginaceae according to recent phylogenic studies (Albach et al., 2005, 2004a, 2004b; Bello et al., 2002; Olmstead et al., 2001; Tank et al., 2006), but was previously included in the family Scrophulariaceae. It is commonly known as Picrorhiza, or Gentian, or Nepalese kutki, or Hellebore in English. The species grows well on rocky crevices on slopes, gravelly areas, open pastures, shrubland with moist acidic soil and high organic matter. It prefers mostly the north facing slopes and some shade of small shrubs for growth and survival (Ghimire et al., 2005; Hong et al., 1998; Pusalkar, 2014). There is a lot of confusion regarding taxonomy of *N. scrophulariiflora*. Name *N. scrophulariiflora* (Pennell) Hong was first given by Pennell (1943) as *Picrorhiza scrophulariiflora* (originally written the spelling of *scrophulariiflora* was written as *scrophulariaeflora*). Based on pollen grains, Hong (1984) designated this species under separate genus *Neopicrorhiza* as *Neopicrorhiza scrophulariiflora* (Pennell) Hong. This name was later accepted as authentic Latin name by Brummitt (1992) and at present taken as accepted name by some (Press et al., 2000; Roskov et al., 2019). However, still use of basionym, *Picrorhiza*, in different publications are also widespread (Pusalkar, 2014; Smit, 2000). Here, we have followed the nomenclature of Annotated Checklist of the Flowering Plants of Nepal (Press et al., 2000) and Catalogue of Life (Roskov et al., 2019) and used the name *Neopicrorhiza scrophulariiflora* (Pennell) Hong (hereafter, *N. scrophulariiflora*).

N. scrophulariiflora is very similar to *Neopicrorhiza minima* R.R. Mill, *Picrorhiza kurrooa* Royle ex Benth and *Picrorhiza tungnathii* Pusalkar (Pusalkar, 2014), but they differ from one another in terms of floral characteristics mainly in stamen size and anther shape. They also differ in their distribution ranges. Phylogenetic analysis based on DNA sequencing suggests that *Neopicrorhiza* and *Picrorhiza* should

be treated as closely related sister taxa but not separate genera (Pusalkar, 2014). *N. scrophulariiflora* is distributed in between 3500-5000m above sea level in Nepal (Ghimire et al., 2005; Press et al., 2000), north east India, China (Southwest Tibet, Western China), Bhutan and northern Myanmar; *N. minima* is endemic to Bhutan. *P. kurrooa* is endemic to western Himalayas (Pakistan, India-Jammu and Kashmir, Himachal Pradesh, Uttarakhand). *P. thungnathii* is probably endemic to Himalchal Pradesh (Lahaul-Spiti and Chamba district) and Uttarakhand state (Rudraprayag and Chamoli districts) (Pusalkar, 2014). Sanskrit name katuka for *N. scrophulariiflora* is widely mentioned in ancient texts.

Due to lack of exact descriptions of the plant, taxonomic identity of Katuka is uncertain, though some believe that katuka is closely species of *N. scrophulariiflora* know as *P. kurrooa* (Dymock et al., 1890). Katuka is mentioned in different ancient Sanskrit texts such as *Charaka samhita* (~900 B.C.), *Susruta samhita* (600 B.C.), *Saligramanighantubusana* (ninth century), *Dhavantri Nighanthu* (eleventh century), *Bhavaprakasa Nighantu* (1558–1559 A.D.) (Dev, 1999; Gurib-Fakim, 2006; Smit, 2000). In those texts, it is mentioned that katuka is bitter in taste and used against fever, urinary, respiratory, blood, skin, gastro-intestinal, liver and heat diseases (Smit, 2000). *N. scrophulariiflora* (syn. *P. scrophulariiflora*) is listed in the China's Pharmacopoeia by Traditional Chinese and Tibetan medicine and is effective in curing fever, jaundice, hemorrhoid and dysentery (CPC, 2005). In Nepal, it is mentioned as non-toxic and beneficial for headache, bile disease, intestinal pain, blood and lung fever, high blood pressure, sore throat, eye disease, gastritis, cough and cold, heart disease, cuts and diarrhea (Ghimire et al., 2001; Lama et al., 2001; Manandhar, 2002). Recent study has mentioned that 45 types of different Ayurvedic medicines contain some portion of *N. scrophulariiflora* that are used against many types of diseases (Kafle et al., 2018).

N. scrophulariiflora contains many important secondary metabolites such as caffeoyl glycoside, cucurbitacin glycoside, cyclopentanoid monoterpenes, hydroquinone glycoside, iridoid glucoside, non-glycosidic iridoid, phenol, phenyl glycoside, phenylpropanoid, phenylethyl glycoside and secoiridoid glycoside, etc. (Sah and Varshney, 2013; Smit, 2000). These metabolites have therapeutic properties and are important against diseases of liver, immunity, cardiovascular,

nervous, dermatological, musculoskeletal, digestive, respiratory and genito-urinary systems (Kafle et al., 2018; Sah and Varshney, 2013; Smit, 2000).

Botanical characteristics

N. scrophulariiflora is a short, hairy perennial rhizomatous herb with about 10 cm height. Rhizomes are pale brown, covered with dead leaves. Leaves basal, alternate, oblanceolate or narrowly spathulate, 2.5–6×0.9–1.8cm, usually 10–20 per rosette; serrate in upper half; surfaces are glabrous or sparingly short-glandular-hairy. Scapes are ascending in nature. Flower is 2–6cm in size and fruit is 4–9cm long. It is densely short-pilose with brownish-white thin hairs. Inflorescence is in terminal raceme, with 8–20 flowers. Bracts are ovate, acuminate with blunt tips. Calyx are purplish green, 5–lobed, lobes are oblanceolate. Corolla are purplish blue, 8–12mm; bilabiate, upper lobe is about 10mm and lower lobe lobes are about 5mm. Stamens are 4 in numbers, didynamous, exserted; anthers are elliptic or oblong to reniform and are about 1mm in size. Capsules are brown, 10–14mm, ovoid, swollen. Seeds are pale brown, reniform, about 1×0.8mm. Rhizomes and roots are intact; grayish brown in colour with numerous circular root scars and short thin fractures and longitudinally wrinkled. *N. scrophulariiflora* flowers from May to August and fruits from August to October (Hong et al., 1998; Ohba et al., 2008). The seeds are dispersed by wind, water and gravity and germinate in May or June (Ghimire et al., 2005).

N. scrophulariiflora has many vernacular names in different geographical regions or different languages and are given in Table 1.

Conservation status

N. scrophulariiflora rhizomes are highly valued and are harvested from the wild and sold in the market (Ghimire et al., 2008; Kafle et al., 2018; Olsen, 2005). There is a huge trade value of katuki with about 66% of international trade originate from Nepal, followed by India (19%) and Bhutan (14%) (Olsen, 2005). Annual trade volume of dry *N. scrophulariiflora* rhizomes during 2015/016 in Nepal was 6076kg and was worth of NRs 8573236 (USD 83235.30) (Kafle et al., 2018). In addition to unregulate over harvesting of the plant for trade, other human-induced factors such as deforestation, forest fires, grazing, habitat encroachment, natural disasters and possibly climate change contribute a lot in depletion of the species (Ghimire et al.,

2005). In Nepal, the species is categorized as banned for collection, export and prioritized for conservation and cultivation (GoN/MoFSC, 1995; MoFSC/DPR, 2006) and as 'vulnerable' (CAMP, 2001). However, there is also a provision that allows the collection of *N. scrophulariiflora* like other forest products in Nepal.

Table No. 1.: Vernacular name

Name	Region/language/system of medicine
Gorki, Honglen	Tibetan
Gorki, Kutaki	Gurung
Hodling, Hong-len, Hunling	Sherpa
Hong len	Amchi
Hung gung	Bhotia
Karu	Punjabi
Katki, Karu	Bengali
Katuka, Katuka-rohini, Rohini, Katuki, Matsyapitta, Tikta, Krsnaveda, Sakuladani	Sanskrit
Katuki	Bengali
Katuko, Katuki	Danuwar
Ko-ohren	Japanese
Kuraki	Tamang
Kutki, Kaduki, Katuki, Katuko	Nepali
Kutki, Kuru	Hindi
Kutki, Kutaki	Lhotsehampkha
len, Puti sin, Ngo-Hoglen	Bhutanese
Picrorhiza, or Gentian, or Kurroa, or Hellebore, Nepalese kutki	English
Putising	Dzongkha
Tikta	Kham
Xuan hu lian	Chinese

When the collection is authorized by District Forest Officers (DFO) by issuing licenses (GoN/MoFSC, 1995) and such licenses generally specify collection area, the

harvesting period, clear identification of species, quantities to be collected and method of harvest (Amatya, 2005). In China, it has been designated as critically 'endangered' (Dong et al., 2007), in Bhutan as 'very rare' species (Mukhia, 2004) and in India as 'vulnerable' (Nayar and Sastry, 1994).

Plant growth and cultivation

N. scrophulariiflora is collected from the wild to meet demands in the regional and international markets. Harvesters are mostly highland animal herders, shepherds or other economically marginalized people in rural areas with a desire to earn good money haphazardly uproot plant in bulk leading to threat of plant existence (Ghimire et al., 2008; Kafle et al., 2018; Olsen, 2005; Olsen and Bhattarai, 2005). Thus, there is an urgent need to know and formulate cultivation techniques in the controlled environmental conditions such as through biotechnological conditions for protecting plant and also fulfill market demands.

The plant normally propagates by seeds and rhizomes. The germination rates are low and there is no persistent seed bank (Ghimire et al., 2008). For cultivation, mature seeds from 2–3 years old mother plants have to be collected during October and sown in nursery during March-April. Rhizome with 3–4cm in length from 2–3 years mature plants should be implanted in nursery in November/December. It grows best in soil with high organic matter (Ghimire et al., 2005; Hong et al., 1998; Pusalkar, 2014) and relatively acidic soil with pH value of 4.8–5.3 (Negi et al., 2015). The plant can also be propagated through rhizome with roots 3–4–more inches in length during rainy season in May-June. Although it has been shown that 50–60% seeds germinate in moisture-maintained beds and 75–100 % in laboratory, only 10–20% of seeds germinate in natural habitat (Ghimire et al., 2008). For plantation of seedling, spacing of 30cm for each plant and 60cm for field lines is well preferred. The best artificial cultivation period for cutting multiplication is for 2–3 years with fitted shading and black-plastic mulching that increases the survival percentages and yield of the plant which very much depend on manuring (Cui et al., 2012). The rhizomes could be harvested after 4–5 years of cultivation (Ghimire et al., 2008) and older the rhizomes are better in secondary metabolites content (Wang et al., 1993). Species is naturally distributed from upper sub-alpine areas to alpine region where the harsh environment condition limits cultivation. In addition

to this, there are not any evidences of successful transplantation for the production of this plant till the date.

Micropropagation

Advancements in scientific knowledge has resulted in development of laboratory techniques using artificial medium for growth of living cells or tissues (Mondal et al., 2013). The *in-vitro* multiplication often known as micropropagation or tissue culture is important for conservation of germplasm of different plants species that are threatened and are economically important (Espinosa-Leal et al., 2018; Kumar and Reddy, 2011). In addition to this, micropropagation could also be used in mass production of plant individuals that can be used to extract different bioactive compounds both for commercial and sustainable use (Mondal et al., 2013). For *in-vitro* micropropagation, different plant parts have been used as explants. They are shoot tips, nodal segments (Bantawa et al., 2010, 2009), leaf (Bantawa et al., 2011a, 2011b) and leaf derivative callus (Bantawa et al., 2010). Bantawa et al. (2009) used woody plant medium (WPM) (Lloyd and McCown, 1981) supplemented with 0.05mg/l 6– benzyladenine (BA) for shoot initiation then (Murashige and Skoog, 1962) (MS) with Bavistin (100) and adenine sulphate (100) was used for shoot proliferation. Rooting was carried out in MS and a-napthaleneacetic acid (NAA, 1mg/l). Rooted were hardened using plastic cups containing virgin soil and sand (9:1) and the survival rate was 97%. Next, plants grown in WPM supplemented with BA were transferred in MS and kinetin (Kin, 0.5mg/l) for shoot proliferation and MS and NAA (1mg/l) for rooting (Bantawa et al., 2010). Multiplication of hoots was formed within 28 days and root initiation started in 15–18 days for which root platelets were acclimatized in sterile virgin soil and sand (9:1) with a 90% survival after 60 days. Acclimatization of well-rooted plantlets was established in cultivation after 12 weeks.

In plant regeneration via callus-mediated organogenesis where *in-vitro* leaf was used as explant Bantawa et al. (2011a, 2011b), WPM supplemented with NAA (0.1mg/l) and Kin (0.05mg/l) was used for shoot initiations. Then WPM and BA (0.1mg/l) was used for shoot proliferation and WPM and NAA (1mg/l) for root initiation that showed root initiation only in 14 days. Survival rate of transferred plant was 90% and after 10 months of hardened plants were distributed to local farmers for planting

proving that tissue culture could be used to multiplicate large numbers of affordable plants. In a next experiment, somatic embryos of *N. scrophulariiflora* were encapsulated in calcium alginate gel matrix with MS supplemented with BA (0.1-0.2mg/l) was used for shoot initiation (Bantawa et al., 2010). MS with L–glutamine and abscisic acid, ABA (0.1–1.0mg/l) were used for shoot proliferation and then for rooting MS mixed with Kin (0.5mg/l) and gibberellic acid, GA_3 (0.5mg/l) was used. High microbial contamination was observed during initial establishment of *in-vitro* cultures. Once hardened and transferred to mixture of autoclaved soil and sand (9:1) showed survival rate of 85% after 5 months. Above mentioned experiments have shown that *in-vitro* micropropagation is important affordable technique to obtain robust plant individuals in large numbers with good contents of bioactive compounds. However, all techniques are still in the process of further developments and till the date there are no data available for using *in-vitro* micropropagation for mass production. Yet, techniques look promising as a small part of plant could reproduce into many replicates. Thus, it needs further investigations to have substantial implementations.

Pest: Neopicrorhiza scrophulariiflora leaves are affected by a types of fungus and the disease is known as Leaf spot.

Ethno-medicinal uses

As mentioned before *N. scrophulariiflora* is used against different ailments/diseases. In the present review, a total of 82 ailments/diseases were treated by this plant and different remedies were categorized into circulatory system, dermatological system, endocrine system, ENT (ear, nose and throat), gastro-intestinal system, hepatic disorder, immunological system, musculoskeletal system, neurological system, reproductive system, respiratory system, urino-genital system, fever and others. Other disease category included antidote against poison including scorpion sting, snake bite and mice or dog bites, body cooling, cough/hiccough, headache, heat, hypotension, mucous problems, gums and teeth problems, typhoid and wounds. Among disease categories gastrointestinal accounted maximum percentage (27%), others (26%), circulatory, dermatological and musculoskeletal systems (7% each), respiratory system (6%), endocrine system and ENT (5% each), neurological, reproductive and fever (4% each) hepatic disorder (2%), and immunological and

urino-genital systems (1% each). Use of roots is quite effective as these contain bioactive compounds in large amount (Srithi et al., 2009). Different preparations were used and they are powder (23%), paste (20%), decoction (16%), mixture (5%), raw (4%) and juice (1%). However, in large documentations most unspecified preparations (31%) are also mentioned.

Phytochemistry

There are different techniques used for isolation of compounds from different plant parts of N. scrophulariiflora. The most common techniques are ionic liquid-based ultrasonic- assisted extraction and ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry, silica gel column chromatography (Wang et al., 2006, 2004) and high performance liquid chromatography (HPLC) (An et al., 2009; Zhu et al., 2008). Total of 124 compounds are reported and are categorized into 17 categories; iridoid (34.7%), phenylethanoid glycoside (20.2%), phenol (9.7%), cucurbitacin glycoside (8.1%), hydroxycinnamate group (5.6%), sugar and phenyl glycoside (4.0% each), phytosterol and ferulic acid and its ester (2.4% each), fatty acid, flavonoid and steroidal glycoside (1.6%), and phytosterol glucoside, alcohol, hydroquinone glycoside, coumarin and Phenylpropanoid (0.8%). Different compounds are mostly extracted from roots/rhizome (An et al., 2009; Zhu et al., 2008) but rarely from stem (Huang et al., 2006) and seeds (Chen et al., 2011). Extracts that were used during isolation process are ethanol hydroxide (Kim et al., 2006a) or ethyl acetate (EtOAc)/methanol hydroxide (MeOH) (Huang et al., 2004a) or 1-BuOH soluble fraction of methanol (MeOH) extracts (Kim et al., 2006b) or ethanol extracts (Hu and Yang, 2005; Wang et al., 1993). The final compounds determined in n-butanol (n-BuOH) layer using HPLC techniques (An et al., 2009; Zhu et al.,2008) or column chromatographic techniques (Hu and Yang, 2005; Huang et al., 2006).

Pharmacology: *N. scraphularifolia* has shown the pharmacological activities such as anti-atherosclerotic activity (Guo et al. (2009), anti-inflammatory activity (Smit et al., 2000), antimicrobial activity (Timsina, 2003), anti-malarial activity (Wang et al., 2013), anti-oxidative activity (Tiwari et al., 2012), hepatoprotective activity(Wang et al., 2006), immunomodulatory property(An et al., 2009; Smit,2000; Smit et al., 2000;

Zeng et al., 2008), nerve growth factor potentiating activity(Li et al., 2000, 1999), renal improvement activity(He et al., 2009; Feng et al.,2010) and others.

This study of *N. scrophulariiflora* reviewed textual and web based information. The study was further dealt with morphological characters, pharmacognostical and phytochemical screening evaluation, climacteric variable like temperature and rainfall at the geographical region; and soil test collected from the habitat of *N. scrophulariiflora.* This data will be compared with the data generated on same parameters after the next 10 years.

Aims & Objectives

The assessment criteria for the identity and purity of the genuine source and the plant material; *N. scrophulariiflora* is established for assurance of quality and reliability. This study has been carried out on the crude drugs *N. scrophulariiflora* rhizomes and their powders to fulfillment of the following objectives as shown below:

1. Authentication, collection and preservation of the samples
2. Organoleptic, macroscopical and microscopic evaluation of the crude drugs and their powders,
3. Foreign matter, loss on drying, total ash value, extractive value, phytochemical screening and finger-printing of the plant material.

Plan of Study:

The present work has been carried out pharmacognostical studies and phytochemical evaluation of the study material; the rhizomes under the following headings:

- Organoleptic and macroscopic characteristics the rhizomes and their powders.
- Physico-chemical evaluation of the rhizome
- Phytochemical screening and quantification

DARUHARIDRA

Berberis aristata DC.

Ayurveda" is a traditional medicine system which was developed during the Vedic times, around 5000 years ago. The herbal remedies described in it take us "back to the nature" which makes use of plants, metals and its oxides termed as "bhasmas" for the treatment of various ailments. The traditional Indian and Chinese medicinal systems reveal that almost every part of this plant has some significant medicinal value. Its roots, stem, bark, leaves, rhizomes and fruits are used in many classical ayurvedic preparations like *Rasaut, Darvyadi kvatha, Darvyadi leha, Darvyadi taila, Rasanjana, Dasanga lepa* and many more. Extracts obtained from the plant find application in pharmaceuticals, neutraceuticals and cosmeceutical preparations. ***Berberis aristata***, also known as **Indian barberry**, "chutro" or **tree turmeric**, is a shrub belonging to the family Berberidaceae and the genus *Berberis*. The genus comprises approximately 450-500 species of deciduous evergreen shrubs and is found in the temperate and sub-tropical regions of Asia, Europe, and America. *B. aristata* is native to the Himalayas in India and in Nepal. It is an erect spinous shrub, often found in small patches on the hilly slopes. This shrub is found growing wild in the sub-Himalayan tract at altitude ranging from 1800-3000 meters. It also grows in the Nilgiri hills in South India and Srilanka(Anonymous, 1998).

There are 12-13 varieties like *Berberis asiatica, Berberis lycium, Berberis vulgaris, Berberis nepalensis,* etc (Mazumder et al., 2011).

Taxonomy of *Berberis aristata*

Kingdome: Plante

Clade : Angiosperms

Order : Ranuculases

Family : Berberidaceae

Genus : *Berberis*

Species : *B. aristata, B. vulgaris, B. lyceum, B. nepalensis, B. asiatica, etc.*

Substitute: *Berberis asiatica* Roxb. ex. DC. (Berberidaceae), known in English as *tree turmeric*, is an important medicinal plant. It is extensively sold in different markets of India as a common substitute to "Daruharidra", that is, *B. aristata*.

Therefore, the current study has been undertaken to establish the data for this species, which will be useful to pharmaceutical industries for the authentication of their commercial samples.

Vernacular name

Indian barberry, tree turmeric

Indian names: *darhaldi* (Bengal), *kashmoi* (Garhwal), *rasont, kashmal* (Himachal Pradesh), *chitra, dar-hald, rasaut, kashmal* (Hindi), *maradarisina, maramanjal,* (Kerala), *daruhald (*Maharashtra), *chitra, chutro* (Nepal), *chitra, kasmal. simlu, sumlu* (Punjab) *mullukala, usikkala* (Tamil Nadu), *daruharidra, darvi, kata, pitadaru, suvarnavarna* (Sanskrit).

Botanical characteristics

Berberis aristata DC belonging to family Berberidaceae commonly called 'Indian barberry' in English and 'Daruhaldi' in Hindi is indigenous to India.

A large deciduous shrub usually is 1.8-3.6 m high. The stem is 4.5 m height and 20 cm diameter, nearly cylindrical, surface rough. Twing whitish or pale yellowish brown, Bark pale brown, closely and rather deeply furrowed, rough, Leaves on long shoots, 3.8-10 cm long, 1.5-3.3 wide, obovate or elliptic, simple or either entire or with spinous toothed margin, base gradually narrowed, with prominent reticulate nerves, glossy dark green above, glossy pale green below but not glaucous beneath petiole or distinct up 4 mm, stout, 4-6 mm long. The fruits are small berry 7-10 mm long, ovoid, blue-black thick pale bloom in colour(BD, 1984). The all parts of plant are economically important and when medicinal value is focused. Parts like wood, stem, root bark and fruit and its extract in traditional medicine from long time.

Conservation status

Several species of the genus Berberis are vulnerable or endangered including Berberis aristata DC. and Berberis asiatica. These plants are reported to be rarely available in their natural habitat.

Current status

The extensive uses of *B. aristata* by different pharmaceutical industries coupled with the recent revival of interest in herbal medicine have led to an ever-increasing demand of this species. It has therefore become essential to search for a possible substitute for this species and to ensure the quality of the raw drug by

pharmacognostic investigations. A detailed pharmacognostic study of *B. aristata* is reported by Srivastava et al. (2001), but to date no detailed pharmacognostic data is available on *B. asiatica*.

Ethno-medicinal uses

The root is extensively used in various indigenous systems of medicine for treating a variety of ailments such as eye and ear diseases, rheumatism, jaundice, diabetes, fever, stomach disorders, skin disease, malarial fever, and as tonic, and so forth (Watt, 1883; Kirtikar & Basu, 1933; Anonymous, 1988).

The bark and root are the medicinal part of the plant. It is used as a single plant remedy or in polyherbal formulations, particularly in organized systems of medicine such as Ayurveda, Siddha, and Unani. Its use in the management of infected wounds has also been described in Ayurvedic classical texts (Sushrut Samhita, 1963). It is also used against pneumococcal infection (Hashmi & Hafiz, 1986; Bhandari et al., 2000). The major alkaloid of the plant has been reported to be berberine (Bhakuni et al., 1968; Rastogi & Mehrotra, 1993), which is known for its activity against cholera (Dutta & Panse, 1962), severe diarrhea (Lahiri & Dutta, 1967), amoebiasis, latent malaria, and for the treatment of oriental sore caused by *Leishmania tropica* (Anonymous, 1988). Ethnomedical investigation carried out by Shah & Joshi (1971) revealed that the tribal Kumaun region use the decoction of root for treating eye troubles and boils. However, Chauhan (1978-79) reported that the decoction is also being word for piles, gastric disorders, and other allied complaints by Tibetans.

The *Berberis aristata* DC extract is called 'Rasaut', used as alternative and deobstruent and are used in skin diseases, menorrhagia, diarrhoea, jaundice and all affections of eyes. In bleeding piles it is administered with butter. Its ointment made with camphor and butter and applied pimples and boiles. The decoction of root bark is used as a wash for unhealthy ulcers, also in malarial fever. The stem is used for diaphoretic, laxative and useful in rheumatism. The of stem of *Berberis aristata* DC have been used in ethno medicine and in many Ayurvedic preparation for several medicinal properties alternative, antibacterial, antidiarrhoeal, ophthalmic, antidiabetic, eye, ear, oral cavity and in skin diseases(AK, 1976).

Traditionally B. aristata is well-known for its properties such as Lekhaniya (reducing toxicity and unnecessary fats), Arshoghna (anti-haemorrhoidal) Stanyasodhana

(lactode purant), Ropana (wound healer), Svedala (promotes sweating), Rasayana (rejuvenative), Kandughna (anti-pruritic) and can also be used for treating skin disorders. B. aristata i.e. *Daruharidra* (resembles in its properties to those of Turmeric i.e. Haridra), hence both the herbs have been mentioned together as *Haridra* dvaya, meaning two Haridras viz. Haridra and *Daruharidra*.

It is an important commodity in the folklore medicine of India used for allergies, metabolic disorders, ophthalmia and other eye diseases and as a laxative. It is one of the 73 plants which are used to treat skin diseases traditionally in Nepal and other surrounding villages (Joshi and Joshi, 2007). A multi- erbal formulation containing B. aristata is used for treating bleeding piles in some rural parts of India(Saraf et al., 2010). Ethnobotanical studies indicate that Rasaut-decoction of B. aristata leaves, is used as an alternative and deobstruent and commonly used to treat skin diseases, menorrhagia, diarrhoea, cholera, jaundice, eye and ear infections, as well as urinary tract infections. Its root decoctions are used to treat eye diseases in Bhotiya communities in the Himalayan ranges in India(Phondani et al., 2010). Furthermore, Malani tribal communities from Himachal Pradesh, India use it to cure skin diseases, jaundice, piles and malaria. Its fruits are eaten as laxative and anti-scorbutic(Sharma et al., 2005). In Garhwal Himalaya, B. aristata is used as a psychomedicine for treating exorcism in children(Tiwari et al., 2010). The plant root is useful in treating jaundice(Acharya and Rokaya, 2005). In Nepal, the plant's fruit and leaf juice are used for treating diarrhoea and dysentery while its bark and root decoction are used to treat jaundice and fever(Kunwar and Adhikari, 2005). Some Himalayan tribes residing in Sikkim and Darjeeling in India use the plant extract as anti-diabetic and anti-hepatopathic(Chhetri et al., 2005).

Phytochemistry

The plant contains a number of important phytochemicals which are alkaloids of the type proto-berberine, isoquinoline, bisbenzyl-isoquinoline and other bioactive constituents like flavonoids and phenolic acids.

The root bark of the plant contains a protoberberine alkaloid; karachine(Blasko et al., 1982b) along with aromoline, oxyberberine, oxyacanthine, berbamine, and berberine chloride(RAHMAN and Ansari, 2011). *B. aristata* flower contains various polyphenolic flavonoids like quercetin, rutin, meratin and acids like Ecaffeic acid and chlorogenic

acid(Sivakumar and KAMACHANDRAN NAIR, 1991). Alkaloids like pakistanine, 1-O-methylpakistanine, pseudopalmatine chloride and pseudoberberine chloride were isolated from the bark of the plant(Saied et al., 2007). Another alkaloid taxilamine was also obtained from this plant(Blasko et al., 1982a). Alcoholic extraction of the powdered bark of B. aristata after concentration and filtration gave berberine, tetrahydropalmatine, tetrahydroberberine palmatine and palmatine chloride or its mixtures(Chakravarti et al., 1950).

Pharmacology:

One of the traditional medicinal plants from ayurvedic system is known as Berberis aristata (Daruharidra) and its herbal formulations are used to treat malaria, bleeding, fever, skin and eye infections, jaundice, diarrhoea and hepatitis for a long time. Its traditional use as anti-microbial, anti-bacterial, anti-pyretic, immunostimulant, laxative, anti-haemorrhagic and anti-inflammatory agent is also well known. Traditional anti-osteoporosis activity of B. aristata was confirmed when ovariectomized (OVX) rats were tested for the aqueous methanol extract of the plant. These findings suggested that the ethnic use can be continued in treatment of osteoporosis, joint pain and menopause(Yogesh et al., 2011). Rasaut also shows anti-bacterial, anti-fungal, anti-inflammatory, analgesic, and anti-pyretic activities(Shahid et al., 2009, Meena et al., 2009, Sati and Joshi, 2011).

This study of *Berberis aristata* reviewed textual and web based information. The study was further dealt with morphological characters, pharmacognostical and phytochemical screening evaluation, climacteric variable like temperature and rainfall at the geographical region; and soil test collected from the habitat of *Berberis aristata.* This data will be compared with the data generated on same parameters after the next 10 years.

Aims & Objectives

The assessment criteria for the identity and purity of the genuine source and the plant material; *Berberis aristata* is established for assurance of quality and reliability. This study has been carried out on the crude drugs *Berberis aristata* rhizomes and their powders to fulfillment of the following objectives as shown below:

1. Authentication, collection and preservation of the samples,

2. Organoleptic, macroscopic and microscopic evaluation of the crude drugs and their powders,
3. Foreign matter, loss on drying, total ash value, extractive value, phytochemical screening and finger-printing of the plant material.

Plan of Study

The present work has been carried out pharmacognostical studies and phytochemical evaluation of the study material; the rhizomes under the following headings:

- Organoleptic and macroscopic characteristics the rhizomes and their powders.
- Physico-chemical evaluation of the rhizome
- Phytochemical screening and finger printing

GUDUCHI

Tinospora cordifolia (Wild.) Miers ex Hook. F. & Thomas

Tinospora cordifolia (*TC*) being a *rasayana* drug from Ayurveda is recommended for a number of diseases and for the promotion of health. It is distributed throughout India as well as in China, Burma and Srilanka. There is evidence that the plant is found in tropics of Africa and Australia (Singh et al., 1984). Its habitat ranges across a wide region in India spreading from Kumaon Mountains to Kanyakumari, the southern tip of India. *Tc* has several vernacular names in various Indian languages: *Gulvel* (Marathi), *Giloe* (Hindi), *Gulancha* (Bengali), *Somida* (Telugu), *Sindal* (Tamil) and *Sittamrytu* (Malyalam). This plant has continued to draw the attention of research workers all over the world for over 50 years.

Tinospora cordifolia (TC) referred to as *Guduchi* (plant which protects from diseases, Sanskrit), has been described in ancient textbooks of Ayurveda including *Sushrut Samhita* and *Charak Samhita*. Other synonyms used for *Gauche* which refer to its various properties and uses, include *Chhinnaruha/Chhinnodbhava* (for the plant's capacity to grow from the cut side), *Vatsadini* (eaten by grazing animals), *Amruta* (imparts immortality), *Rasayana* (capacity to improve quality of *rasa*, the primordial tissue which in turn strengthens all other tissues), *Chakrangi/Chakralakshana* (for its wheel-like appearance), *Jwaranashi/Jwarari* (potent antipyretic), *Vayastha* (prevents ageing), *Amrutsambhava* (ambrosia), *Bhishakpriya* (favourite of physicians), *Saumya* (not harmful) and *Tikta* (bitter taste). Ayurveda recommends that the plant grown on Mango (*Mangifera indica*) and Neem (*Azadirachta indica*) trees is the best for medicinal use. May (high summer month in India) is the best season for collection of the plant specimens. While harvesting, care should be taken to select the stems having a girth equal to thumb size. Different dosage formulations of *Tc* along with their specific method of preparation and dosages have been described in Ayurveda. These include *Swaras* (juice from the fresh stem: 10–20 mL/day), *Kalka* (paste of fresh stem: 10 g/day), *Churna* (powdered dry stem: 1–3 g/day), *Kwatha* (hot water extract from ground dried stem: 20–30 mL two to three times a day), *Fant* (hot water infusion: 10–20 mL/day), *Arishta* (stable processed formulation from a decoction of *Tc* containing self-generated alcohol), *Satwa* (sediment starchy extract of the stem: 750

mg to 2 g/day) *Ghana* (solidified aqueous extract: 500 mg to 1 g three to four times a day), along with lipid formulations of *Tc* processed in *ghee* or oil (*Guduchi Ghrita*: 10–20 g/day)

and *Guduchi Taila* (for external application). Apart from these, multi-ingredient formulations containing *Tc* have been described in traditional Ayurvedic textbooks, some of which are routinely used by practitioners (e.g. *Chandraprabhavati*, *Kaishor guggul*, *Abhayadi kwatha*, *Rasnadi Kwatha* etc).

According to Ayurveda, every drug has certain attributes namely, *Rasa* (taste), *Guna* (property), *Vipak* (metabolite), *Veerya* (potency), *Prabhav* (inate and specific property) and *Karma* (action). Though it is not possible to interpret all these terminologies in contemporary medical terms, the properties of *Tc* can be explained based on these Ayurvedic attributes. *Tc* itself possesses a bitter, pungent and astringent taste but it acts by virtue of its *madhur* (sweet) *vipak*. The bitter taste is said to improve metabolic activity, even at a cellular level. All the three tastes destroy toxic metabolic products and purify tissues. *Rasayana* drugs with *madhur vipak* are considered to be immunostimulants (Wagner, 1999). *Tc* has *snigdha* and *laghu guna*. Its effects on the reproductive system are attributed to its *snigdha guna* and *madhur vipak*. Agents with *laghu guna* usually create a feeling of wellbeing and enthusiasm.

Its *veerya* is considered as *ushna* and therefore it helps in digestion, increases appetite and helps in the dissipation of heat and waste products from the body. *Tc* has *tridoshashamak* actions and therefore can be used for patients irrespective of their constitution (*pitta*, *vata* or *kapha*). Three major pharmacological properties of *Tc* are *deepan* (ability to facilitate metabolism), *jwaranashan* (to alleviate fever) and *rasayana*. The latter property imparts health, prolongs life, enhances memory and intelligence, preserves youth, improves skin colour and texture, improves the quality of the voice, strengthens the function of special senses and increases sexual functions in terms of sexual urge as well as fertility. It is also said that a person taking *rasayana* drugs develops the power to bring all the spoken words to reality (Upadhyaya, 2000). *Tc* has a special affinity for *rakta* (blood), *meda* (adipose tissue) and *shukra dhatu* (reproductive elements). The main target organs for *Tc* are kidney, liver and spleen(Vaidya, 1998, Shastri, 1999).

Other properties of *Tc* include *jeevaniya*, *balya* and *vayasthapaniya*. The literal description of these terms indicates that *Tc* promotes life, imparts strength (to the tissues) and establishes and maintains youthfulness.

By virtue of these properties, *Tc* is advocated in Ayurveda for various clinical conditions. It relieves thirst and is used as an appetizer and digestive agent. It is recommended for gastrointestinal diseases including dyspepsia, flatulence, gastritis, jaundice, splenomegaly and hemorrhoids. It is used in diarrhea. It has a role in the treatment of metabolic disorders (*prameha*) including diabetes and kidney disorders. It is antispasmodic, analgesic, antipyretic and antiinflammatory. It is given for intermittent fevers and infective conditions such as typhoid, malaria, filariasis and leprosy. It also has anthelmintic properties. It is prescribed for urinary disorders, skin diseases and eye diseases. It is also used in gout and rheumatoid arthritis. It has cardiotonic, hematinic, expectorant, antiasthmatic and aphrodisiac actions. The well-ground whole plant is applied on fractures. It is a general tonic to be used in convalescence(Vaidya, 1998, Shastri, 1999, Tripathi, 2001a, Tripathi, 2001b).

Botanical characteristics

Medicinal plants have been used to treat human illness since time immemorial. *Guduchi* or *Giloe* is considered as *Tinospora cordifolia* Miers belonging to family Menispermaceae. It is Amrita, Gauche in Sanskrit, Gurjo in Nepali, Giloe in Hindi. It is distributed throughout the tropical areas upto 1000 feets in altitude of Indian subcontinent of India, Pakistan, Sri Lanka, Burma, Africa, Austalia and China(Panchabhai et al., 2008), Bangladesh(Hossain et al., 2009), Thailand, Philippines, Indonesia, Malaysia, Borneo, Vietnam(Panchabhai et al., 2008, Singh et al., 2003). It is an indigenous; and has been documented in the ancient Ayurveda literatures for diabetics and immune-modulatory properties. It is a large glabrous, deciduous, shady climbing shrub with succulent corky grooved stems with long filiform or slender pendulous fleshy aerial roots from the branches, bark is creamy white to grey; leaves are membranous, subdeltoid, cordate; flowers are small and yellow or greenish yellow; seeds are curved like moon shaped and fruits are fleshy and single seeded. Flowers grow during the summer and fruits during the winter(Kirtikar KR, 1975, Anonymous, 1976).

Traditional uses

Plant-derived medicines have been part of traditional health care in most parts of the world for thousands of year(Palombo and Semple, 2001). The plant juice has traditionally been used for the treatment of fever(Panchabhai et al., 2008)

Ayurveda and folklore

T. cordifolia is widely used in folk medicine and Ayurvedic system of medicine as a tonic, vitalizer and as a remedy for diabetes and metabolic disorders(AK, 1954, Hossain et al., 2009, Chopra RN, 1958b). Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, anti-spasmodic, anti-inflammatory, anti-arthritic, hepatoprotective, anti-allergic and anti-diabetic properties. The plant is used in ayurvedic medicine as "Rasayanas" to improve the immune system and the body resistance against infections. It is also known by the name magical herb due to its property to cure a number of diseases(Srivastava, 2011). In ancient traditional Ayurvedic system of India, it is a constituent of several remediation used for various treatment such as general debility, dyspepsia and urinary diseases (Ahmad et al., 2009).

It is well reputed in traditional system of medicine to treat various aliments such as fevers, inflammations, skin infections and urinary infections(Kirtikar KR, 1975).

Tinospora cordifolia commonly known as Guduchi or Amrita, a traditional herbal medicine, is used as a remedy for fever, diabetes, dyspepsia, jaundice, and skin diseases(Sinha et al., 2004).

Various Ayurvedic texts and Nighantu (lexicons/Ayurvedic materia medica) have described its anti-diabetic usages under various names *viz*. *Pramehaghna*, *Pramehahara*, *Mehaghna* and *Mehahara*[5-9](YT, 2008, YT, 2004, Tripathi, 1997, Chunekar KC, 2006, Shastri, 2008). Ayurvedic Pharmacopoeia of India has also cited its antidiabetic utility(Anonymous., 2001). Tribals of Korkus (Melghat, Maharashtra, India) have been using the herb for polyuria, diabetes and fever(Tambekar et al., 2009). In Bhava Prakash, the plant is considered as bitter tonic and curative against chronic diarrhea and dysentery(Chunekar KC, 2006). It is categorized as "*Rasayana*"(Mishra, 2008) and used for its anti-inflammatory,[4,5] immunomodulatory,[6] anti-allergic,[7]. It is one of the frequently used drugs to treat the *Madhumeha*, *Pandu*, *Kamala*, *Amlapitta*, *Grahani*, *Kustha*, *Jirna Jwara* and *Viswamjwara*, *Trishna*, *Shool* and *Yakritavikara (Patgiri et al., 2014)*.

Phytochemistry

The phytochemical constituents of *T. cordifolia* include aliphatic compound, alkaloids, steroids, glycosides, sesquiterpenoid, polysaccharides, different types of fatty acids and essential oils(Panchabhai et al., 2008, Patel and Mishra, 2011). The preliminary phytochemical analysis carried out by Hussain et al. showed that *T. cordifolia* contains saponins, tannins, terpenoids, flavonoids, alkaloids and glycosides(Hussain et al., 2015), which conformed with descriptions of the plant's phytochemical constituents as described in the literature(Panchabhai et al., 2008).

Singh et al. studied preliminary phytochemical screening of aerial part of the plant and reported a number of chemical constituents such as alkaloids, steroids, glycosides, terpenoids, flavonoids and polysaccharides(Singh et al., 2003).

The active adaptogenic constituents present in *Tinospora cordifolia* are diterpene compounds including tinosporone, tinosporic acid, cordifolisides A to E, syringen, the yellow alkaloid, berberine, giloin, crude giloininand, a glucosidal bitter principle as well as polysaccharides, including arabinogalactan polysaccharide(Shanish Antony et al., 2010).

HPTLC standardization of Tinospora cordifolia using Tinosporaside(Sethi, 1996) was found that the Rf of Tinosporaside was about 0.58 and content of Tinosporaside was found to be 0.05% w/w in the sample(Singh et al., 2003).

Palmatine is a quaternary protoberberine alkaloid. It is typically yellow in color and reported as the most important pharmacological active constituents of a number of plants, such as *Tinospora cordifolia*(Ali and Dixit, 2013, Giri et al., 2006)

Pharmacology:

The plant hea been used for hundreds of years in Ayurvedic medicine with no reported toxicity. Tinospora cordifolia is widely used as a tonic, vitalizer and as a remedy for metabolic disorders(AK., 1954, Chopra RN, 1958a). The plant showed various medicinal properties such as antidiabetic(Gupta, 1967, Stanely et al., 2000, Grover et al., 2000, Grover et al., 2001, Stanely Mainzen Prince and Menon, 2003, Mainzen Prince et al., 2004, Shalam et al., 2006, Purandare and Supe, 2007), diabetic cataract(Rathi et al., 2002), diabetic food ulcer(Purandare and Supe, 2007), different types of infections(Sohni and Bhatt, 1996, Samy, 2005), gastrointestinal related complications(Bafna and Balaraman, 2005), Analgesic(Hossain et al., 2009), Anti-

Inflammatory and Anti-Pyretic Activities(Hussain et al., 2015), hypolipidemic(Prince et al., 1998, Mainzen Prince et al., 2004, Prince and Menon, 2003, Kar et al., 2003), antihepatotoxic(Peer and Sharma, 1989, Bishayi et al., 2002, Pandit et al., 2003, Tasaduq et al., 2003, Bhar et al., 2005), immunomodulatory(Kapil and Sharma, 1997, Manjrekar et al., 2000, Thattet and Dahanukar, 1989), antipyretic(Vedavathy and Rao, 1991), antiulcer(Sarma et al., 1995, Sarma et al., 1996), anticancer(Chauhan, 1995, Manjrekar et al., 2000, Jena et al., 2003, Diwanay et al., 2004, Ali and Dixit, 2013), inhibition of lipid peroxidation(Prince and Menon, 1999), antioxidant activities(Mathew and Kuttan, 1997, Stanely Mainzen Prince and Menon, 2001, Singh et al., 2003), antibacterial activity(Jeyachandran et al., 2003), amelioration of CNS toxicity(Singh et al., 2003) radioprotective(Pahadiya and Sharma, 2003), antifertility activity(Gupta and Sharma, 2003), antiallergic(Devprakash et al., 2011, Sunanda et al., 1986), alpha glucosidase inhibition(Chougale et al., 2009), Sleeping time(Hossain et al., 2009) It increases body weight, total haemoglobin and hexokinase activity.

The notable medicinal properties reported are anti-periodic, anti microbial, antiarthritic, anti-leprotic, ant-malarial and antineoplastic activities (Krishna et al., 2009, Panchabhai et al., 2008, Upadhyay et al., 2010, Sood et al., 2012).

Analgesic, Anti-Inflammatory and Anti-Pyretic Activities:

The analgesic effects of *T. cordifolia* extract were assessed by using the acetic acid-induced writhing test, hot plate test and tail-flick test. The carrageenan test was performed to assess anti-inflammatory potential, and anti-pyretic activity was evaluated by the brewer's yeast- nduced pyrexia method. The results showed that the *T. cordifolia* extract exhibited significant analgesic effects in a dose-dependent manner in the three pain models tested. The extract also exhibited significant anti-inflammatory effects in the carrageenan-induced inflammation test and antipyretic effects in the brewer's yeast-induced pyrexia test in dose- -dependent manner compared to the effects observed in the control group animals(Hussain et al., 2015). In this study, it can be concluded that *T. cordifolia* extract has strong analgesic, anti-inflammatory and anti-pyretic effects.

Hossain et al. (2009) investigated analgesic activities of methanol extract of the aerial parts of *Tinospora cordifolia.* Analgesic activity of the crude extract at the dose of 200 and 400 mg/kg b.w. was evaluated for its central and peripheral pharmacological

actions using hotplate and tail flick tests and acetic acid-induced writhing test respectively in mice. The extract produced a significant ($p < 0.05$-0.001) increase in pain threshold in hotplate and tail flick tests in a dose dependent manner. In acetic acid-induced writhing test the extract at both doses produced significant ($p < 0.001$) inhibition of writhing reaction but maximum inhibition (65.01%) of writhing was found at 400 mg/kg dose compared to the reference drug Diclofenac-Na at the dose of 10 mg/kg b.w. (77.07%)(Hossain et al., 2009) .

Goel et al. carried out to evaluate analgesic effect of commercially available extract of Guduchi (*T. cordifolia*) from Himalaya Drug Company, Bangalore. Albino rats were divided randomly in three groups of six rats each. Group 1 (control) received distilled water orally, group 2 (test) received *T. cordifolia* extract in dose of 300 mg/kg orally and group 3(standard) received Pentazocine in dose 10mg/kg intraperitoneally. Analgesic activity was evaluated using hot plate and abdominal writhing method. All the observations were analysed statistically using student's t-test. *T. cordifolia* extract significantly ($p<0.05$) increased the response time and decreased the number of writhes in hot plate method and abdominal writhing method respectively, on comparison with the control group(Goel et al., 2014). The above findings suggest that this commercially available extract of Guduchi (*T. cordifolia*) possess analgesic activity. This analgesic activity probably involves peripheral as well as central mechanisms as the extract showed analgesic activity in both hot plate and abdominal writhing method.

In a previous study the water extract of the stem of Neem-Giloe [The *T. cordifolia* that grows on Azadirachta indica (neem)] had a statistically significant and dose dependent mild analgesic activity. It also potentiated analgesic effect of morphine(Pendse et al., 1977).

Another study showed that the aqueous extracts of *Tinospora cordifolia* (AETC) had significant analgesic and anti-inflammatory activities(Siddalingappa et al., 2012).

Guduchi Ghana is one of the unique Ayuvedic classical preparations which is prepared from aqueous of extract of *Guduchi* (*Tinospora cordifolia* Miers.) stem. Patgiri et al. evaluated comparative anti-infl ammatory activity of classically prepared and market sample of *Guduchi Ghana*. Both samples were evaluated for anti-inflamatory activity using carrageenan induced paw edema model in rats. Animals were divided in three

groups, having six animals in each. Group A received test drug, Group B received market sample at a dose of 50 mg/kg orally, while Group C (control group) received tap water. In this study, reduction in edema was observed in Group A and B at 3 h interval by 33.06% and 11.71% respectively. Group A showed significant effects ($P < 0.05$) in comparison to control group. Thus, these experimental results have shown anti-inflammatory activity of *Guduchi Ghana(Patgiri et al., 2014)*.

Utpalandu et al. studied the anti-inflammatory effect of Zingiber officinale, Vitex negundo and Tinospora cordifolia on carrageenin induced hind paw oedema and cotton pellet granuloma in rats. In this study, hind paw oedema was produced by subplanter injection of carrageenin and paw volume was measured plethysmometrically at«SQ»0«SQ» and «SQ»3«SQ» hours intervals after injection. Cotton pellet granuloma was produced by implantation of 50(1mg sterile cotton in each axilla underether anaesthesia. The animals were treated with Zingiber officinale, Vitex negundo, Tinospora cordifolia and the standard drugs acetylsalicylic acid and phenylbutazone. It was found that Zingiber officinale, Vitex negundo and Tinospora cordifolia produced significant anti-inflammatory effect in both acute and subacute models of inflammation. In acute inflammation, effect of Tinospora cordifolia was more than acetylsalicylic acid. In subacute inflammation, the results of these drugs were less than phenylbutazone. It was concluded that Zingiber officinale, Vitex negundo and Tinospora cordifolia possess anti-inflammatory effects in both acute and subacute inflammation(Utpalendu et al., 1999).

Pendse et al. screened for anti-inflammatory, analgesic and antipyretic actions of the water extract of the stem of Neem Giloe (Tinospora cordifolia), an Indian Indigenous plant in albino rats and immunosuppressive effect in albino rabbits. It significantly inhibited acute inflammatory response evoked by carrageenin in a doss of 50 mg/100g given orally and intra-peritoneally. In chronic inflammation produced by croton-oil in granuloma pouch technique, 20 mg/100 g of the water extract significantly inhibited granulation tissue response; the reduction in exudative response and increase in the weight of adrenal glands were not significant. A significant inhibition of primary and secondary phases was observed in adjuvant-induced arthritis. It significantly inhibited antibody formation by typhold «DQ»H«DQ» antigen. A mild analgesic effect of its own as well as potentiation of morphine

analgesia were possessed by the extract but it was devoid of antipyretic effect(Pendse et al., 1977).

Philip et al. carried out the study regarding the mechanism of anti-inflammatory potential of T. cordifolia at the molecular level. In vitro evaluations were conducted in RAW264.7 macrophages which were preincubated with chloroform extract of T. cordifolia (CETC) and subsequently stimulated with LPS. The expressions of COX-2, TNF-a and iNOS genes were analysed by SQRT-PCR and Western blot, cytokines (IL-6, IL-1b and PGE2) levels by ELISA, NF-jB activation and p38 MAPK phosphorylation by Immunoblot and confocal imaging. Anti-inflammatory potential of CETC was validated further in a rat model of carrageenan-induced hind paw edema. It was found that the LPS-induced upregulation of proinflammatory biomarkers was significantly prevented by CETC, without inhibiting COX-1. CETC- and LPS-incubated cells showed reduced phosphorylated p38 MAPK levels, and higher levels NF-jB were retained in cytoplasm. Rats pretreated with CETC showed a statistically significant decrease in paw oedema ($P \leq 0.05$). It can be concluded that the above findings encourage strongly to focus on CETC to develop anti-inflammatory drugs with lower degree of inhibition to the constitutively expressing COX-1(Philip et al., 2018).

Antibacterial and antifungal activity

Patil et al. conducted a study on aqueous, methanol, ethanol and acetone extract of *Tinospora cordifolia* stem and leaves extract for antifungal activity against *Candida albicans, Aspergillus niger, Aspergillus fumigatus, Microsporum gypseum and Trichophyton rubrum* and for antibacterial activity against bacterial pathogens. The acetone extracts had wide range of antibacterial activity against bacterial pathogens than the ethanol and methanol extract, where aqueous extract was slightly higher antibacterial activity than ethanol extract.

Plant extracts of *Tinospora cordifolia* (TC) have been reported to have potential against microbial infections. The anti-bacterial activity of Tinospora cordifolia extracts has been assayed against various Gram positive and Gram negative organisms. The antimicrobial activity of TC stem extracts was investigated against bacteria causing UTIs viz. uropathogens, Escherichia coli and Staphylococcus aureus. The study conducted using disc diffusion method showed that all three solvent extracts of TC

reveal different antibacterial activity against both uropathogenic isolates with decreasing order as ethanolic (maximum) > methanolic (moderate) >
aqueous (poor). The larger zones of inhibition exhibited by *Tinospora cordifolia* extract against *A. niger* may be due to the presence of variety of active compounds. This is well known, since tannins and saponins are important plant metabolites which is responsible for their antimicrobial activity. From the results obtained, the stem extract of *Tinospora cordifolia* showed antifungal activity among the entire fungal organism(Patil et al., 2017).

Sandhu et al. studied the antimicrobial activity of *Tinospora cordifolia.* The results of antimicrobial activity revealed that T. cordifolia stem extracts were very effective against *Serratia marcescens, E. coli, Streptococcus thermophilus, Fusarium oxysporium, Aspergillus niger* while these extracts showed very less inhibition against Trichoderma reesei. This plants can be a source of useful drugs but further studies are required to isolate the active component from the crude plant extract for proper drug development(Amit et al., 2013).

The anti-microbial activity of *T. cordifolia* was observed in root, stem and leaf extracts on pathogenic microorganisms (Jeyachandran et al., 2003, Samy, 2005).

Anti-arthritic activity

Paval et al. carried out a study on antiarthritic activity of ethanol extract of Tinospora cordifolia in Wistar rats using Freund's complete adjuvant and Bovine type II collagen to induce arthritis. In this study various haematological and histopathological parameters were used to assess the effectiveness of the treatment. These were supported by histological study of the affected ankle joints using haematoxylin and eosin. The study results suggested that oral dosage of 150 mg/kg body weight exhibited anti-arthritic activity in Freund's adjuvants arthritic rats. In collagen induced arthritic rats, the dosage of 100 mg/kg body weight showed anti-arthritic activity. It was concluded that the Tinospora cordifolia demonstrates a significant anti-arthritic activity(Paval et al., 2011)

CNS depressant activity

Hossain et al. (2009) investigated neuropharmacological activities of methanol extract of the aerial parts of *Tinospora cordifolia* using rodent behabioural models; hole cross to evaluate motor activity, open field to evaluate exploratory behaviour and

thiopental sodium-induced sleeping time to evaluate sedative potential of the extract. The extract significantly (p < 0.05-0.001) decreased motor activity and exploratory behavior of mice in hole cross and open field test respectively. The extract also produced rapid onset and maximized the duration of sleeping time when administered with thiopental sodium. Results of this study suggest that the aerial part of *T. cordifolia* possesses significant CNS depressant activity (Hossain et al., 2009).

Alpha glucosidase inhibition: Chougale et al., (2009) studied the crude ethyl acetate, dichloromethane (DCM), chloroform and hexane extracts of *Tinospora cordifolia.* Among them, 15 mg of the DCM extract was most effective in that showed 100 % inhibition of the alpha glucosidase whereas salivary amylase was inhibited to the extent of 75 % and pancreatic amylase to 83 %. On giving a maltose load of 2mg / g along with 0.3 mg / g body weight of the DCM Tinospora stem extract, a decrease was revealed in the hyperglycemic shoot up in normal and diabetic animals by 50 and 58 % respectively as compared to the controls(Chougale et al., 2009). Hence, the extract was found to inhibit alpha glucosidase in a non-competitive manner. Inhibitors of alpha glucosidase have potential use in the treatment of diabetes mellitus. The stem extract of *Tinospora cordifolia* was evaluated for inhibition of the enzyme. The extract was also found to inhibit the salivary and pancreatic amylase and therefore can effectively reduce an increase in postprandial glucose level. TC has been demonstrated to possess inhibitory action against alpha glucosidase from rat intestine, pancreatic amylase and salivary amylase. It also has a good potential to control the increase in the post prandial glucose level as observed in the oral glucose tolerance test.

Antidiabetic and its complications

Various preparation of Stem, leaf and roots have shown effects on Diabetic retinopathy(Agrawal et al., 2012), diabetic cataract(Rathi et al., 2002), diabetic neuropathy and gastropathy(Nadig et al., 2012, Jaspan et al., 1983), diabetic nephropathy(Grover et al., 2001, Nagaraja, 2007, Joladarashi et al., 2012), diabetic ulcers(Purandare and Supe, 2007), Protection against brain, heart, liver and kidney damage in chronic diabetes(Rawal et al., 2004a, Rawal et al., 2004b, Mary et al., 2003), hyperlipidemia(Stanely Mainzen Prince and Menon, 2003, Prince et al., 1998),

weight loss(Stanely Mainzen Prince and Menon, 2003, Stanely et al., 2000), cardioprotective activity(Rao et al., 2005, Nagaraja Puranik and Kammar, 2008), Impotency and genitourinary troubles(Rai and Gupta, 1967, Gupta and Sharma, 2003), Diabetic osteoporosis and anti-arthritic(Kapur et al., 2008, Utpalendu et al., 1999) Gastrointestinal protection in old age (Kamble et al., 2008, Sharma et al., 2015), Role in gestational diabetes(Shivananjappa, 2012), Suppression of oxidative stress(Sai and Srividya, 2002, Vincent et al., 2004), Immunomodulation to ameliorate diabetes(Kapil and Sharma, 1997, Kalikar et al., 2008, Harrison and Honeyman, 1999), Stress buster(Mitra, 2008)

Clinical evidences

The effect of the aqueous leaf digest (10 g/200 mL water) on postprandial blood glucose levels in type-2 diabetics was determined. The herb is found to exhibit a significant ability to reduce blood sugar levels in human subjects(Sai and Srividya, 2002). Its hypoglycaemic potential was substantiated by a similar response observed in another study, wherein two Ayurvedic dosages form *viz*. *Guduchi Ghana* (solidified aqueous extract) and *Guduchi Satva* (sedimented starchy aqueous extract) which exerted significant hypoglycemic and antihyperglycaemic activity along with significant relief in signs and symptoms of type-2 diabetics. Statistically, *Guduchi Ghana* was proved to be more effective than *Guduchi Satva* to control glycemic level(Sharma et al., 2012). Two or more clinical studies reported on Kwatha (decoction) and Churna (fine powder) of *Guduchi*, also supports its antidiabetic potential(Chakraborty, 2012, Sharma et al., 2015). Another clinical study has shown that *Guduchi* plays an important role in normalization of altered liver functions (alanine transaminase, aspartate transaminase) and the herb was found to be safe for therapeutic usage (dose: 500 mg/d, duration: 21 d, subjects: healthy individuals); thus via improving the function of hepar, the herb regulates the carbohydrate and lipid metabolism(Karkal and Bairy, 2007). All these studies corroborate with the results of earlier animal studies and establish its use as a safe anti-diabetic agent in Ayurvedic system of medicine.

Amelioration of CNS toxicities of L-DOPA:

The gold standard drug for the treatment of Parkinson's disease is L-DOPA, but various studies have proved that the treatment with L-DOPA leads to the death of

surviving dopaminergic neurons in the CNS(Prasad et al., 1999). In this study, Singh et al. (2003) have approached to counteract the toxicities of L-DOPA therapy by co-administration of *Tinospora cordifolia* crude powder. On the zero day each animal was given with an intraperitoneal (ip) injection of 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) (20mg/kg) and after 48 hours the animals were treated with L-DOPA or L-DOPA plus *Tinospora cordifolia* crude powder upto 30 days. At the end of the study period we were evaluated the level of anxiety, grip strength and mitochondrial Complex-I activity. The results revealed that the coadministration of *Tinospora cordifolia* crude powder protected the dopaminergic neurons when compared with Sham operated control group(Singh et al., 2003). Hence, the study concluded that the treatment with *Tinospora cordifolia* crude powder could reduce the toxicities of L-DOPA therapy for Parkinson's disease.

Jayachandran et al. studied the antibacterial activity of the aqueous, ethanol and chloroform extracts from the stems of Tinospora cordifolia using using disc diffusion method against Escherichia coil, Proteus vulgaris, Enterobacter faecalis, Salmonella typhi (Gram-negative), Staphylococcus aureus and Serratia marcesenses (Gram-positive). He found the results that the ethanolic extract has significant antibacterial activity against tested bacteria(Jeyachandran et al., 2003). This present study justifies the claimed uses of Tinospora cordifolia in the traditional system of medicine to treat various infectious diseases.

Antidiarrhoeal and antiulcer activities

The use of plant as remedy for diarrhea and ulcer is well documented in Ayurvedic system of medicine. However, pharmacological evidence does not exist to substantiate its therapeutic claims of the classics. Kaur et al. investigated the antidiarrheal and antiulcer activity of ethanolic and aqueous extracts of *T. cordifolia* in rats. The antidiarrheal activity of *T. cordifolia* extracts was evaluated by castor oil and magnesium sulfate induced diarrhea using parameters such as onset of diarrhea, number of wet stools, total number of stool and weight of total number of stools. The antiulcer activity of extracts was investigated using ethanol and pylorus ligation induced ulcer. Furthermore, tissue antioxidant parameters such as reduced glutathione, catalase activity and lipid peroxidation level were also investigated. *Tinospora cordifolia* extracts were more efficacious in reducing number of total stools

in both the models of diarrhea and showed a dose dependent antidiarrheal effect. The antiulcer activity of the extracts was confirmed by a reduction in ulcer index along with the decrease in gastric volume, total acidity, and an increase in pH of gastric content in both the models. The obtained results have established a pharmacological evidence for the folkloric use of the *T. cordifolia* as antidiarrhoeal and antiulcer agent(Kaur et al., 2014).

Acute toxicity

Female Wister rats of weight (180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and TCCP was administered orally at a dose of 2000 mg/kg in 0.3% CMC. Then the animals were observed for mortality and morbidity at 0, 1/2, 1, 2, 4, 6, 8, 12, and 24 hours. Feed was given to the animals after 4 hours of dosing and body weight was checked 6 hours after dosing. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. The animals were observed twice daily for 14 days and body weight was taken. The same experiment was repeated once again on 3 female rats (preferably female) as there was no observable clinical toxicity for the animals on the phase 1 study and thus the LD_{50} of Tinospora cordifolia stem powder was determined as above 2000mg/kg body weight(Singh et al., 2003). From acute toxicity study, 200 mg/kg (1/10 of tested dose) of TCCP was selected as dose(Agarwal et al., 2002).

A toxicity study carried out by Hussain et al. (2015) according to methods described by Oduola et al., with slight modifications(Oduola et al., 2010). The mice were divided into 5 groups. The first group was kept as a control group and was given 10 mL/kg p.o. of normal saline; the second, third, fourth and fifth groups were treated with aqueous methanolic extract of *T. cordifolia* at doses of 1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 4000 mg/kg respectively. All the groups were closely observed for toxic effects for 24 h. (Hussain et al., 2015).

Acute toxicity study: An acute oral toxicity evaluation of CETC was performed as per the guidelines of Organization for Economic Cooperation and Development (OECD). Wistar rats (both male and female) weighing 150–200 g were divided into two groups of six each. First set received 5% Tween 80 (control), and the second set received CETC 2 g/kg suspended in 5% Tween 80 orally. Animals were then observed systematically for any changes in skin, eyes, body weight, nature of feeding, body

temperature, behaviour and nature of excretions as a mark of acute toxicity initially for 48 h and then continued up to 14 days. Rats were then sacrificed, and blood was withdrawn for the estimation of biochemical parameters including albumin, globulins, lipid profile, bilirubin creatinine and enzymes (aspartate transaminase (SGOT), alanine transaminase (SGPT) and alkaline phosphatase. A small portion of liver was also taken for histopathological examination. A high dose of CETC of 2000 mg/kg body weight was administered to the test group. There were no obvious toxic or deleterious symptoms and mortality suddenly or up to the end of observation period. During this period, a normal body weight gain as well as usual behaviour was observed. The liver histopathological and serum biochemical analyses also showed no significant toxicological changes in the levels of parameters between the vehicle-treated and CETC-treated rats(Philip et al., 2018).

Molecular action

Among these berberine ameliorated renal dysfunction in streptozotocin-induced diabetic rats, which was accompanied by inhibition of renal aldose reductase and reduction of oxidative stress (Liu et al., 2008). Anti-tumor promoting action of berberine is attributed to its antioxidant property(Thirupurasundari et al., 2009). of above points, we selected *Tinospora cordifolia* crude powder (TCCP) which is wide reported for potent antioxidant10 and free radical scavenging activities for reducing the toxicities of L-DOPA therapy in experimental PD.

Palmatine is a close structural analog of berberine that has been shown to exhibit significant antitumor activity against HL-60 leukemic cells(Kuo et al., 1995).

Antidiabetic activities

The isoquinoline alkaloid rich fraction from stem, includes palmatine, jatrorrhizine, and magnoflorine which have been reported for insulin mimicking and insulin releasing effect both *in vitro* (using rat pancreatic β-cell line, RINm5F) and *in vivo* (Patel and Mishra, 2011). Another isoquinoline alkaloid 'berberine' has been tested and used successfully in experimental and human diabetes. It lowers elevated glucose level as effectively as metformin. It also inhibits FOXO1, which integrates insulin signaling with mitochondrial function, thus improving hepatic metabolism during insulin resistance and metabolic syndrome. By adenosine monophosphate-activated protein kinase activation, it decreases the blood sugar and cholesterol level and

maintains the blood pressure (Zhang et al., 2008, Yin et al., 2008, Cheng et al., 2009, Sharma and Batra, 2013). Besides, tinosporin, isocolumbin, palmatine, tinocordiside, cordioside and β-sitosterol compounds present in stem and root which are also reported to possess antidiabetic, antihyperlipidemic and antioxidant properties(Gupta, 1967).

Anticancer activity

Ali et al. studied palmatine, an alkaloid from *Tinospora cordifolia* by using response surface methodology (RSM) and study its anticancerous property against 7,12-dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. In this study, the effect of three independent variables, namely, extraction temperature, time, and cycles was investigated by using central composite design. A single topical application of DMBA (100 μg/100 μL of acetone), followed 2 weeks later by repeated application of croton oil (1% in acetone three times a week) for 16 weeks, exhibited 100 percent tumor incidence (Group 2). The highest yield of alkaloid from *Tinospora cordifolia* could be achieved at 16 hours of extraction time under 40°C with 4 extraction cycles. Alkaloid administration significantly decreases tumor size, number, and the activity of serum enzyme when compared with the control (Group 2). In addition, depleted levels of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase and increased DNA damage were restored in palmatine treated groups. The data of the present study clearly indicate the anticancer potential of palmatine alkaloid in DMBA induced skin cancer model in mice(Ali and Dixit, 2013).

Nutritional supplementation

Along with rich protein and dietary fibre contents, appreciable levels of major and minor elements namely Zn, Mn, Cl, K, Ca, Ti, Cr, Fe, Co, Ni, Cu, Br, and Sr are found in this herb, which act as micronutrients for health restoration and alleviate degenerative processes in diabetes(Geeta and Kumari, 2013, NILE and Khobragade, 2009). Crude values for food content in *Tinospora* include high fibre (15.19%), sufficient protein (4.5%-11.2%), sufficient carbohydrate (61.66%), and low fat (3.1%). Nutritive value is 292.54 calories per 100 g. It has high potassium (0.845%) (regulatory function of nerve impulses), high chromium (0.006%) (regulation of carbohydrate utilization pathophysiological alterations in diabetes), sufficient iron (0.28%) (to improve haematopoietic functions especially in diabetic nephropathy where

erythropoietin release from kidney is compromised), and sufficient calcium (0.131%) (regulatory functions in nervous, cardiovascular, and musculoskeletal systems)(Mutalik and Mutalik, 2011).

Drug interactions

Although *Tinospora* is less likely to have drawbacks of the conventional drugs used for diabetes, the concepts of herb-drug interactions should also be kept in mind. No negative herb-drug interaction is reported till date, but more studies in this area remain yet to be accomplished. Concurrent administration of *Tinospora* with metformin showed beneficial pharmacokinetic as well as pharmacodynamic interaction leading to enhancing antihyperglycemic and antihyperlipidemic activities (Patwardhan, 2012). Plant alkaloid berberine has been shown to boost the effects of metformin and 2,4-thiazolidinedione, and can partly replace the commercial drugs, which could lead to a reduction in toxicity and side effects of the latter(Prabhakar and Doble, 2009). In Ayurveda, decoction of *Tinospora* stem is used as a medium of '*Shodhana*' process (relates to combining a substance with another substance to enhance its activity and to help counter some of its unwanted effects) to purify *Guggul* (*Commiphora wightii*), which is an vital component of various Ayurvedic antidiabetic formulations. *Tinospora* enhanced the activity of *Guggulu*. When used alone, the effect of *Guggulu* significantly decreased. Use of *Tinospora* combinations has a potential basis for clinically desirable drug interactions (Kamble et al., 2008, Lather et al., 2011, Caranasos et al., 1985).

This study of *T. cordifolia* reviewed textual and web based information. The study was further dealt with morphological characters, pharmacognostical and phytochemical screening evaluation, climacteric variable like temperature and rainfall at the geographical region; and soil test collected from the habitat of *T. cordifolia.* This data will be compared with the data generated on same parameters after the next 10 years.

Aims & Objectives

The assessment criteria for the identity and purity of the genuine source and the plant material; *T. cordifolia* is established for assurance of quality and reliability. This study has been carried out on the crude drugs *T. cordifolia* rhizomes and their powders to fulfillment of the following objectives as shown below:

1. Authentication, collection and preservation of the samples,
2. Organoleptic, macroscopical and microscopic evaluation of the crude drugs and their powders,
3. Foreign matter, loss on drying, total ash value, extractive value, phytochemical screening and finger-printing of the plant material.

Plan of Study

The present work has been carried out pharmacognostical studies and phytochemical evaluation of the study material; the rhizomes under the following headings:

- Organoleptic and macroscopic characteristics the rhizomes and their powders.
- Physico-chemical evaluation of the rhizome
- Phytochemical screening and finger printing

CHAPTER-III: RESEARCH METHODOLOGY

This study had two sections including field survey and experimental (analytical). The methodology of each was described one by one as follow;

3.1.Field Survey:

3.1.1. **Study design:** This study was descriptive cross-sectional

3.1.2. **Study type:** both qualitative and quantitative

3.1.3. **Sampling method:** Stratified random sampling method/household survey was carried out to gather related information.

3.1.4. **Site Selection:** Based on the literature search and interactive meetings with different experts; potential study sites representing to hill, mountain and terrain regions of Nepal was determined for the field visit and data collection process. Manang (high altitude), Dhading (mountain) and Parsa (low land) were selected as a study sites.

3.1.5. **Method of data collection:** Data was collected interviewing individuals, households, senior citizens, shepherds, forest guards, and hunters with structured questionnaires. Some of the tools and techniques were;

3.1.5.1.**Literature search** Extensive literature review through internet search and published resources regarding the impacts of environmental changes on medicinal plants and biodiversity conservation were retrieved through the internet search.

3.1.5.2.**Secondary data** Secondary data on the use pattern and availability of Ayurvedic medicinal plants was carried out in the sites. Climate change and related data was collected from Government of Nepal.

3.1.5.3.**Individual interview** A semi-structured questionnaire was used to conduct the personal interviews and consultation with diverse stakeholder's viz, local people, traditional herbal healers, medical doctors, medicinal plant growers and collectors, researchers, academicians, members of Vaidyas association, and non-government organizations viz. target organizations including government sector or organizations working for Ayurveda and other traditional medicines, herbal medicines; I/NGO working in preservation, promotion of herbal resources; educational and research institutions

regarding medicine and herbal resources; professional organizations of traditional medicine practitioners; private health service research, educational and manufacturing companies; community forestry-related organizations.

3.1.5.4. **Interactive discussion** Workshop, village-level meetings, and group discussions were held with diverse stakeholders to know the impacts on medicinal plants from environmental changes, conservation practices and strategies.

3.1.5.5. **Case study**: Based on the field visit and consultation with respondents and stakeholders, case studies representing from all ecological regions was prepared for the noticeable impacts from climatic or environmental changes on medicinal plants. Field observations, informal meetings, discussions, and consultations was employed to collect information about folklore uses of medicinal plants; traditional knowledge based healing practices and local livelihood. In particular, elderly people, forest guards, shepherds, and women representing different ethnic groups, castes, and occupations was encouraged to participate. They were asked about the changes, impacts, and adaptation practices of climate change through historical timelines. Their observations and experiences on traditional knowledge was triangulated and used for cross-checking with previous.

3.1.5.6. **Livelihood Vulnerability Index (LVI Survey:** An ethnobotanical survey was undertaken to collect information from traditional healers, shephards, local users, elderly persons, Pradhana, etc. Data was collected through the use of questionnaires and personal interviews during field trips in the study site. The Livelihood Vulnerability Index (LVI) developed by Hahn et al. (2009) has been comprised of nine major components: (i) socio-demographic profile, (ii) livelihood strategies, (iii) social networks, (iv) health, (v) food, (vi) water, (vii) Knowledge and skill, (viii) finance and (xiv) natural disasters and climate variability (Table-5). For each component relevant sub-components will be identified during key informant interviews. The Livelihood Vulnerability Index (LVI) designed by the DFID (1999) sustainable livelihood framework approach will be used for calculating

3.1.6. Study site

The study sites were included three communities from districts in Annapurna landscape such as Manang, Dhading and Parsa (Figure 1.). The Annapurna landscape covers highland Manang district and lower land Parsa district. It is the basin of Maryangdi river.

Dhunebeshi municipality, Dhading is geographically situated approximately between longitudes 27° 45′ 36″ N and 85° 14′ 24″ E at 1320 m in height. Kharka in Dharapani Municipality, Manang is geographically located approximately between 28°31'54.7"N and 84°26'59.0"E and at height 25,823 feet. Madhuwan Mathaul, Parsa district is geographically located approximately between longitudes 27°12'59.8"N 84°51'11.7"E at 420 m height.

Figure-1: Study sites: Dhunebeshi municipality; Dhading, Dharapani municipality; Manang, Madhuwan Rural municipality, Parsa

The main factors that define the vulnerability of households and communities to the impacts of climate variability and change include exposure, sensitivity, and adaptive capacity(IPCC, 2007a). In this study, exposure refers to the nature and degree to which agriculture-based livelihood systems are susceptible to significant climate variation(IPCC, 2001). Exposure indicators selected for the three study sites characterize the frequency of water-induced hazards and variation in past temperature and precipitation (Table 6). The value of each of these three parameters was extracted for each studied village based on the coordinates collected during the

household survey. Historical hazard data could not be extracted for each household or village as they were reported for the whole area and damage was distributed across the studied villages. Therefore, the same value was used as the exposure sub-index in the vulnerability calculation.

Sensitivity is the degree to which an agriculture-based livelihood system is affected by or responsive to climate stimuli. In this study, sensitivity included loss of property (land or crop) to climate-related hazards over the last 10 years. Similarly, income from agriculture and animal husbandry, the severity of climate-related hazards to agriculture and food security, distance of the house from the hazard zone, conflict over the use of water resources in the community, crop production trends in the past 10 years, and land type owned by the household represent its sensitivity. We used the sustainable rural livelihoods framework to analyze the livelihood vulnerability index (LVI) of the communities in the study areas. LVI of a household is taken to be an emergent property of the nine types of livelihood assets: socio-demographic, physical, human, natural, financial, social, food, water and knowledge and Skills. Detailed information on the indicators of LVI used in this study is given in Annex-1.

3.1.7. Sampling and data collection

Dharapani municipality; Manang, Madhuwan Mithaul; Parsa and Dhunebeshi municipality; Dhading were three communities purposively selected from three districts of three provinces; Gandaki province, Province 2 and Bagmati province of Nepal. For HHs survey, respondents from sample HHs were selected randomly, and approximately 100 HHs in each community were surveyed. The final sample size was 300 with completed questionnaires.

The HH survey was conducted in July 2020 to August 2020 by 10 specialists who were properly trained in an intensive training session before the questionnaire pretest. The respondents were HH heads or their spouses, farmers were mainly attached with herbal farming and forestry for at least 10 years. A fully structured questionnaire was used to gather information on socio-demographics, livelihoods, social networks, health, food and water security, natural disasters, and climate variability. The survey was conducted in local language. Interviews were conducted by field staff from Central Department of Environment and Climate Change, Tribhuvan University and local volunteers. Survey question was based on the subcomponents or indicators as

presented in Table and figures. We also conducted at least two focus group discussions (FGDs) in each community with each group including 7–10 HH heads from different social groups, community's leaders, village leaders to selecting realistic and compatible indicators to the socioeconomic uniqueness of the studied community. Meteorological forecasting and hydrological data of 10 years were collected from Department of Meteorology and Hydrology, Babarmahal, Kathmandu, Nepal.

3.1.8. Data analysis

One set of analysis were undertaken viz. calculation of a balanced weighted average Livelihood Vulnerability Index (LVI) for Dhading, Manang and Parsa districts in 2020 A.D. Additionally, t-tests were used to compare the mean scores of Dhading, Manang and Parsa's main components and subcomponents.

3.1.8.1. Constructing livelihood vulnerability index (LVI)

This study applied the methodology developed by Hahn (Hahn et al., 2009) and Shah (Shah et al., 2013) to calculate level of vulnerability to climate variability. Nevertheless, and as the study focused on medicinal plants suggest, modifications have been made to adapt it to our specific case study. We added two sub components besides the fifty-nine that contained the LVI developed by Hahn et al. (2009). Housing and land tenure component was separated into two distinct components to better understand the sensitivity of HHs to each of these factors. Knowledge and skills was added to the LVI as a means of capturing the human vulnerability of HHs to climate change. The following sections provide an overview of the LVI, 9 major components, and 59 indicators of livelihood vulnerability used in this study, which are presented in Table 1. In this approach, each of the 9 main components is a combination of 59 subcomponent indicators. Each indicator or main components are viewed as having an equal contribution in the overall sum(Sullivan et al., 2002).

3.1.8.2. Method of LVI Calculation

A livelihood vulnerability index was used to assess relative exposure, vulnerability of the communities and sensitivity. This method computes a vulnerability index by aggregating data for a set of indicators. It helps identify indicators or determinants for targeting interventions and programs(Czúcz et al., 2009, Eakin and Bojórquez-Tapia, 2008).

3.1.8.3. Calculating the livelihood vulnerability index (LVI)

LVI indicators are calculated through four steps (Hahn et al., 2009). Each indicator was normalized (rescaled from 0 to 1). After raw data are transformed into appropriate measurement units, each subcomponent was standardized by the following equation:

$Index_s$ = (Observed value – minimum)/(Maximum – Minimum) ……. (1)

After each was standardized, the subcomponents were averaged using Eq. (2) for calculating the standardized scores of each main component:

$$M = \frac{\sum_{i=1}^{n} \text{index}_{si}}{n} \quad \text{..... (2)}$$

Table -1: Major Components and Sub-components of LVI

Major Components	SN	Sub-components	Unit
1. Socio-demographic	1	Dependency ratio	ratio
	2	Female headed HH	percent
	3	Age of house head	Average
	4	HH where head has not attended school	Percent
	5	HH size	Average
	6	HH farming experience	year/average
	7	HH herb collection experience	year/average
2. Livelihood Strategies	8	HHs with members working in a different community or migrating	Percent
	9	HHs fully dependent on agriculture as a source of income	Percent
	10	Agricultural livelihood diversification index	1/#livelihood, average
	11	Herbs livelihood diversification index	1/#livelihood, average
	12	HHs who did not get the expected price for crops	Percent
	13	Semi pacca or kachha houses	Percent
	14	Total land cultivated	Acres, average
3. Social networks	15	Neighbours visiting in a month	Times
	16	Respondents feel can talk to others about private matters	Members
	17	Respondents reportedly willing to borrow money in need	Members
	18	Respondents reporting that other people can't be trusted	Percent
	19	Respondents are not willing to help you in case of necessity	Percent
	20	Respondents generally untrustful in matters of lending or borrowing money	Percent
	21	Number of friends and relatives who would over help during need	Members, average
	22	Times you have helped a friend or relative	Times, average

		when in need	
	23	HHs where none is affiliated with any institution	Percent
4. Health	24	HHs where someone had to miss work or school due the illness in the past year	Percent
	25	HHs reducing food intake in bad times	Percent
	26	Life expectancy index	Years
5. Food	27	HHs dependent on family farm for food	percent
	28	Number of months when the HHs trouble providing food	Months, average
	29	Crop diversity Index	1/# crops, average
	30	Herb diversity Index	1/# crops, average
	31	Number of times the HH needed to purchase food	Times, average
	32	Time to drinking water	Minutes, average
	33	Time to market	Minutes, average

Table -1: *(Continued)*

Major Components	SN	Sub-components	Unit
6. Water	34	HHs with borrewell fail	percent
	35	HHs with access to water source	percent
	36	HHs without consistent water supply	percent
	37	HHs reporting depletion of their natural water source	percent
	38	HHs with risk investment	Percent
	39	HHs with vulnerability risk	Percent
7. Natural Vulnerability and Climate Variation	40	HHs reporting less rain in the past 10 years	percent
	41	HHs reporting more droughts in the past 10 years	percent
	42	HHs reporting more floods in the past 10 years	percent
	43	HHs reporting unusual rains in the past 10 years	percent
	44	HHs reporting temperature increase in the past 10 years	percent
	45	HHs with the same crop choice than in previous years	percent
	46	HHs reporting less rain this year than the average of rainy seasons	percent
	47	HHs with agricultural problems	percent
	48	HHs without adaptations to climate or weather problems	percent
	49	Previous herbs cultivation	percent
	50	Problems with herbs cultivation	percent
8. Knowledge and Skills	51	HHs without TV	percent
	52	HHs without Radio	percent
	53	HHs not participating in knowledge exchange with others	percent
	54	HHs perceiving lack of education	percent

	55	Year of schooling	Number, average
9. Finance	56	HHs with more expenditures than income	percent
	57	HHs member working outside (not farming)	percent
	58	HHs with money loans	percent
	59	HHs who had not repaid loans	percent

where M is one of the 13 major components; $index_{si}$ represents the subcomponents; indexed by i, that make up each major component; and n is the number of subcomponents in each major component. Finally, LVI score was generated by combining the weighted averages of all the major components (Eq. 3). To ensure that all main components contribute equally to the overall LVI, the weights of each main component are determined by the number of subcomponents of which it is comprised (Sullivan et al., 2002).

$$\mathrm{LVI} = \frac{\sum_{i=1}^{12} w_{Mi} M_i}{\sum_{i=1}^{12} w_{Mi}} \quad \text{..... (3)}$$

where LVI is the vulnerability index for one of the communes, equals the weighted average of the 9 major components; wMi the weights of each major component, which are determined by the number of subcomponents that make up each major component. The range of LVI lies between from 0 (least vulnerable) to 0.5 (most vulnerable).

3.2. Experimental (Analytical) study

3.2.1. Soil collections: Soil samples for the future reference will be collected from each sampling sites from depth of 15 cm according to authentic methods (Saxena 1989). Physiochemical parameters such as water holding capacity, moisture content, total nitrogen, available phosphorus, available potassium, pH, total organic matter will be analyzed by standard methods (Gupta, 2000).

3.2.2. Specimen collection Samples of Guduchi (*Tinospora cordifolia*), Chutro (*Berberis aristata*) and Kutaki (*Picrorhiza neoscrophulariflora*) will be collected to study morphological (Macroscopic and Microscopic) and phytochemical profiling, from Parsa, Dhading and Manang districts respectively. Herbarium specimens will be collected from for the morphological records and kept in the Department of Ayurveda and Alternative medicine, Teku for the future record.

3.2.3. Requirement of chemical/reagents/instruments

Table No. 2: Chemical/reagents

SN	Name of Chemical/reagents	SN	Name of Chemical/ reagents
1.	Gelatin	9.	
2.	FAA	10.	Acetic acid Glacial
3.	Ethyl Alcohol	11.	Distilled water
4.	Tertiary Butanol	12.	Glycerine
5.	Xylene	13.	1% Chloral hydrate
6.	Clove oil	14.	DPX

Table No. 3: Instruments

SN	Name of Instruments	SN	Name of Instruments
1.	Light Microscope	9.	Slides
2.	Digital Camera fitted with Microscope	10.	Cover slips
3.	Tissue Processor	11.	Needles
4.	Blades	12.	Brush
5.	Foreceps	13.	Dryer
6.	L –Molds or Plastic block stand	14.	Embedding Center
7.	Scales	15.	Cryo Center
8.	Water tank	16.	Incubator

3.2.4. Storage of collected medicinal plants materials As per WHO guidelines, plant samples were kept in Formalin-ethyl alcohol and acetic acid solution (FAA) prepared in distilled water (10%:50%:5% + 35%) and other plant materials will be stored at room temperature(WHO, 2007).

3.2.5. Physiochemical, organoleptic, macroscopic and microscopic parameters of plants materials Physiochemical parameters such as Ash value, Extractive value, melting point, boiling point, foreign organic matters, colour, odour, shape, size, taste, macroscopic and microscopic examination of plants materials will be evaluated as per WHO guidelines for assessing quality of herbal medicines(WHO, 2007).

3.2.6. Heavy metals analysis: The most common toxic heavy metals viz. Mercury, Arsenic, Lead and Cadmium were analyzed by Atomic Absorption of Spectroscopy (AAS). The results were presented in Table no. 22 & 33)

3.2.7. Microbial loads: Raw materials of botanical origin are more susceptible to microbial contaminations. It was performed to find the contamination (Table no. 21, 32 & 42)

3.2.8. Pesticides residues: Medicinal herbs are cultivating for maximum profit. So cultivators use to protect plant from insects and use pesticides. It has been included to investigate pesticides in herbal medicines (Table no. 22, 33 & 43)

3.2.9. Aflatoxin analysis: The toxin produced by certain species such as *Aspergillus flavus* and *Aspergillus parasiticus* were analyzed by enzyme-linked immunoassay (ELISA). The results was presented in Table no. 22,33 & 43.

3.2.10. Phytochemical Quantification Plant materials was cleaned and subjected for cold extraction in methanol by percolation method. The extract from Guduchi, Daruharidra and Kutaki were estimated in terms of Berberine, Berberine and Picrosides II and Kutcosides respectively High Performance Liquid Chromatography (HPLC) (WHO, 2007, Gyawali and Kim, 2012). The results were presented in Table no.23,24,34, 35, 44 & 45.

Place of Work

Pharmacognostical studies of both the specimens (rhizomes of ***T. cordifolia***) were carried out at Department of Dravyagun, Ayurveda Campus, IOM, TU, Department of Plant Resources and Department of Food and quality control.

Method/Procedures

- Organoleptic method: The process of drugs (rhizomes and their powders) evaluation using by means of organs of sense and included sensory characters like colour, odour, taste, texture, size, shape and fracture.
- Macroscopic method: The process of evaluation of the drugs (rhizomes and their powders) by morphological characters is referred as macroscopic study. External appearances, measurements of length and breadth, transverse cut appearances were observed and recorded.

Physico-chemical Parameters

Organoleptic parameters

Organoleptic characters of hydroethanolic extracts were evaluated observing its characteristics by sense of organs (*Panchagyanendriya Pariksha*). It is, in Ayurveda, known as *Pancha gyanendriya Pariksha*. The characters like color, odor, taste and consistency of both extracts were noted (Table No 16,).

Foreign mater

The parts of the organs or organs other than the required are called foreign organic matter. These may include moulds, earthy material, animal excreta; and each crude drug has its own limits for presence of foreign matter.

Procedure: 10 gm (W) each dried samples of ***T. cordifolia*** rhizomes were taken. Foreign matter viz. earth, weeds, vegetative parts were separated; and the samples were weighed (W_1). The percentage of foreign matters was calculated by the formula as described below; and result was recorded (Table No 16, 26 & 37).

$$\text{Percentage of Foreign Matter} = \frac{W - W_1}{W} \times 100$$

Loss on drying

Estimation of the amount of water and volatile contents in a crude drug when the drug is dried under specified conditions. The moisture content of a crude drug is responsible for decomposition of crude drug due to chemical change or microbial attack.

Procedure: Rhizomes of each plant materials were cut into smaller pieces. Two dried and cleaned conical flasks (250 Ml) were weighed; and accurately weighed (10 gm) rhizomes of each plant materials were kept in each conical flask (W). The weights of conical flasks were recorded with and without plant materials both. The flasks were heated in hot air oven at 105 °C; taking weight of flasks after each hour interval for 6 hours. The flasks were taken out from oven and stoppered immediately. Then the stoppered flasks were cooled in desiccator for 30 minutes before weighing for every reading. It was heated until two consecutive reading were found similar, or might differ by 0.25% or constant (W_1). The percentage of Loss on Drying was calculated by the formula as described below; and and result was recorded (Table No 17, 27 & 38).

$$\text{Percentage of Loss on Drying (LoD)} = \frac{W - W_1}{W} \times 100$$

Determination of ash values

This test was conducted to evaluate the percentage of inorganic salts, carbonates, phosphates, silicates, soil, etc., naturally occurring in the drug or adhering to it or deliberately added as a form of adulteration. The ash remaining, following ignition of herbal materials was determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash (WHO, 2011). The *total ash* method is

designed to measure the total amount of material remaining after ignition. This included both "physiological ash", which was derived from the plant tissue itself, and "non-physiological" ash, which was the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. *Acid-insoluble ash* was the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. *Water-soluble ash* was the difference in weight between the total ash and the residue after treatment of the total ash with water.

i. Total ash

Procedure: Accurately weighed 2 gm powder of each air-dried material were placed in a previously ignited and tared crucible (silica). The powder of materials was spread in an even layer and ignited it by gradually increasing the heat to 500–600°C until it was white, indicating the absence of carbon. Then the crucibles were cooled in desiccators for about 30 minutes and weighed. Total ash was calculated in mg per g of air-dried material as below; and result was recorded (Table No18, 28 & 39).

$$\text{Total Ash(\%)} = \frac{\text{Wt. of Crucible + Ash) – Wt. of Crucible}}{\text{Wt. of Sample}} \times 100$$

ii. Acid-insoluble ash

Procedure: About 25 ml of dilute hydrochloric acid (approx. 70 g per litre) was added into the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. After boiling, the watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate and ignited to constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes, and then weighed without delay. Acid-insoluble ash was calculated in mg per g of air-dried material as below; and result was recorded (Table No 18, 28 & 39).

$$\text{Acid-insoluble Ash} = \frac{\text{(Wt. of Crucible + Residue) – Wt. of Crucible}}{\text{Wt. of Sample}} \times 100$$

iii. Water-soluble ash

Procedure: About 25 ml of water was added in the crucible containing the total ash and boiled for 5 minutes. The insoluble matter was collected on an ashless filter-paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450 °C.

$$\text{Acid-insoluble Ash} = \frac{\text{Total Ash} - \text{Acid-insoluble Ash}}{\text{Weight of Sample}} \times 100$$

The weight of the residue was in miligram subtracted from the weight of total ash. The content of water-soluble ash was calculated in mg per g of air-dried material; and result was recorded (Table No 18, 28 & 39).

pH Values

PH is defined as a figure expressing the acidity or alkalinity of a solution on a logarithmic scale on which 7 is neutral, lower values are more acid and higher values more alkaline. The pH is equal to $-\log_{10} c$, where c is the hydrogen ion concentration in moles per liter.

Procedure: 1 gm and 10 gm of each powder (mesh size 35) of ***T. cordifolia*** was dissolved with 100 ml distilled water at room temperature (25°C) in 250 ml conical flasks respectively. Both mixtures of each were stirred after every 10 minute for 1 hour, and then were filtered in graduated conical flasks of 100 ml respectively. There was 1% and 10% solutions of ***T. cordifolia*** for PH readings and was recorded (Table No. 19, 30 & 40).

Extractive Values

Extraction may be defined as the removal of soluble constituents from a solid or liquid or semi-solid with means of suitable solvent. It may be defined as the treatment of the plant or animal tissues with appropriate solvent, which would dissolve the medicinally active constituents. Extraction is the method of removal of a soluble fraction in the form of a solution from an insoluble matrix with the help of a suitable solvent.

Extractives are concentrated preparations of vegetable or animal drugs obtained by removal of menstrum, evaporation of all or nearly all solvent, and the weight is called extractive values. In this study, coarsely powders of both plants rhizomes were consecutively treated with petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water to obtain cold, hot and successive extractive values.

i. Cold extractive values:

This is a type of maceration process used for the removal of soluble constituents from a solid or liquid or semi-solid by means of suitable solvents. Coarse powder of ***T. cordifolia*** rhizomes were separately macerated with petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water in individual conical flasks.

Procedure: Accurately weighed 10 gm (W) of coarse powder of ***T. cordifolia*** rhizomes were kept each in 6 conical flasks consecutively. Approx. 150 ml of every menstruum (solvents; Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Methanol and Water) were poured in every conical flask consecutively. The moistened drugs were stirred for 10 -15 minutes in every hour for 6 hours and then left in contact with menstrua for 24 hrs. During this period, menstruum dissolved the active constituent of the drug and became almost saturated with it. They were filtered, kept in conical flasks (Weight of flask, W_1) and evaporated on Rota vapor. The whole processes were repeatedly done for three times to obtain maximal solutes (active compounds) in extracts. Finally, the extracts were lyophilized to remove moisture and weighed (Weight of flask + dried filtrate, W_2) to calculate cold extractive values (CEV) as follow; and result was recorded (Table No 20,31 & 41).

$$\text{Cold Extractive Value} = \frac{W_2 - W_1}{W} \times 100$$

A. Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts.

The solid ingredients, ***T. cordifolia*** (each 500 gm, W) were moistened with an appropriate amount of the hydro-alcoholic (1:4) menstruum and allowed to stand for approximately 4 hours in a well closed container, after which the mass was packed with 2 liters of hydro alcohol (Solvent; EtOH: H2O = 4:1) i.e. four times of the quantity of raw crude powder. Then the mixture was stirred with a cleaned stick in an interval of 1 hour for 6 hours every day, and left for 48 hours. The mixture was poured in a large container, filtered using watman filter paper, and dried on Rota vapor (Weight of Conical flask, W_1). The whole process was repeatedly done for three times to obtain maximum solutes (active compounds) in extracts. Finally, the extracts were lyophilized to remove moisture, weighed (Weight of Conical flask + extract, W_2) and

calculated the extractive value using the following formula; and result was recorded (Table No. 20, 31 & 41).

$$\text{Extractive Value (Percolation method)} = \frac{W_2 - W_1}{W} \times 100$$

CHAPTER-IV: RESULTS

Soil Test Climate Change on Soil Properties: The samples of soil collected from Dhunebeshi municipality, Dhading, Dharapani (Kharka-Nache) rural municipality, Manang and Madhuwan-Mithaul rural municipality, Parsa showed the highest acidity in pH value of soil sample collected from Dhading district whereas the least acidity in pH value of soil sample collected from Parsa district shown in Table 4.

Table -4: Soil test of samples collected from Dhading, Manang and Parsa Districts

SN	Parameters	Dhading	Manang	Parsa
1.	pH	5.2 (Acidity)	5.3 (Acidity)	6 (Acidity)
2.	Organic matter	1.37 % (Minimal)	10.48 % (Plenty)	7.20 % (Plenty)
3.	Nitrogen	0.07% (Minimal))	0.52% (Plenty)	0.36% (Plenty)
4.	Phosphorus	18.3 kg/ha (Minimal)	0 kg/ha (Nil)	361.8 kg/ha (Plenty)
5.	Potash	59.8 kg/ha (Minimal)	66.1 kg/ha (Minimal)	518.9 kg/ha (Plenty)

The organic matter (10.48%) was found the highest in soil sample collected from Manang district; meanwhile the soil sample collected from Dhading district was found the least (1.37%) among the samples collected from the selected districts in this study.

Figure-2: Soil collected from Madhuwan, Parsa (A), Soil collected from Kharka (Nache), Manang (B) & Soil collected from Dhunebeshi, Dhading (C)

The organic matter mostly represents the carbon content of the soil. One of the most important component of soil is nitrogen which is considered essential for plant growth.

It was recorded the least (0.07%) at Dhading and the highest (0.52%) at Manang district. On the other hand, the essential element, Phosphorus in the soil sample was recorded absent (0 kg/ha) from Manang and the plenty (361.8 kg/ha) from Parsa district. The soil sample from Dhading district was minimum (18.3%). Similarly, Potash in the soil samples was plenty (518.9% kg/ha) collected from Parsa. It was minimal in the soil samples that was 59.8kg/ha and 66.1kg/ha in Dhading and Manang respectively. Soil testing laboratory recommended supplementation of the essential components reported minimal or absent.

Calculation of Livelihood Vulnerability Index (LVI)

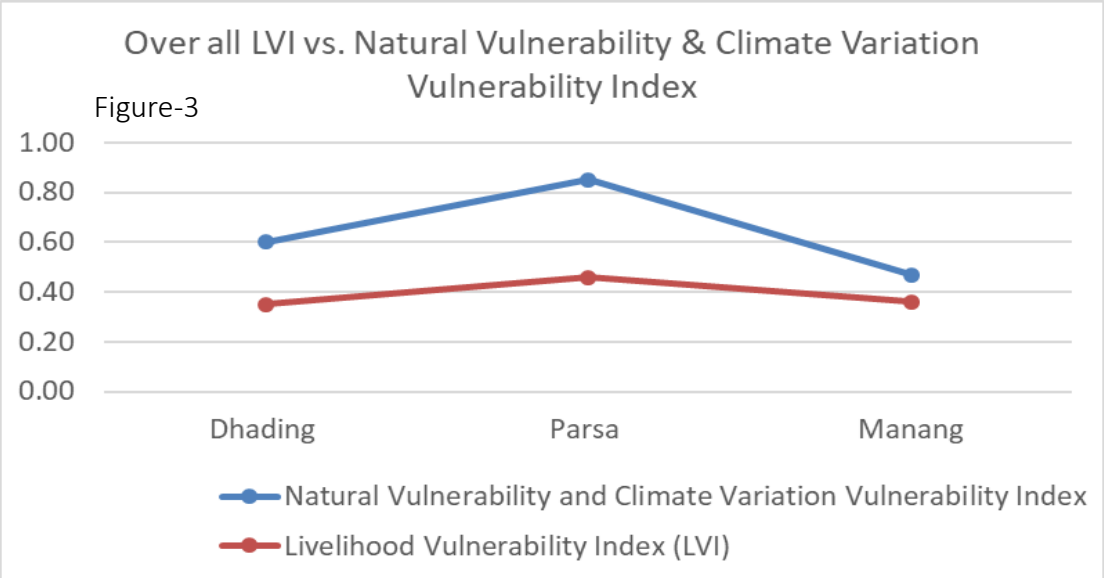
The results of LVI values of all 9 major components calculated from 59 subcomponents of Dhading, Manang and Parsa districts have been presented collectively in a spider diagram in figure 4 and Table 5. In the spider diagram, it is indicated that an increment of scale in 0.1 unit from 0 is less vulnerable at the center of the web; to 0.9 is most vulnerable at the outside edge.

Table-5: Calculation of Livelihood Vulnerability Index

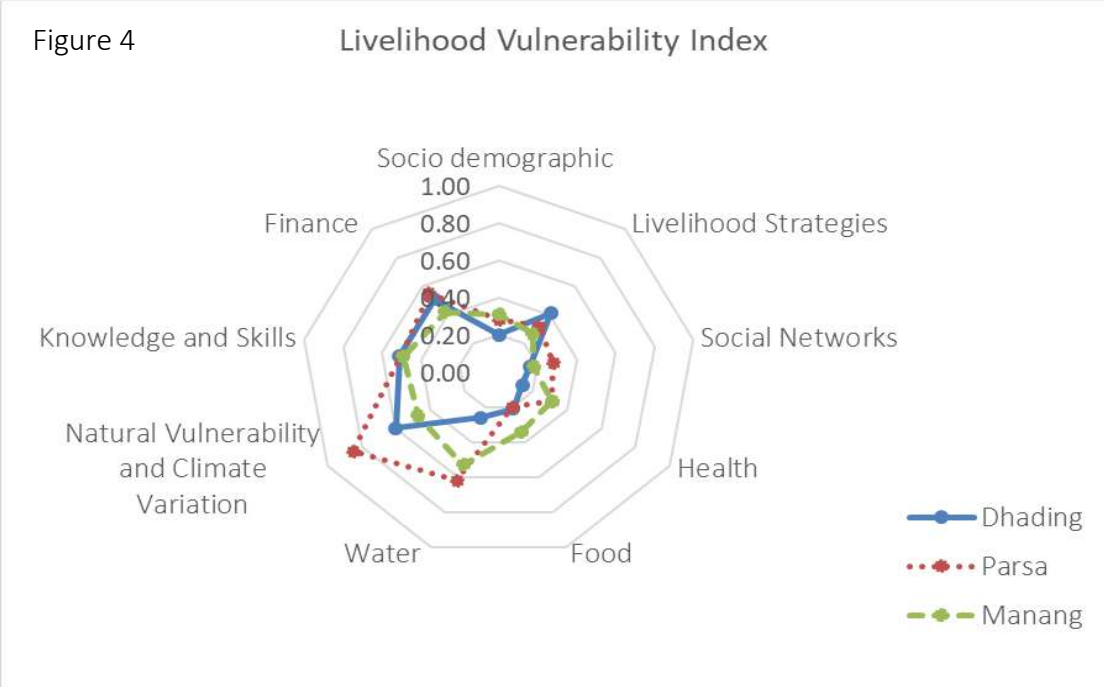
SN	9 Major Components of LVI	Dhading	Parsa	Manang
1.	Socio demographic Vulnerability Index	0.20	0.28	0.31
2.	Livelihood Strategies Vulnerability Index	0.41	0.32	0.27
3.	Social Networks Vulnerability Index	0.16	0.28	0.18
4.	Health Vulnerability Index	0.14	0.31	0.31
5.	Food Vulnerability Index	0.21	0.2	0.34
6.	Water Vulnerability Index	0.26	0.62	0.53
7.	Natural Vulnerability and Climate Variation Vulnerability Index	**0.60**	**0.85**	**0.47**
8.	Knowledge and Skills Vulnerability Index	0.51	0.5	0.49
9.	Finance Vulnerability Index	0.51	0.55	0.42
	Livelihood Vulnerability Index (LVI)	**0.35**	**0.46**	**0.36**

Overall, Dhading (0.35) and Manang (0.36) had a lesser LVI than Parsa district (0.46). The diagram reflects well that Madhuwan Mithaul, Parsa district was more vulnerable in most components, in particular livelihood strategies, social networks, health, water, natural vulnerability and climate variation and finance; whereas Dhunebeshi municipality, Dhading district is slightly more vulnerable in socio-demographic,

natural and climate variability, skill and knowledge, finance, food, health natural disasters than Dharapani rural municipality, Manang district.



Dharapani rural municipality, Manang district is slightly more vulnerable in food and water than Madhuwan Mithaul, Parsa and Dhunebeshi municipality, Dhading districts.



Natural vulnerability and climate variation was found the highest (0.85) at Madhuwan Mithaul, Parsa district and the lowest (0.47) at Dharapani, Manang.

Trend Analysis of Rainfall and Temperature:

Trend Analysis of Rainfall

Water resource has the prime concern for any future planning and development including flood control, flood protection and sustainable watershed management. The rainfall available in the watershed is key factor for determining the availability of water to fulfil the different demand mainly for agriculture, hydropower water supply, industry, etc. The timely availability of water influences the agriculture sector, food security and energy sector. Global climate changes affect the long-term rainfall pattern causes availability of water and may danger of occurrence of serious drought and flood. The high instantaneous rainfall in monsoon(June-August) may have shortage of water

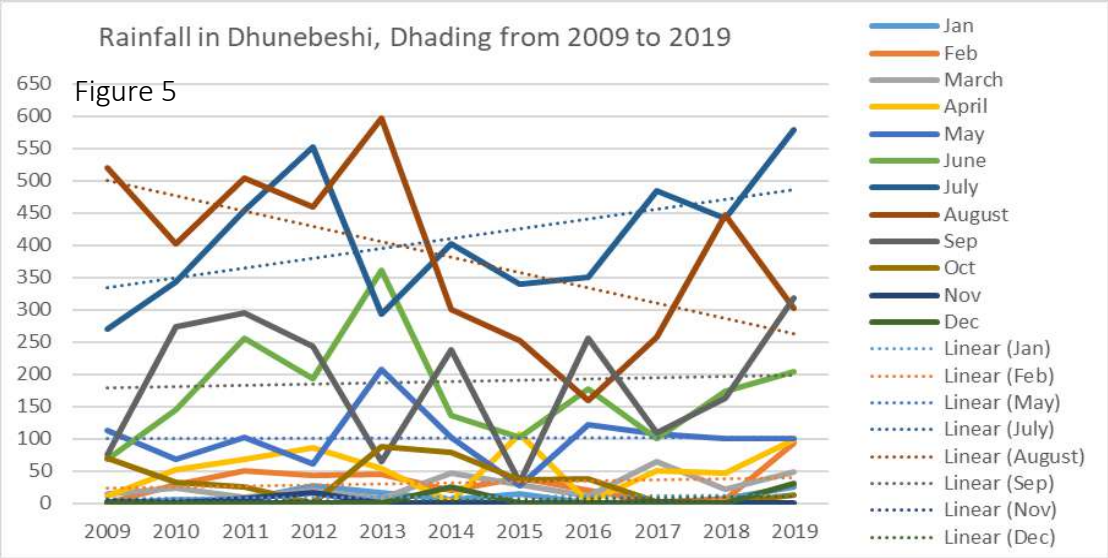
Rainfall at Dhunebeshi municipality (Dhading) during 2009-2019: Rainfall was found inconsistent during this period (2009-2019). The average rainfall was found increasing in order from 95.33 mm (2009) to 144.59 mm (2013); and then was found decreasing from 144.59 mm (2013) to 81.75 mm (2015). It was again found increasing in order from 81.75 mm (2015) to 150.99 mm (2019). It shows fluctuation in rainfall yearly. In average, it can be concluded that rainfall is higher in recent years than previous one shown in Table 6).

Table-6: Average rainfall in Dhunebeshi (Dhading) during 2009-2019 AD.

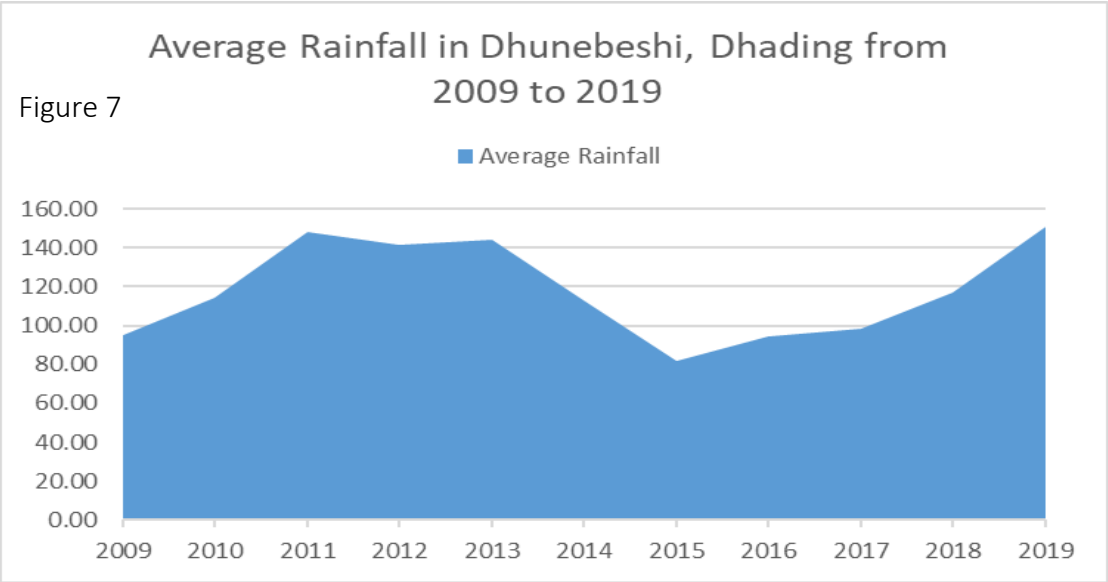
Year	N	Minimum	Maximum	Mean	Skewness	Kurtosis	Std. Deviation
2009	12	0.00	520.40	95.33	2.30	5.38	154.42
2010	12	0.00	402.60	114.48	1.22	-0.02	143.88
2011	12	0.00	503.40	147.83	1.13	-0.17	182.96
2012	12	0.00	552.10	141.59	1.49	1.13	187.21
2013	12	0.00	596.10	144.31	1.57	2.01	186.17
2014	12	0.00	403.00	113.22	1.25	0.58	132.94
2015	12	0.00	339.90	81.75	1.79	2.43	107.21
2016	12	0.00	351.20	94.63	1.13	0.32	118.68
2017	12	0.00	484.90	98.28	2.12	4.73	143.27
2018	12	0.00	446.00	117.15	1.50	1.05	164.70
2019	12	0.00	578.30	150.99	1.59	2.40	172.50

It was also found that rainfall was recored accelerating in month of July and decelerating in the month of August of monsoon season 2009 to 2019. Similarly, average rainfall was slightly accelerated in the month of May (Pre-monsoon); and in

the month of February (Winter) in this period. The rainfall in the rest months was rarely remained change of this period shown in Figure 5.



Skewness is the measure of asymmetry in frequency distribution about the mean predominantly positive skewness of average value 1.42 indicating annual precipitation in the region is asymmetric and it lies to the right of mean i.e. Right skewed. Kurtosis is the measure of peakedness or flatness of frequency distribution having value of + 1.66 indicating mesokurtic shape shown in Figure 7.



All the statistical parameters for annual and seasonal basis are given in Table 6 above.

Rainfall in at Simara (Bara) during 2015-2019: Rainfall was found inconsistent during this period (2009-2019). The average rainfall was found increasing in order from 109.36 mm (2009) to 152.63 mm (2011); and then was found to be decreased heavely (74.43 mm) in 2012. It was again found increased in 2013; and then

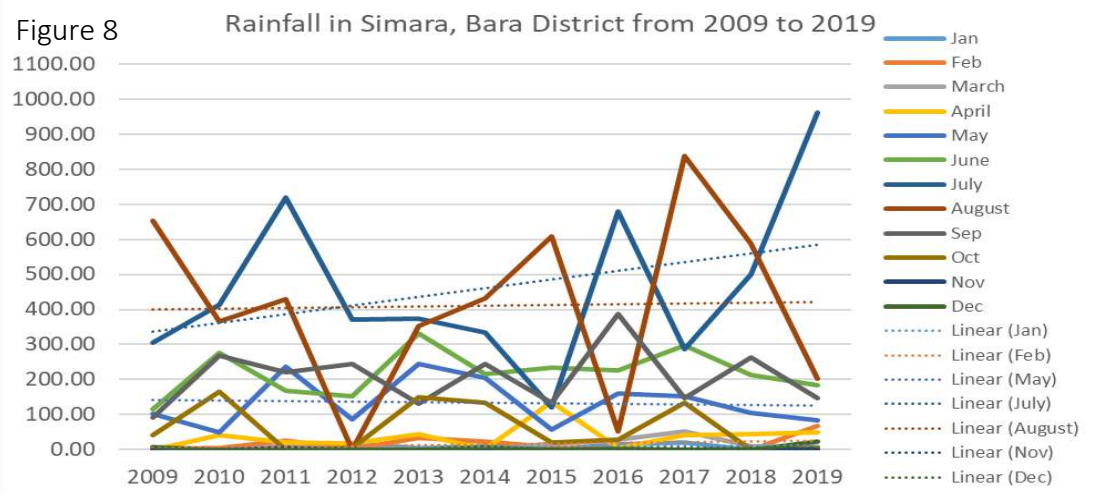
decreased gradually upto 2015. In 2016 and 2017, rainfall was slightly increased from 132.29 mm to 163.93 mm respectively. It was again fallen down to 143.14 mm in 2018 and was slightly arisen to 144.84 mm in 2019. It shows frequently fluctuation in rainfall during this period. In average, it can be concluded that rainfall is inconsistent in this area during this period shown in Table 7.

Table-7: Average rainfall in Simara (Bara) during 2009-2019 AD.

Year	N	Minimum	Maximum	Mean	Skewness	Kurtosis	Std. Deviation
2009	12	0.00	653.60	109.36	2.44	6.21	192.92
2010	12	0.00	412.60	131.65	0.77	-1.11	157.93
2011	12	0.00	719.10	152.63	1.76	2.89	224.66
2012	12	0.00	370.00	74.43	1.75	2.36	121.00
2013	12	0.00	374.00	138.68	0.62	-1.40	148.97
2014	12	0.00	432.60	133.68	0.78	-0.57	150.94
2015	12	0.00	608.60	111.28	2.46	6.76	173.56
2016	12	0.00	679.80	132.29	1.99	3.75	209.85
2017	12	0.00	839.00	163.93	2.39	6.48	237.18
2018	12	0.00	587.00	143.14	1.42	0.83	208.09
2019	12	0.00	963.00	144.84	3.04	9.84	267.38

It was also found that rainfall was recorded accelerating in month of July and remained stagnent in the month of August of monsoon season during 2009 to 2019.

Figure 8



Similarly, average rainfall was slightly deacelerated in the month of May (Pre-monsoon); and slightly accelerated in the month of February (Winter) in this period. The rainfall in the rest months was rarely remained change of this period shown in Figure 8.

Skewness is the measure of asymmetry in frequency distribution about the mean predominantly positive skewness of average value 1.62 indicating annual precipitation

in the region is asymmetric and it lies to the right of mean i.e. Right skewed. Kurtosis is the measure of peakedness or flatness of frequency distribution having value of 3.00 indicating mesokurtic shape shown in Figure 9.

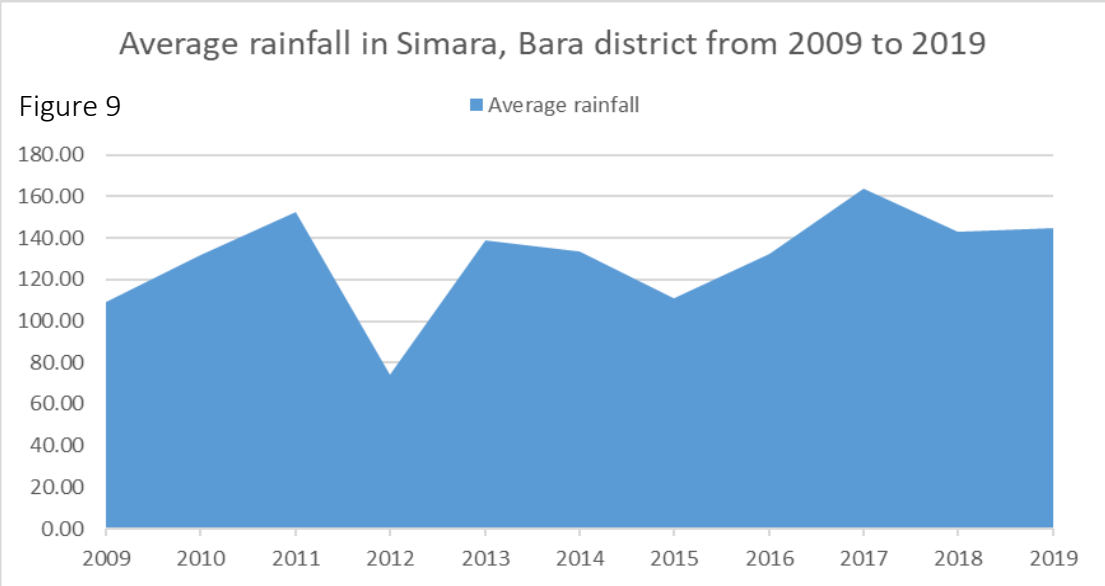


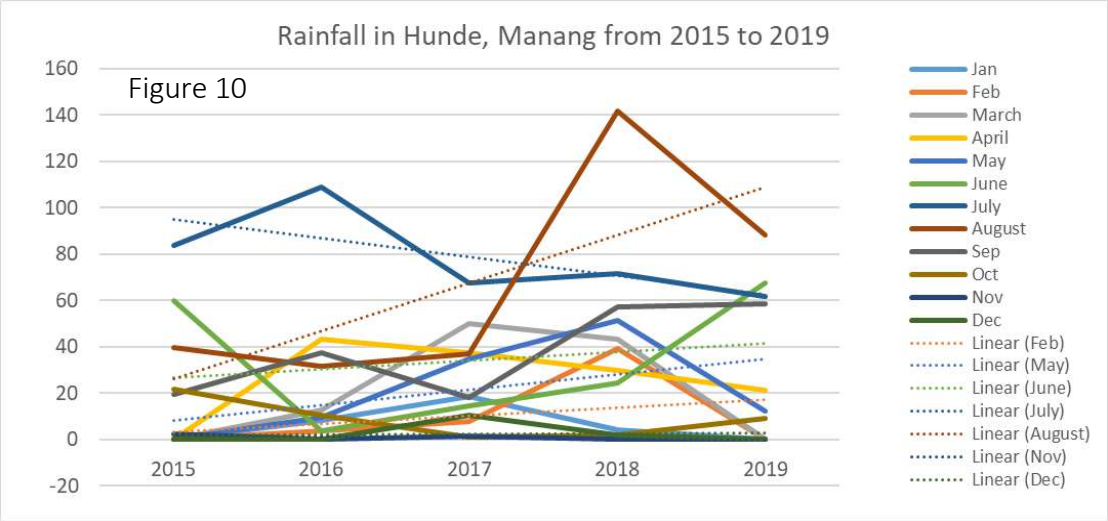
Figure 9

Rainfall in Dharapani (Manang) during 2015-2019: Rainfall was found inconsistent during this period (2015-2019). The average rainfall was found up and down during this period. In average, the lowest rainfall (67.60 mm) was recorded in 2017; and the highest (141.00 mm) was recorded in 2018. increasing in order from 95.33 mm (2009) to 144.59 mm (2013); and then was found decreasing from 144.59 mm (2013) to 81.75 mm (2015). Hence, it can be concluded that rainfall is inconsistent in Hunde higher in recent years than previous one shown in Table 8.

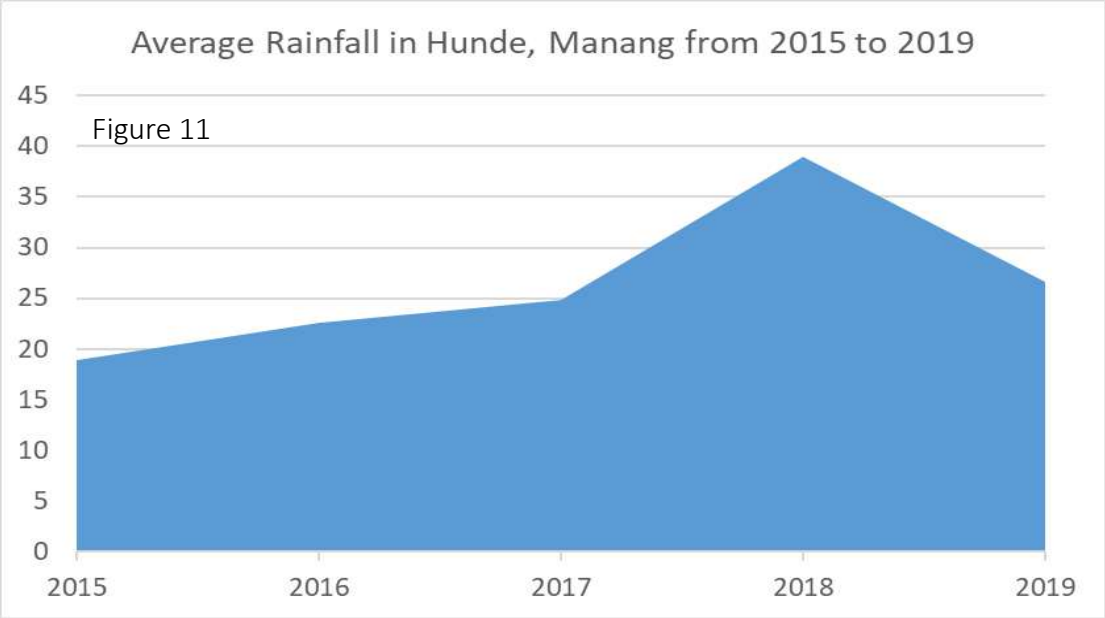
Table-8: Average rainfall at Hunde (Manang) during 2015-2019 AD.

Year	N	Minimum	Maximum	Mean	Skewness	Kurtosis	Std. Deviation
2015	7	0.00	83.50	32.39	0.73	-0.47	30.66
2016	12	0.00	108.90	22.54	2.26	5.71	30.95
2017	12	1.00	67.60	24.89	0.78	-0.05	20.60
2018	12	0.00	141.00	38.63	1.55	3.18	40.09
2019	8	0.00	88.10	39.90	0.17	-1.83	32.88

It was also found that rainfall was recorded accelerating in month of August and deacelerating in the month of July of monsoon season during 20015 to 2019 shown in Figure 10.



Skewness is the measure of asymmetry in frequency distribution about the mean predominantly positive skewness of average value 1.09 indicating annual precipitation in the region is asymmetric and it lies to the right of mean i.e. Right skewed. Kurtosis is the measure of peakedness or flatness of frequency distribution having value of 1.31 indicating mesokurtic shape shown in Figure 11.



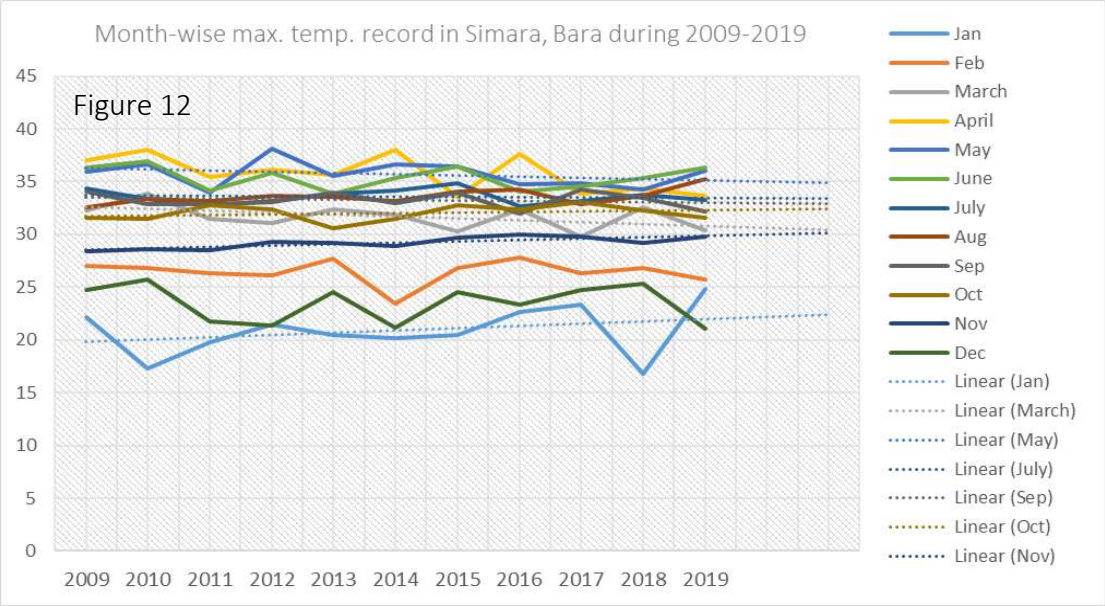
Trend Analysis of Temperature

The preliminary data analysis was carried out to find the statistical parameters (mean, standard deviation, skewness and kurtosis) of annual maximum temperature series for the period 2009-2019. Maximum temperature in the area varies between 17.30°C (January 2010) to 38.10°C (May-2012) where mean annual maximum temperature is 36.59°C with standard deviation 5.06°C. The skewness of average value -1.05 indicating annual maximum temperature in the region is asymmetric and it lies to the left of mean i.e. left skewed. Kurtosis value of 0.41 represents the platykurtic shape of annual data distribution. All the statistical parameters for annual average temperature is shown in Table 9.

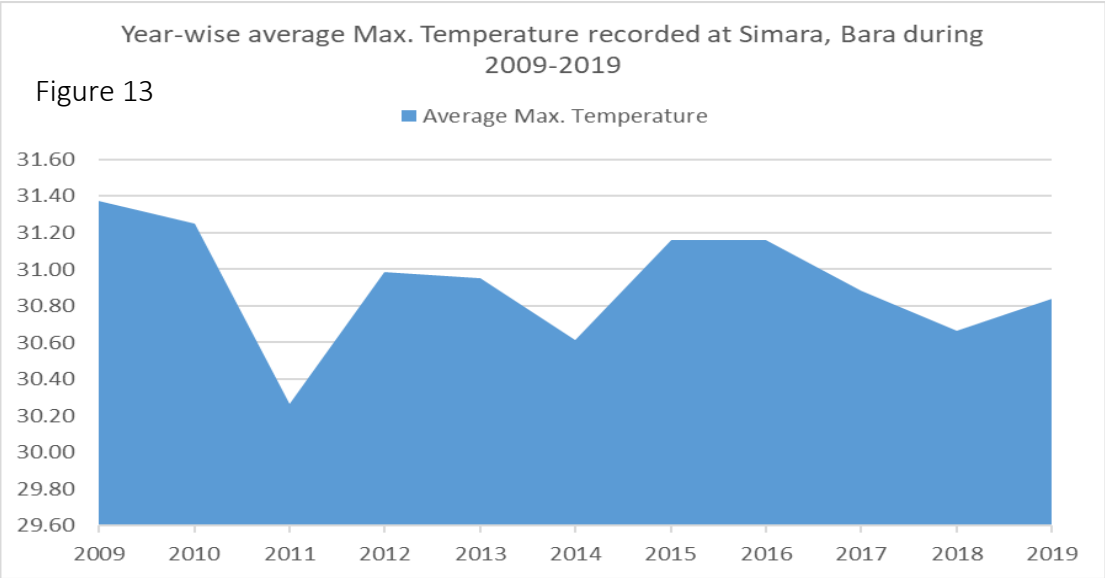
Table-9: Annual max. temperature recorded at Simara (Bara) during 2009-2019 AD.

Year	N	Minimum	Maximum	Mean	Std. Dev.	CV	Skewness	Kurtosis
2009	12	22.20	37.00	31.38	4.79	22.92	-0.74	-0.53
2010	12	17.30	38.00	31.25	5.86	34.31	-1.25	1.76
2011	12	19.80	35.40	30.27	5.10	25.98	-1.23	0.34
2012	12	21.40	38.10	30.98	5.45	29.75	-0.81	-0.30
2013	12	20.50	35.60	30.95	4.70	22.06	-1.21	0.81
2014	12	20.20	38.00	30.62	5.97	35.64	-0.79	-0.64
2015	12	20.50	36.40	31.16	4.99	24.85	-1.05	0.33
2016	12	22.60	37.60	31.16	4.54	20.58	-0.91	0.20
2017	12	23.30	34.80	30.88	4.07	16.60	-0.91	-0.66
2018	12	16.80	35.30	30.67	5.40	29.11	-1.80	3.24
2019	12	21.10	36.30	30.84	4.78	22.83	-0.86	-0.07
Annual		20.52	36.59	30.92	5.06	25.88	-1.05	0.41

The results shown in Table 9 indicated a rising trend in month of May, August and September, whereas significant falling trends showed in the months of March and July and significant rising trend in the month of November to January. The annual average maximum temperature in the Bara district showed an increasing trend. An increasing trend in the winter and pre-monsoon season will lead to increase in annual mean temperature and consequently decrease the crop period, however, a maximum temperature during monsoon may auger the soil moisture content. A decreasing monsoon maximum temperature will result in less evaporation loss causing to rise in the ground water table. Figure 12 depicts the maximum temperature variability during the period 2009-2019.



The skewness of average value -1.05 indicates annual maximum temperature in the region is asymmetric and it lies to the left of mean i.e. left skewed. Kurtosis value of 0.41 represents the platykurtic shape of annual data distribution shown in Figure 13.



Annual maximum temperature recorded at Dhunebeshi (Dhading) during 2009-2019:

The preliminary data analysis was carried out to find the statistical parameters (mean, standard deviation, skewness and kurtosis) of annual maximum temperature series for the period 2009-2019.

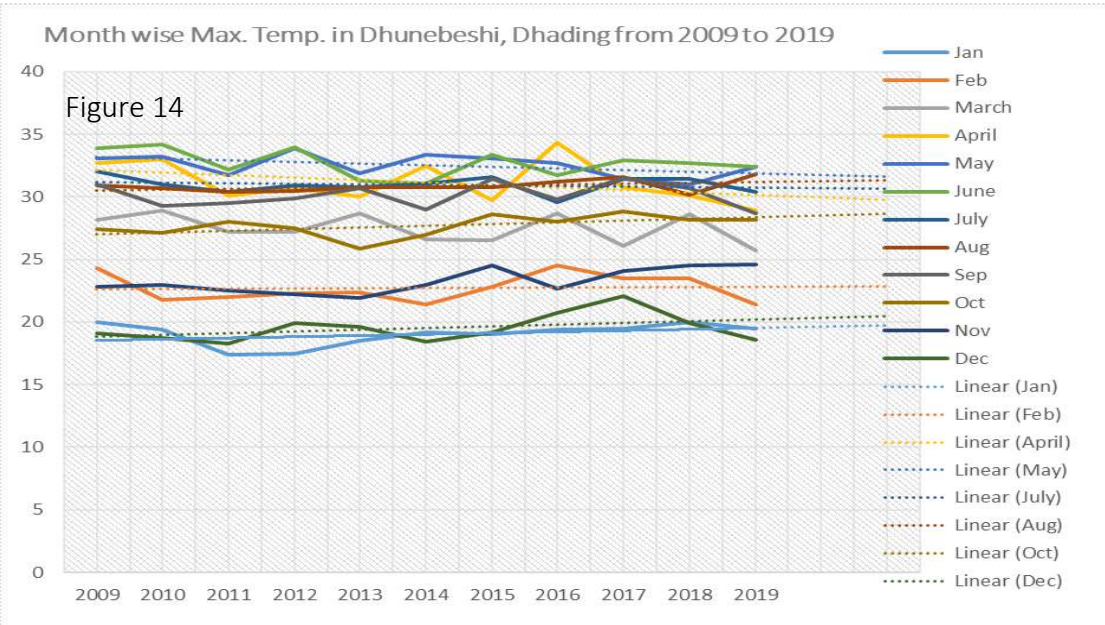
Maximum temperature in the area varies between 17.40°C (January 2011) to 34.30°C (April-2016) where mean annual maximum temperature is 33.21°C with standard deviation 5.05°C. The skewness of average value -0.63 indicating annual maximum temperature in the region is asymmetric and it lies to the left of mean i.e. left skewed.

Kurtosis value of 1.04 represents the platykurtic shape of annual data distribution. All the statistical parameters for annual are shown in Table 10.

Table-10: Annual max. temp. recorded at Dhunebeshi (Dhading) during 2009-2019

Year	Minimum	Maximum	Mean	Std. Dev.	CV	Skewness	Kurtosis
2009	19.10	33.90	27.95	5.23	27.33	-0.62	-1.10
2010	18.70	34.20	27.53	5.49	30.11	-0.51	-1.24
2011	17.40	32.20	26.64	5.23	27.34	-0.81	-0.85
2012	17.50	34.00	27.21	5.49	30.16	-0.52	-0.99
2013	18.50	31.90	26.88	4.97	24.74	-0.67	-1.32
2014	18.40	33.40	26.96	5.27	27.78	-0.51	-1.24
2015	19.00	33.40	27.55	5.12	26.21	-0.65	-0.93
2016	19.40	34.30	27.78	4.85	23.54	-0.57	-0.90
2017	19.50	32.90	27.81	4.54	20.59	-0.63	-1.12
2018	19.90	32.70	27.57	4.47	19.95	-0.83	-0.73
2019	18.60	32.40	26.88	4.92	24.21	-0.58	-1.01
Average	18.73	33.21	27.34	5.05	25.63	-0.63	-1.04

The results shown in Table 10 indicated a rising trend in month of August and October, whereas significant falling trends showed in the months of April and May and significant rising trend in the month of December to January. The annual average maximum temperature in the Dhading district showed an increasing trend shown in Figure 14.

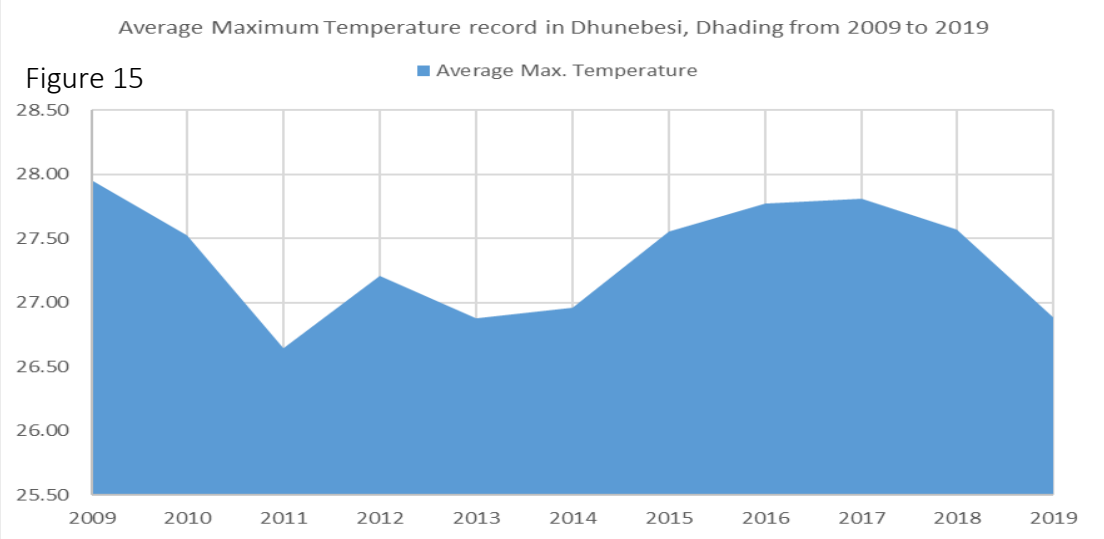


An increasing trend in the winter and pre-monsoon season will lead to increase in annual mean temperature and consequently decrease the crop period, however, a maximum temperature during monsoon may auger the soil moisture content. A decreasing monsoon maximum temperature will result in less evaporation loss

causing to rise in the ground water table. Figure 14 depicts the maximum temperature variability during the period 2009-2019.

The skewness of average value -0.63 indicates annual maximum temperature in the region is asymmetric and it lies to the left of mean i.e. left skewed. Kurtosis value of -1.04 represents the platykurtic shape of annual data distribution shown in Figure 15.

Figure 15



Maximum temperature recorded at Hunde (Manang) during 2015-2019:

The preliminary data analysis was carried out to find the statistical parameters (mean, standard deviation, skewness and kurtosis) of annual maximum temperature series for the period 2015-2019.

Maximum temperature in the area varies between 5.50°C (January 2016) to 20.20°C (June-2016) where mean annual maximum temperature is 13.72°C with standard deviation 4.88°C.

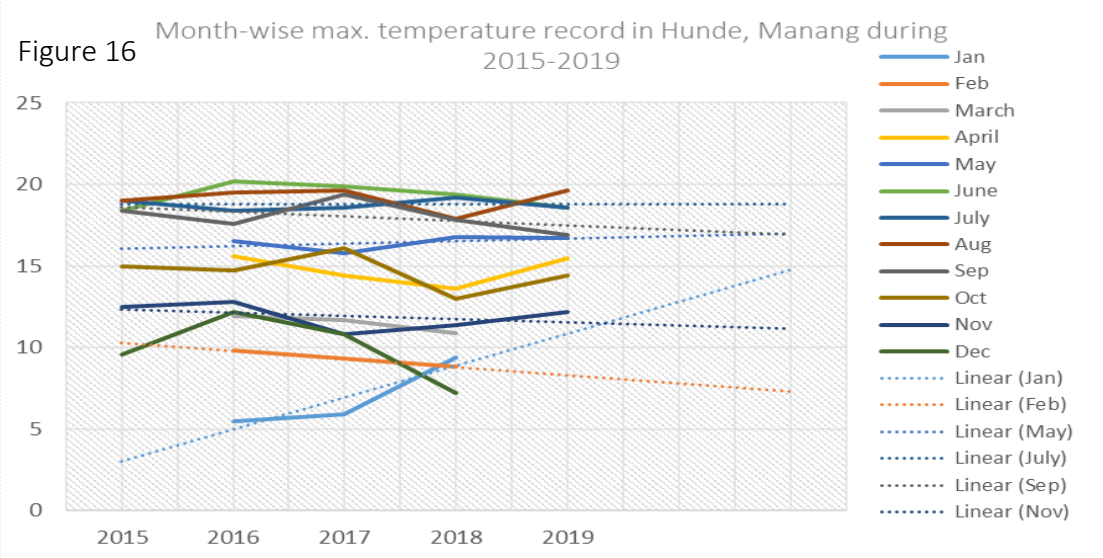
Table-11: Annual max. temp. recorded at Hunde (Manang) during 2015-2019

Year	Minimum	Maximum	Mean	Std. Dev.	CV	Skewness	Kurtosis
2015	0.00	19.00	9.33	8.68	75.37	-0.09	-2.09
2016	5.50	20.20	14.56	4.32	18.69	-0.67	0.15
2017	5.90	19.90	14.36	4.63	21.48	-0.35	-1.02
2018	7.20	19.40	13.78	4.32	18.65	-0.04	-1.56
2019	12.20	19.60	16.56	2.46	6.07	-0.63	-0.20
Average	6.16	19.62	13.72	4.88	28.05	-0.36	-0.95

The skewness of average value -0.36 indicating annual maximum temperature in the region is asymmetric and it lies to the left of mean i.e. left skewed. Kurtosis value of -0.95 represents the platykurtic shape of annual data distribution. All the statistical parameters for annual are shown in Table 11.

The results shown in Table 11 indicated a rising trend in month of May and January, whereas significant falling trends showed in the months of September, November and February. The annual average maximum temperature in the Manang district showed an increasing trend in pre-monsoon and winter season shown in 16.

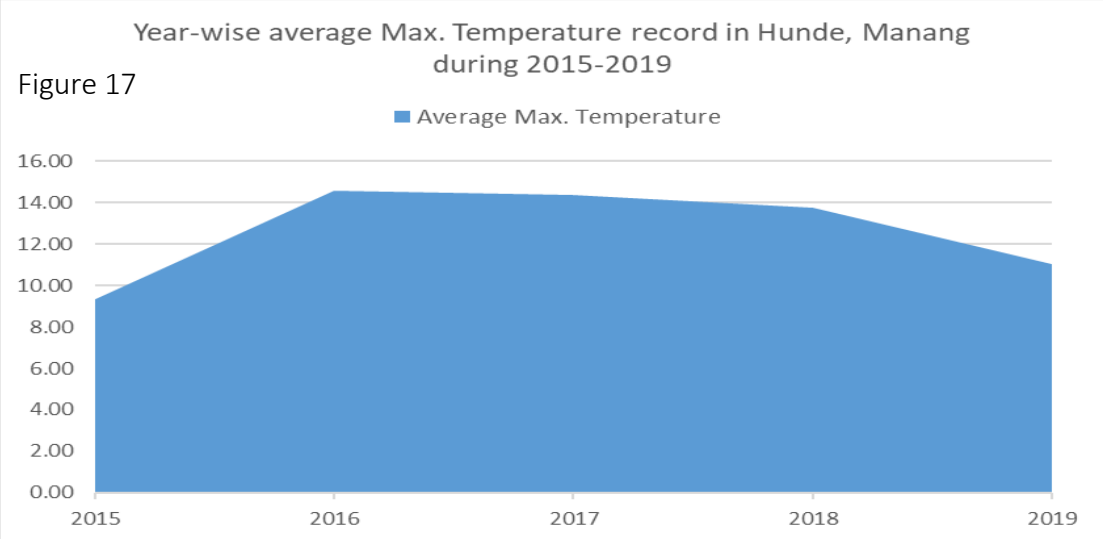
Figure 16



An increasing trend in the winter and pre-monsoon season will lead to increase in annual mean temperature and consequently decrease the crop period, however, a maximum temperature during monsoon may auger the soil moisture content. Figure 16 depicts the maximum temperature variability during the period 2009-2019.

The skewness of average value -0.36 indicates annual maximum temperature in the region is asymmetric and it lies to the left of mean i.e. left skewed. Kurtosis value of -0.95 represents the platykurtic shape of annual data distribution shown in Figure 17.

Figure 17



Minimum Temperature recorded at Simara (Bara) during 2009-2019: The preliminary data analysis was carried out to find the statistical parameters (mean, standard deviation, skewness, kurtosis and coefficient of variation) of annual minimum temperature series for the period 2009-2019. Minimum temperature in the Chitwan Anapurna Landscape (CHAL) varies between 5.40°C (January 2018) to 26.50°C (July-2009) where mean annual maximum temperature is 26.15°C with standard deviation 7.05°C.

Table-12: Annual min. temp. recorded at Simara (Bara) during 2009-2019

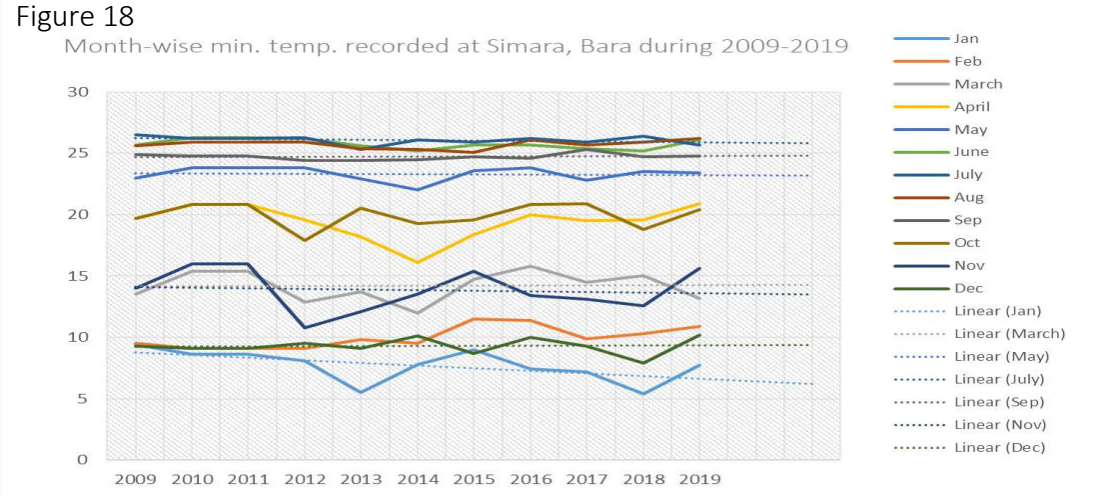
Year	Minimum	Maximum	Mean	Std. Dev.	CV	Skewness	Kurtosis
2009	9.30	26.50	18.40	6.90	47.63	-0.24	-1.76
2010	8.60	26.30	18.90	7.02	49.27	-0.49	-1.44
2011	8.60	26.30	18.90	7.02	49.27	-0.49	-1.44
2012	8.10	26.30	17.88	7.40	54.80	-0.13	-1.93
2013	5.50	25.60	17.71	7.34	53.82	-0.37	-1.51
2014	7.80	26.10	17.62	6.92	47.95	-0.08	-1.79
2015	8.70	25.90	18.53	6.57	43.20	-0.32	-1.51
2016	7.40	26.20	18.77	6.88	47.28	-0.42	-1.46
2017	7.20	25.90	18.29	7.11	50.60	-0.37	-1.61
2018	5.40	26.40	17.94	7.52	56.48	-0.42	-1.36
2019	7.70	26.20	18.76	6.88	47.36	-0.40	-1.55
Average	7.66	26.15	18.33	7.05	49.79	-0.34	-1.58

The results shown in Table 12 indicated a rising trend of annual mean minimum temperature in the Bara district. An increasing trend with highly significant minimum temperature in the premonsoon and post-monsoon season will increase the duration of the summer season and enhance the global warming(Parthasarathy and Dhar, 1974), however, a lesser significant rising minimum temperature during monsoon and winter may decrease the length of crop period without reaching to its maturity hence reduce the yield. An increasing minimum temperature will support the global warming causing climate change(Kharmeshu, 2012). This suggests that temperature trend shows rising and due to this rising temperature, other climatic variables may experience affected in the hydrologic processes and surrounding environment of the district (Rao, 1993). The minimum temperature variability during the period 2009-2019 is presented in Figure 18. It also indicated the linear trend line overlaid on the time series for different months and seasons.

The results shown in Figure 18. indicated a rising trend in the months of May, June and August, October and December, whereas in the months of January, March, July

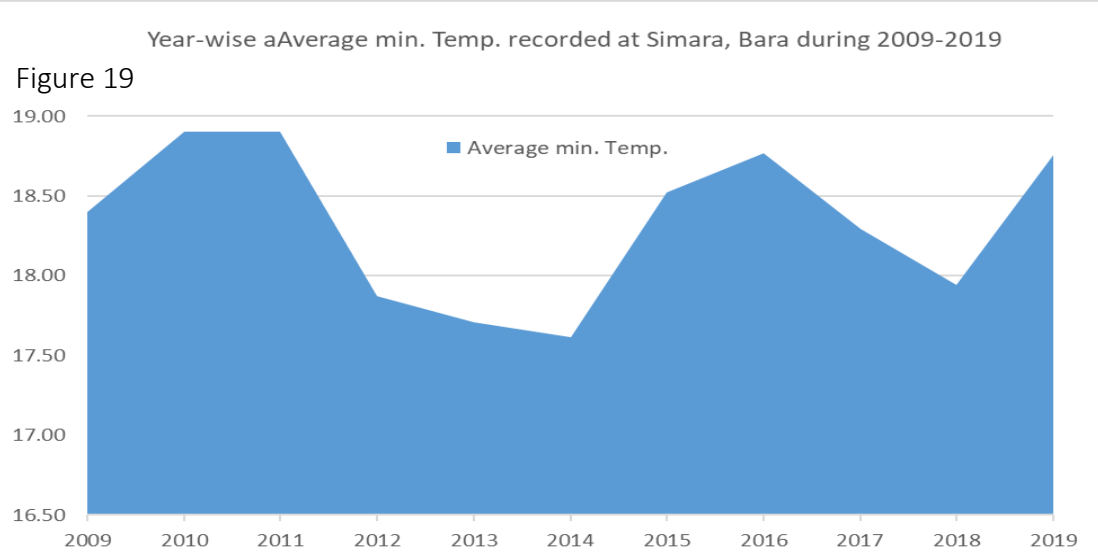
and September was found falling trends from 2009 to 2019. The annual mean minimum temperature in the Bara district showed an increasing trend.

Figure 18



The predominantly negative skewness of average value -0.34 indicating annual minimum temperature in the region is asymmetric and it lies to the left of mean. Kurtosis of annual frequency distribution -1.58 indicating Platykurtic shape. All other statistical parameters for annual basis are depicted in Table 12 and as shown in Figure 19.

Figure 19



Annual min. recorded temperature at Dhunebeshi (Dhading) during 2015-2019:

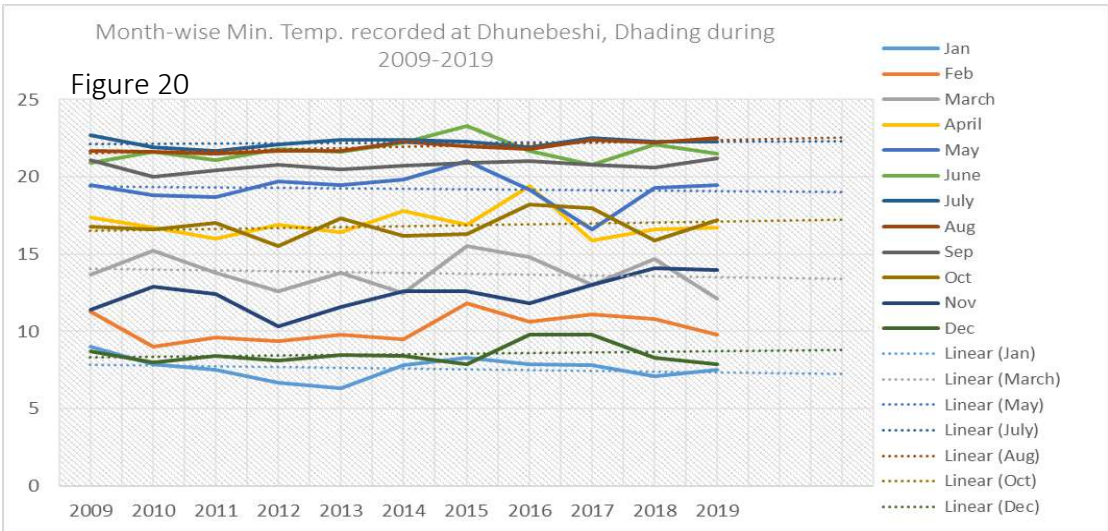
The preliminary data analysis was carried out to find the statistical parameters (mean, standard deviation, skewness, kurtosis and coefficient of variation) of annual minimum temperature series at Dhunebeshi (Dhading) for the period 2009-2019. Minimum temperature in the Chitwan Anapurna Landscape (CHAL) at Dhuneneshi

varies between 6.30°C (January 2013) to 23.30°C (June-2015) where mean annual maximum temperature is 22.34°C with standard deviation 5.42°C.

Table-13: Annual min. temp. recorded at Dhunebeshi (Dhading) during 2009-2019

Year	Minimum	Maximum	Mean	Std. Dev.	CV	Skewness	Kurtosis
2009	8.70	22.70	16.18	5.16	-0.26	-1.62	8.70
2010	7.90	21.90	15.85	5.32	-0.46	-1.28	7.90
2011	7.50	21.70	15.68	5.26	-0.36	-1.42	7.50
2012	6.70	22.10	15.47	5.83	-0.24	-1.69	6.70
2013	6.30	22.40	15.78	5.65	-0.41	-1.34	6.30
2014	7.80	22.40	16.02	5.64	-0.27	-1.64	7.80
2015	7.90	23.30	16.57	5.47	-0.38	-1.25	7.90
2016	7.90	21.90	16.51	5.24	-0.51	-1.49	7.90
2017	7.80	22.50	15.98	5.06	-0.15	-1.32	7.80
2018	7.10	22.30	16.17	5.38	-0.44	-1.04	7.10
2019	7.50	22.50	16.02	5.63	-0.36	-1.46	7.50
Average	7.55	22.34	16.02	5.42	-0.35	-1.41	7.55

The results shown in Figure 20 indicated a rising trend of annual mean minimum temperature in the month of April, August and December; falling trends in the month of March and January in the Dhading district. An increasing trend with highly significant minimum temperature in the premonsoon and post-monsoon season will increase the duration of the summer season and enhance the global warming. The annual mean minimum temperature in the Bara district showed an increasing trend.



The predominantly negative skewness of average value -1.41 indicating annual minimum temperature in the region is asymmetric and it lies to the left of mean. Kurtosis of annual frequency distribution 7.55 indicating leptokurtic shape. All other statistical parameters for annual basis are depicted in Table 13 and also shown in Figure 21.

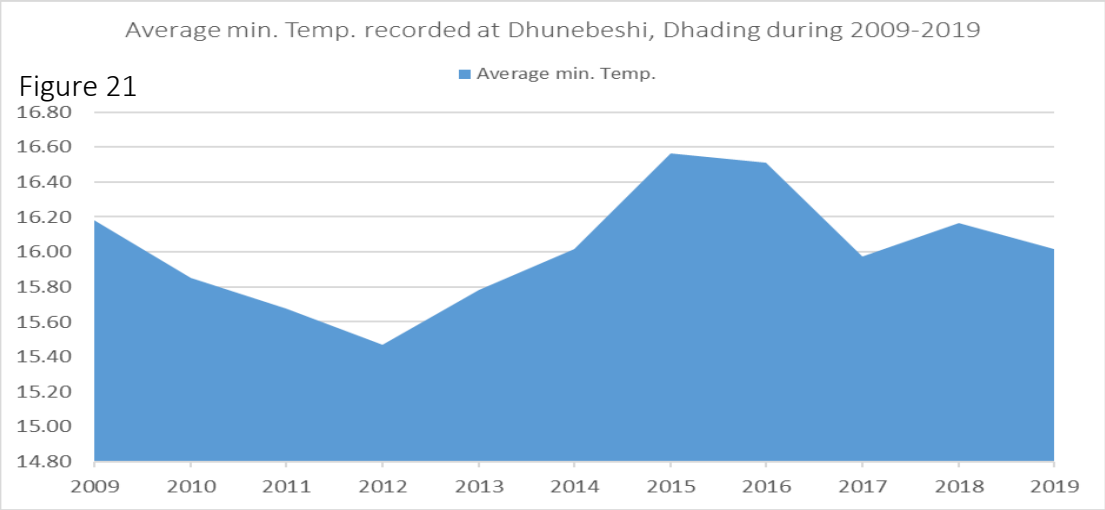


Figure 21

Annual minimum temperature recorded at Hunde (Manang) during 2015-2019:

The preliminary data analysis was carried out to find the statistical parameters (mean, standard deviation, skewness, kurtosis and coefficient of variation) of annual minimum temperature series for the period 2015-2019.

Table-14: Annual min. temp. recorded at Hunde (Manang) during 2015-2019

Year	Minimum	Maximum	Mean	Std. Dev.	CV	Skewness	Kurtosis
2015	-8.20	10.00	1.89	5.45	29.76	0.06	-0.42
2016	-9.90	10.10	2.10	6.82	46.45	-0.48	-1.28
2017	-10.40	8.60	-0.25	7.02	49.26	-0.10	-1.63
2018	-11.40	8.10	-1.77	6.89	47.53	0.24	-1.47
2019	-4.10	8.60	2.66	5.38	28.94	-0.05	-2.41
Average	-8.80	9.08	0.93	6.31	40.39	-0.07	-1.44

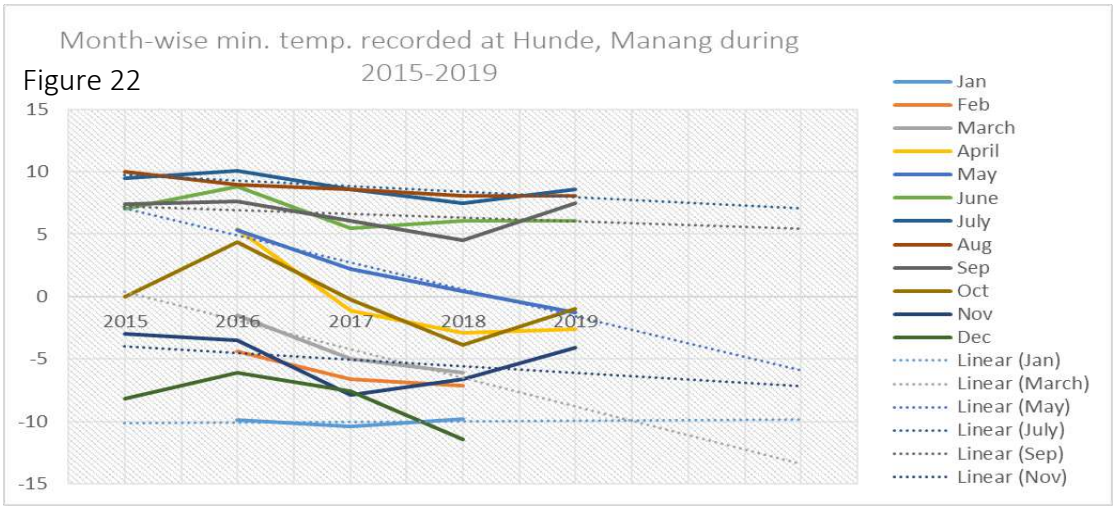
Minimum temperature in the Chitwan Anapurna Landscape (CHAL) varies between -11.4°C (December 2018) to 10.10°C (July-2016) where mean annual maximum temperature is 9.08°C with standard deviation 6.31°C.

The results shown in Table 14 indicated a falling trend of annual mean minimum temperature in the Manang district. A decreasing trend with highly significant minimum temperature in the premonsoon and post-monsoon season will increase the duration of the winter season and enhance the extreme cold and threats to various plants and animals.

The results shown in Figure 22 indicated a falling trend of annual mean minimum temperature in the month of Jan, March, May, July, September and November in the Manang district. A falling trend with highly significant minimum temperature in the

premonsoon and post-monsoon season will increase the duration of the winter season and affects the life cycle of many plants and animals leaving in the district.

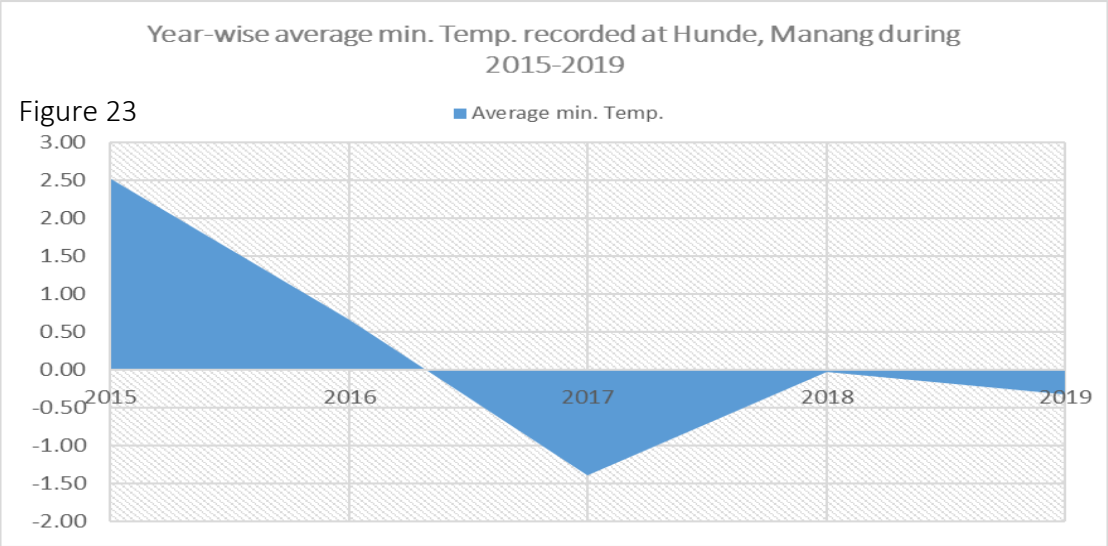
Figure 22



The annual mean minimum temperature in the Manang district showed a falling trend.

The negative skewness of average value -0.07 indicating annual minimum temperature in the region is asymmetric and it lies to the left of mean. Kurtosis of annual frequency distribution -1.44 indicating platykurtic shape. All other statistical parameters for annual basis are depicted in Table 14 and also shown in Figure 23.

Figure 23



PHARMACOGNOSTICAL STUDY

Plant distribution and sample collection:

1. ***Neopicrorhiza scrophulariiflora*** (Pennell) Yang.: Authentic specimens of Kutaki (*Neopicrorhiza scrophulariiflora*; NPS) rhizomes was collected from Kharka (Nache), Dharapani rural municipality, Manang District of Nepal, an altitude of 4200 masl in October of 2020 (Figure No. 24). The collected rhizomes were fresh and matured shown in Figure No 25 and 25.1

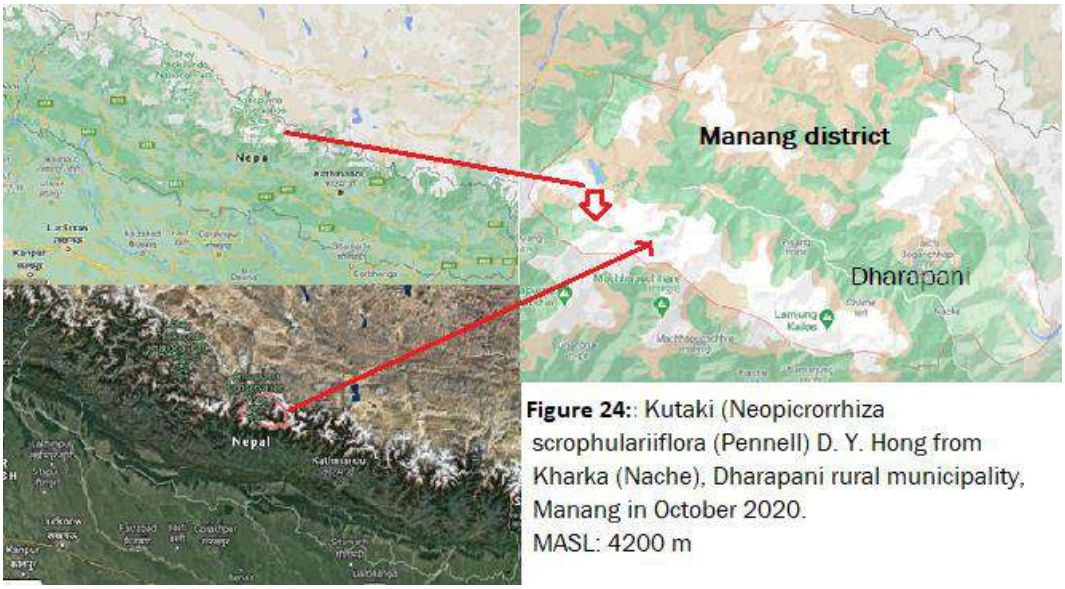


Figure 24:: Kutaki (Neopicrorrhiza scrophulariiflora (Pennell) D. Y. Hong from Kharka (Nache), Dharapani rural municipality, Manang in October 2020.
MASL: 4200 m

2. ***Berberis aristata*** DC.: Authentic specimens of Daruharidra (*Berberis aristata*; BA) root was collected from Dhunebeshi Municipality ward no. 5, Dhading District of Nepal, an altitude of 1315 masl in October of 2020 (Figure No 26).

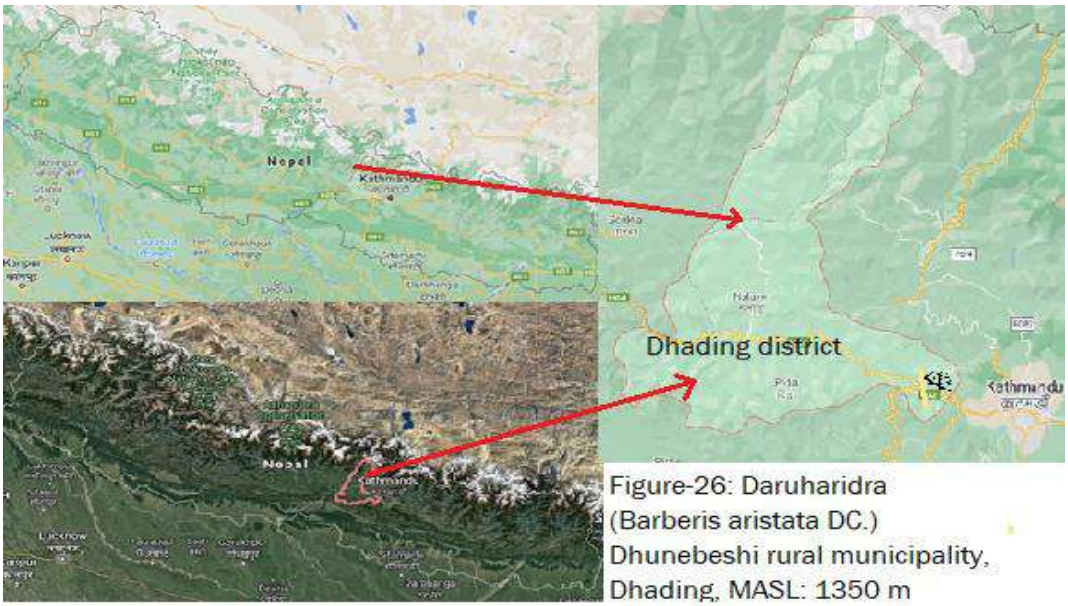


Figure-26: Daruharidra (Barberis aristata DC.) Dhunebeshi rural municipality, Dhading, MASL: 1350 m

The collected rhizomes were fresh and matured shown in Figure No 27.

3. ***Tinospora cordifolia*** Wild.: Authentic specimens of Guduchi (*Tinospora cordifolia*; TC) stem was collected from Mathuwan Mithaul rural municipality, Parsa district of Nepal; an altitude of 1315 masl in October of 2020 shown in Figure No. 28. The collected stems were fresh and matured (Figure No. 29).

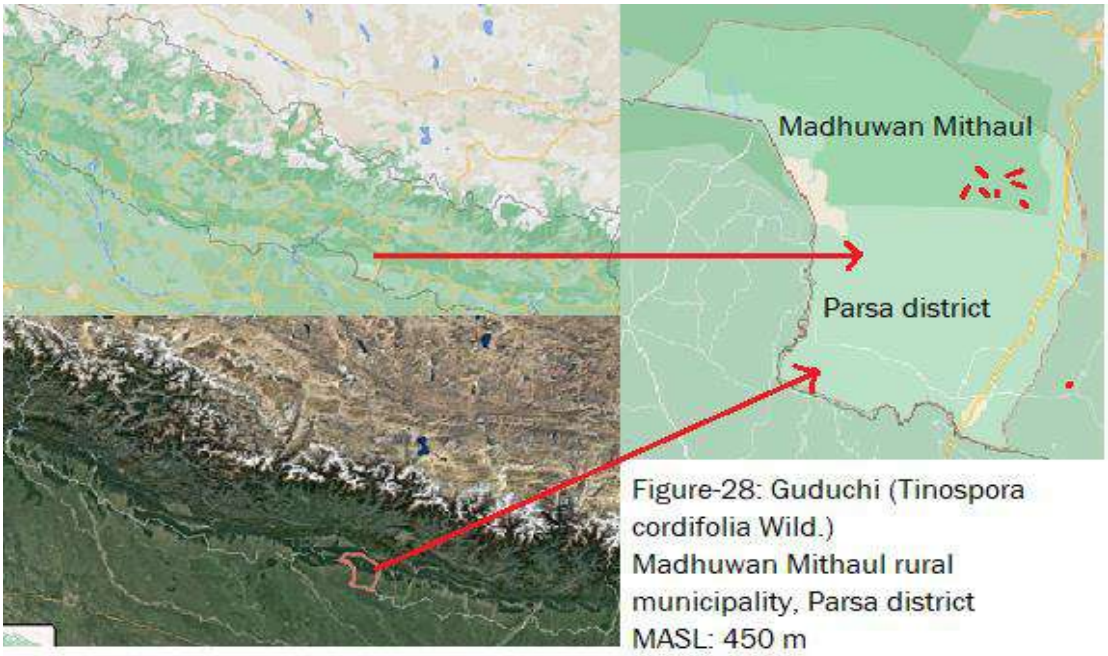


Figure-28: Guduchi (Tinospora cordifolia Wild.)
Madhuwan Mithaul rural municipality, Parsa district
MASL: 450 m

Sample authentication:

The authenticity of these plants were established by comparison with the sheets in the herbarium of Ayurveda Campus, Kirtipur, Kathmandu, Nepal, consulted taxonomist at Department of Botany, TU and also with the description given by Hooker (1882).

Accession Number; NPL-AYC-PR0; 234, 235 & 236

Preservation of fresh collected samples:

Formaldehyde: ethyl alcohol: acetic acid (10%:50%:5% + 35% water) was used with almost any plant material intended for anatomical or morphological study. Specimens samples was left in it after collection from field. It can be preserved for almost indefinitely without appreciable damage. The minimum time of fixation was about 72 hours (Johansen, 1940).

Deposition of herbarium sheet:

The authentic herbarium sheet of the sample plants was deposited at herbarium of Ayurveda Campus, Kirtipur, Kathmandu, Nepal; and the same at Department of Ayurveda and Alternative Medicine (DOAA).

Kutaki

Neopicrorhiza scrophullarifolia (Pennell) D. Y. Hong

Morphological characters: N. scrophulariiflora is a perennial herbs grows in alpine region of eastern Himalaya.

Figure-25: Dried rhizomes (A), Plant with flowering stalk (B), Vegetative growth (C) and Flower (D)

Whole plant (stem) of 10-60 cm creeping, rootstock stout, covered with old leaf-bases above (A); florescence, cymes (B); leaves: 2-6 cm, oblanceolate and narrowed below to a winged leaf-stalked (C); flower: Dark blue purple, in a dense cylindrical head, borne on astout stem arising from a rosette conspicuously toothed leaves (B & D). Corolla 1.5 cm, a long 3-lobed upper lip and a short lower lip; stamens and style exserted; calyx hairy, nearly as long as the corolla-tube, calyx-lobes 5, lanceolate blunt; fruiting stem 5-10 cm; capsule 6-10 mm

Organoleptic parameters:

The rhizomes and its powder characters were separately evaluated by organoleptic characters like surface, colour, odour, taste, etc.

Table-15: Organoleptic parameters

Colour:	Dark grey rhizomes are crowned with reddish-brown small scales
Odour:	Slightly irritable.
Taste:	Acrid, strongly bitter.
Sound:	Not applicable
Shape:	Elongated, Zig-zag, and cylindrical.
Size:	Rhizomes are 2.5 to 7.5 cm in length.

The traditional Ayurvedic method for identification of drugs like *Nama, Rupa, Guna,* etc. was also studied separately.

Rupa (Structure): Matasyasakala (Scaly covers over the surface), Chakrangi (Circular ring in transverse cut surface)

Rasa (Taste): Katvi (Unpalatable)

Guna (Properties): Katuka (Cholerectic action)

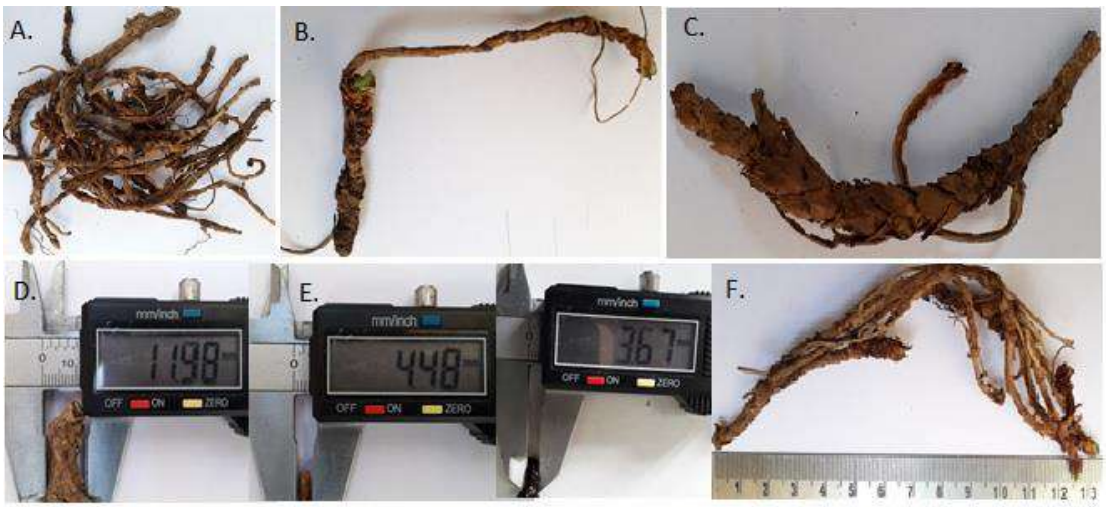
Rhizomes: Kandaruha, Rohini, Katurohini, Asokarohini

Macroscopic examination

Rhizome: - 2.5-8 cm long and 4-8 mm thick, subcylindrical, straight or slightly curved, externally greyish-brown, surface rough due to longitudinal wrinkles, circular scars of roots and bud scales and sometimes roots attached, tip ends in a growing bud

Figure-25.1.: Neopicrorrhiza scrophulariflora Roxb. rhizomes: Dried sample of rhizome (A), dichotomously branched rhizomes (B & C), matted (D) and naked (F) rhizomes with rootstock, leaves remnant (E), rootstock (G), aerial shoot (H).

Figure-25.2:



Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong: Rhizomes (A), Single rhizome showing young one (B), Rhizome with leave scars (C), Matured rhizome diameter (D), Young rhizome diameter (E), Young rhizome diameter in mm and Rhizome length (F) in cm.

surrounded by tufted crown of leaves, at places cork exfoliates exposing dark cortex; fracture, short; odour, pleasant; taste, bitter.

Root: - Thin, cylindrical, 5-10 cm long, 0.05-0.1 cm in diameter, straight or slightly curved with a few longitudinal wrinkles and dotted scars, mostly attached with rhizomes, dusty grey, fracture, short, inner surface black with whitish xylem; odour, pleasant; taste, bitter. Herbarium seet attached to Annex-4.

PHYSICO-CHEMICAL EVALUATION

Determination of Foreign Matter

The foreign matter was found 4% in NPS rhizomes and presented in table no. 16. It is the higher than the recommended value (<2%) in Ayurvedic Pharmacopiea of India.

Table No. 16: Determination of Foreign Mater in NPS Rhizomes

Samples for	Percentage of Foreign Matter
	Dried Rhizomes of NPS (%)
Foreign Matter	4

Determination of Loss on Drying (LoD)

The percentage of loss on drying was observed 1.96% in NPS rhizomes and shown in table no 17. It is recommended value in Ayurvedic Pharmacopiea of India.

Table No 17: Determination of Loss on Drying in NPS rhizomes

Samples for	Percentage Loss on Drying
	Dried rhizomes of NPS (%)
Loss on Drying	1.96

Determination of Total Ash, Acid-insoluble Ash and Water-soluble Ash

The percentage total ash (0.11%), acid-insoluble (0.01%) and water-soluble ash (0.14%) values obtained from NPS rhizomes shown in Table No 18. It is the less than the recommended values for total ash (<7%) and acid-insoluble (<2%). Water soluble value is not mentioned in Ayurvedic Pharmacopiea of India.

Table No. 18: Determination of Total Ash Values NPS rhizomes powder

Parameters	Values in Percentage (w/w)
	Dried Rhizomes of NPS (%)
Total ash	0.11
Acid-insoluble ash	0.01
Water-soluble ash	0.14

Determination of PH Values

The pH values of 1% solution of NPS rhizomes powder was observed for 5.60 and shown in the table No. 19 below. It isn't mentioned by Ayurvedic Pharmacopiea of India.

Table No. 19: Determination of PH Values of NPS rhizomes powder

Solution of Powder Drugs	PH expressed in Mean
	NPS rhizomes powder solution
1% Solution (w/v)	5.60

EXTRACTIVE VALUES

Cold Extractive Value: Extraction process of NPS rhizomes was carried out in distilled water (aqeous) and ethyl alcohol.

Percolation Method: The extractive values for NPS rhizomes powder in the solvent such as ethanol and distilled water were found to be 21.25% and 24.64% respectively shown in Table No. 20. Ayurvedic Pharmacopiea of India recommendes more than 10% and 20% in ethanolic and aqueous slovents respectively.

Table No. 20: Extractive values of NPS rhizomes

Solvents	Extractive Values (Percolation Method) % Yield
	NPS rhizomes powder solution
Ethanol	21.25
Aqueous	24.64

MICROBIAL LOADS:

Cold extract of NPS rhizome powder was carried out evaluating total microbial and fungal loads and further was investigated for specific microbial load in the sample by liquid froth agar method. Total count of microbial and fungal loads was less than detectable limit (LTDL) and 16902 CFU/gm respectively. E. coli and Salmonella spp including others mentioned in table no. 21 were found LTDL in the sample. The WHO limit of microbial load was recommended for total microbial and fugal load to be not exceeded Max. 10^7 CFU/gm and Max. 10^4 CFU/gm respectively. In the same way, API

(2016) has recommended limit of total microbial load and total fungal count to be not exceeded Max. 10^7 CFU/gm and Max. 10^3 CFU/gm in raw materials respectively.

Table No. 21: Microbial load in NPS rhizomes powder

Parameters	Microbial load (CFU/gm)		
	Dried Kutaki rhizomes	Acceptable Limit	
		WHO (2007)	API (2016)
Total bacterial count	LTDL	Max. 10^7	Max. 10^7
Total fungal count	16902	Max. 10^4	Max. 10^3
Clostridium spp.	NT	Absent	Not mentioned
Enterobacteria	NT	Max. 10^3	Not mentioned
Escherichia coli	NT	Max. 10^2	Absent
Pseudomonas aeruginosa	NT	Not mentioned	Absent
Salmonella spp.	NT	Absent	Absent
Shigella spp.	NT	Absent	Not mentioned
Staphylococcus aureus	NT	Not mentioned	Absent

The limit for E. coli in API should be absent and Max. 10^2 CFU/gm in WHO; Salmonella spp. should be absent in both API and WHO guidelines. API hasn't limit for Clostridium spp., Enterobacteria and Shigella spp.; and Pseudomonas aeruginosa and Staphylococcus aureus must be absent in the herbal raw materials. Furthermore, WHO hasn't determined limit for Pseudomonas aeruginosa and Staphylococcus aureus in its guidelines. However, other microbial of the table should be free from raw materials shown in the table 21.

HEAVY METALS, PESTICIDES AND AFLATOXINS: The heavy metals commonly recommended for evaluation of safety use are Mercury, Lead, Cadmium and Arsenic. Among the pesticides, 4,4, DDT and Parathion are most commonly recommended for evaluation of residues in raw materials. In the sample of NPS rhizome powder, heavy metals; Arsenic, Cadmium, Lead and Mercury were found 0.14, 0.08, 0.65 and 0.08 respectively; and pesticides such as 4,4 DDT and Parathion were found absent. These contaminants are accumulated in the various tissues of the body and ultimately causes damage to the organs. It indicates that fresh raw materials and collected from remote area may be less contaminated with heavy metals and pesticides shown in the table 22.

Table No. 22: Heavy Metals, Pesticides and Aflatoxins in NPS rhizomes powder

Parameters	Heavy Metals, Pesticides and Aflatoxins				
	Heavy metals (ppm)	Pesticides (mg/kg)	Aflatoxins (ppb)	Acceptable Limit	
				WHO (2001)	API (2016)
Mercury (Hg)	0.08			0.03	1
Lead (Pb)	<0.65			10	10
Arsenic (As)	0.14			3	3
Cadmium (Cd)	<0.08			0.3	0.3
4,4, DDT		ND		1	
Parathion		ND		0.5 mg/kg	NA

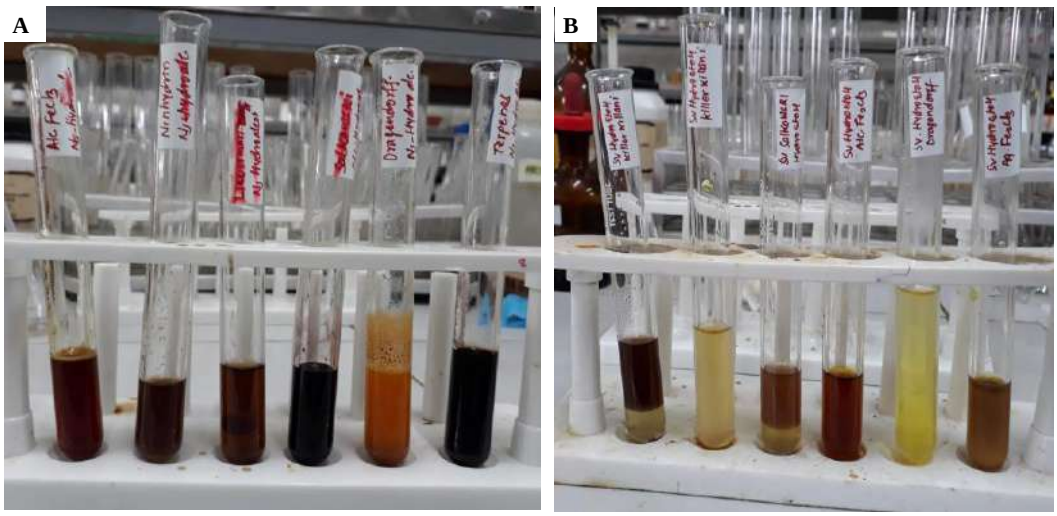
Note: ND = Not Detected in the test

Generally remote area is less contaminated by anthropogenic activities. However, the findings of heavy metals in NPS sample collected from 4200 m altitude of Manang district is alarming to climate change results from Environmental pollution.

PHYTOCHEMICAL STUDY

Preliminary Phytochemical screening (Qualitative test): Aqueous extracts of NPS exhibited presence of carbohydrate, glycosides, phenols, flavonoid, terpenoid, saponin and sterol in the sample extract. However, alkaloid and tannin were not found in the aqueous extract of NPS.

Figure No 26: Phytochemical screening



Phytochemical screening: Hydroethanolic (A) Aquous (B) extracts of NPS rhizomes

Carbohydrate, protein, flavonoid, sterol and terpenoid were moderately shown in the extract. Protein, saponin and phenol were found in trace amount (Table 23).

Table No. 23: Phytochemical screening of NPS rhizomes extracts

Class of Compounds	Aqueous
Glycosides Phenols	+ve
Flavanoids	++ve
Alkaloids	-ve
Carbohydrates	++ve
Sterol/steroid	++ve
Terpenoid	++ve
Proteins	+ve
Glycoside	++ve
Saponin	+ve

DARUHARIDRA

Berberis aristata

Morphological characters: B. aristata is a shrub or small tree grows in sub-tropical region of Nepal.

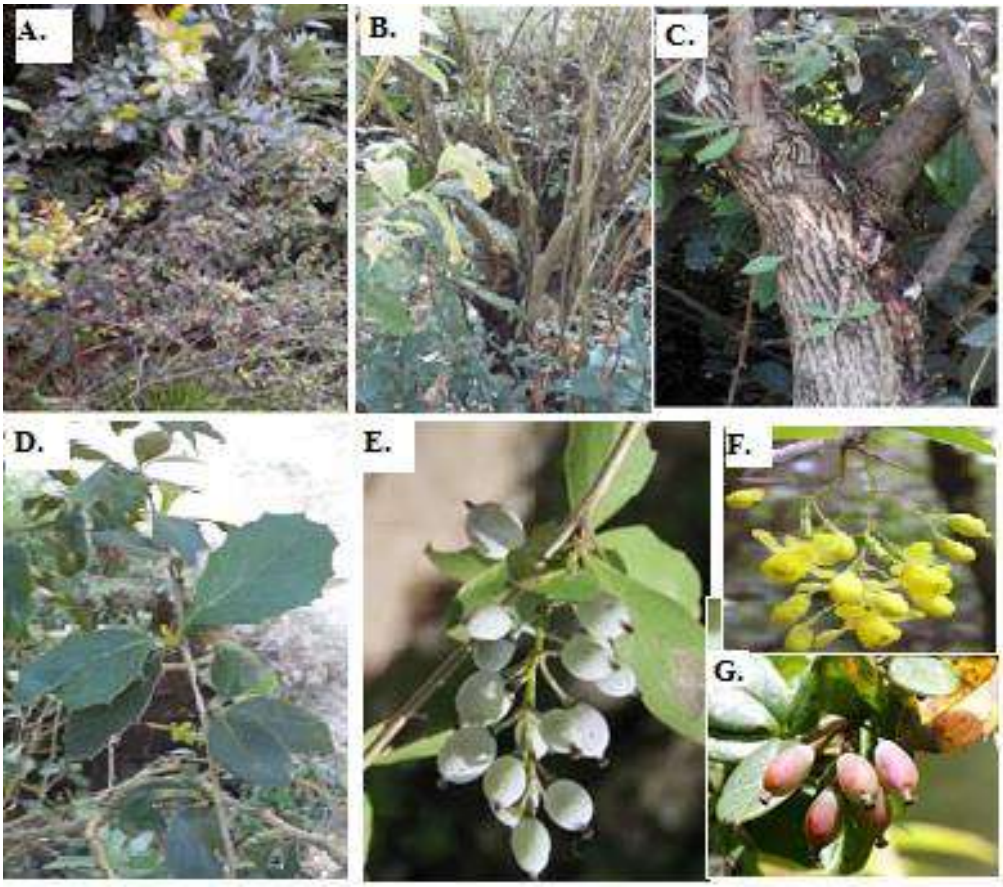


Figure-28: Bush of Daruharidra (A), Plant with stem (B), Woody stem (C), Leaf (D), Fruit green (E), Flower (F) and Fruit ripen (G)

A shrub usually 2 m. with arching pale yellow branches; spines absent or with 1-3 branches of 4-10 mm (A, B & C). Leaves: usually elliptic often spineless leaves; 2.5 - 5cm(D); Florescence, cymes (F); Flower: Short-stalked clusters of numerous yellow flowers much longer than the leaves; 6 mm long (F); Fruit: dark red, finally blue-purple, 6-8mm; style very short (E & G); Seed: small, globose.

Morphological characteristics of *B. aristata*

The morphological characters of the species were found as mentioned below:

Habit:- Perennial shrub, upto 15 m in height found in sub-tropical region.

Leaf:- Simple, sub sessile, broad, obovate, spines on leaf margin, opposite.

Inflorescence:-Raceme

Flowers: - Yellow in colour, axillary in cluster

Fruit: - bright red, ovoid and globulose

Seeds: - Several seeds

Organoleptic parameters:

The root and its powder characters were separately evaluated by organoleptic characters like surface, colour, odour, taste in table no. 24.

Table-25: Organoleptic parameters

Colour:	Outer- whitish grey, inner yellow
Odour:	Not detected
Taste:	Acrid
Sound:	NA
Shape:	Cylindrical; nodular
Size:	Variable

The traditional Ayurvedic method for identification of drugs like *Nama, Rupa, Guna,* etc. was also studied separately.

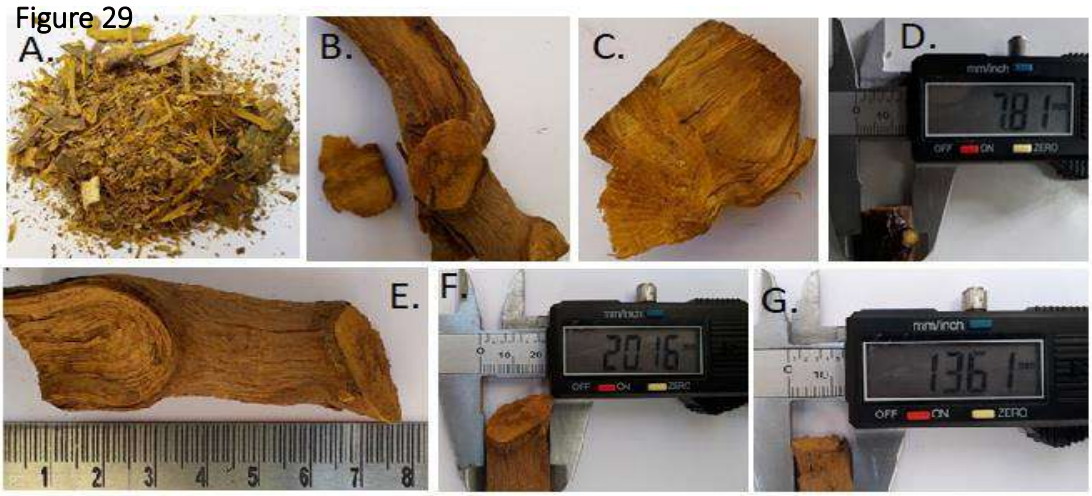
RUPA (Structure): *Daruharidra* (Yellow like Haridra), Rohini (Yellow in colour)

GUNA (Properties): *Krimihara* (Anthelmintic), *Visodhani* (Blood purifier)

Macroscopic examination

Macroscopic characteristics: Drug available in pieces of variable length and thickness, bark about 0.4 - 0.8 cm thick, pale yellowish-brown, soft, closely and rather deeply furrowed, rough, brittle, xylem portion yellow, more or less hard, radiate with xylem rays, pith mostly absent, when present small, yellowish- rown when dried, fracture short in bark region, splintery in xylem; taste, bitter.

Figure 29



Berberis aristata DC. root: Bark (A), Root (B), Root cut piece (C), Root small piece diameter (D), Root cut length (E), Thick Root diameter (F) and Medium root diameter (G) in mm

PHYSICO-CHEMICAL EVALUATION

Determination of Foreign Matter

The foreign matter was found 3% in BA root and presented in table no. 26. It is the higher than the recommended value (<2%) in Ayurvedic Pharmacopiea of India.

Table No. 26: Determination of Foreign Mater in BA root

Samples for	Percentage of Foreign Matter
	Dried Rhizomes of BA (%)
Foreign Matter	3

Determination of Loss on Drying (LoD)

The percentage of loss on drying was observed 1.45 % in BA root and shown in table no 27. It isn't recommended value in Ayurvedic Pharmacopiea of India.

Table No 27: Determination of Loss on Drying in BA root

Samples for	Percentage Loss on Drying
	Dried rhizomes of BA (%)
Loss on Drying	1.45

Determination of Total Ash, Acid-insoluble Ash and Water-soluble Ash

The percentage total ash (0.109 %), acid-insoluble (0.005%) and water-soluble ash (0.223%) values obtained from BA root shown in Table No 28. It is lesser than the recommended values for total ash (<14%) and less than the recommended acid-insoluble (<5%) value; and water soluble (not mentioned) in Ayurvedic Pharmacopiea of India.

Table No. 28: Determination of Total Ash Values BA root powder

Parameters	Values in Percentage (w/w)
	Dried Rhizomes of BA (%)
Total ash	0.109
Acid-insoluble ash	0.005
Water-soluble ash	0.223

Determination of PH Values

The pH values of 1% of BA root powder were observed for 6.50 shown in the table No. 30 below. It isn't mentioned by Ayurvedic Pharmacopiea of India.

Table No. 30: Determination of PH Values of BA root powder

Solution of Powder Drugs	PH expressed in Mean ± SEM
	BA root powder solution
1% Solution (w/v)	6.5

EXTRACTIVE VALUES

Cold Extractive Value: Extraction process of BA root was carried out in distilled water (aqeous).

Percolation Method: The extractive values for BA root powder in the solvent such as ethanol and distilled water were found to be 1.79% and 7.83% respectively shown in Table No. 31. Ayurvedic Pharmacopiea of India recommendes more than 8% and 6% in aqueous solvents and ethanolic respectively.

Table No. 31: Extractive values of BA root

Solvents	Extractive Values (Percolation Method) % Yield
	BA root powder solution
Ethanol	1.75
Aqueous	7.83

MICROBIAL LOADS:

Cold extract of BA root powder was carried out evaluating total microbial and fungal loads and further was investigated for specific microbial load in the sample by liquid froth agar method. Total count of microbial loads was less than detectable limit (LTDL) whereas total fungal count was 21983 in the sample. E. coli, Salmonella spp., Clostridium spp., Enterobacteria, Pseudomonas aeruginosa, Shigella spp. and Staphylococcus aureus were not tested in the sample because of LTDL of total microbial count. The WHO limit of microbial load was recommended for total microbial and fugal load to be not exceeded Max. 10^7 CFU/gm and Max. 10^4 CFU/gm respectively. In the same way, API (2016) has recommended limit of total microbial

load and total fungal count to be not exceeded Max. 10^7 CFU/gm and Max. 10^3 CFU/gm in raw materials respectively as shown in Table 32.

Table No. 32: Microbial load in BA root powder

Parameters	Microbial load (CFU/gm)		
	Dried BA root	Acceptable Limit	
		WHO (2007)	API (2016)
Total bacterial count	LTDL	Max. 10^7	Max. 10^7
Total fungal count	NT	Max. 10^4	Max. 10^3
Clostridium spp.	NT	Absent	Not mentioned
Enterobacteria	NT	Max. 10^3	Not mentioned
Escherichia coli	NT	Max. 10^2	Absent
Pseudomonas aeruginosa	NT	Not mentioned	Absent
Salmonella spp.	NT	Absent	Absent
Shigella spp.	NT	Absent	Not mentioned
Staphylococcus aureus	NT	Not mentioned	Absent

The limit for E. coli in API should be absent and Max. 10^2 CFU/gm in WHO; Salmonella spp. should be absent in both API and WHO guidelines. API hasn't limit for Clostridium spp., Enterobacteria and Shigella spp.; and Pseudomonas aeruginosa and Staphylococcus aureus must be absent in the herbal raw materials. Furthermore, WHO hasn't determined limit for Pseudomonas aeruginosa and Staphylococcus aureus in its guidelines. However, other microbial of the table should be free from raw materials shown in the table 32.

HEAVY METALS, PESTICIDES AND AFLATOXINS: The heavy metals commonly recommended for evaluation of safety use are Mercury, Lead, Cadmium and Arsenic. Among these heavy metals, it was observed that 0.04 ppm, 0.08 ppm, 0.08ppm and 0.08 ppm were mercury, lead, arsenic and cadmium respectively. Among the pesticides, 4,4, DDT and Parathion are most commonly recommended for evaluation of residues in raw materials. Aflatoxin is one of the most important toxin found in old raw materials. In the sample of BA root powder, pesticides such as 4,4 DDT and Parathion were found not detected. Total aflatoxins were also absent in the sample shown in the table 33. These contaminants are accumulated in the various tissues of the body and ultimately causes damage to the organs. It can be said that fresh raw materials and collected from remote area may not be contaminated with heavy metals, pesticides and aflatoxins.

Table No. 33: Heavy Metals, Pesticides and Aflatoxins in BA root powder

Parameters	Heavy Metals, Pesticides and Aflatoxins				
	Heavy metals (ppm)	Pesticides (mg/kg)	Aflatoxins (ppb)	Acceptable Limit	
				WHO (2001)	API (2016)
Mercury (Hg)	<0.04			0.03	1
Lead (Pb)	<0.08			10	10
Arsenic (As)	<0.08			3	3
Cadmium (Cd)	<0.08			0.3	0.3
4,4, DDT		ND		1	
Parathion		ND		0.5 mg/kg	NA

Note: ND = Not Detected in the test

Generally, area nearby metropolitan city is more affected by primary pollutants. However, the findings of heavy metals in BA sample collected from 1315 masl of Dhading district has less affected than the sample collected from remote area.

PHYTOCHEMICAL STUDY

Preliminary Phytochemical screening (Qualitative test): Aqueous extracts of BA root exhibited abundantly presence of glycosides, phenols, alkaloids whereas terpenoid, saponin and phenol were moderately present in the extract sample. Protein and tannin were not detected whereas carbohydrate was present in trace amount in the extract.

Figure 30

However, these class of compounds alkaloids was strongly exhibited their presence in extracts.

Table No. 34: Phytochemical screening of BA root extracts

Class of Compounds	Aqueous
Phenols	++ve
Flavanoids	+++ve
Alkaloids	+++ve
Carbohydrates	+ve
Sterol/steroid	+ve
Terpenoid	++ve
Proteins	-ve
Glycoside	+++ve
Saponin	++ve

Sterol was exhibited trace in amount in the extracts shown in Table 34 above.

GUDUCHI

Tinospora cordifolia

Morphological characters: T. cordifolia is a perennial climber grows in tropical region of Nepal.

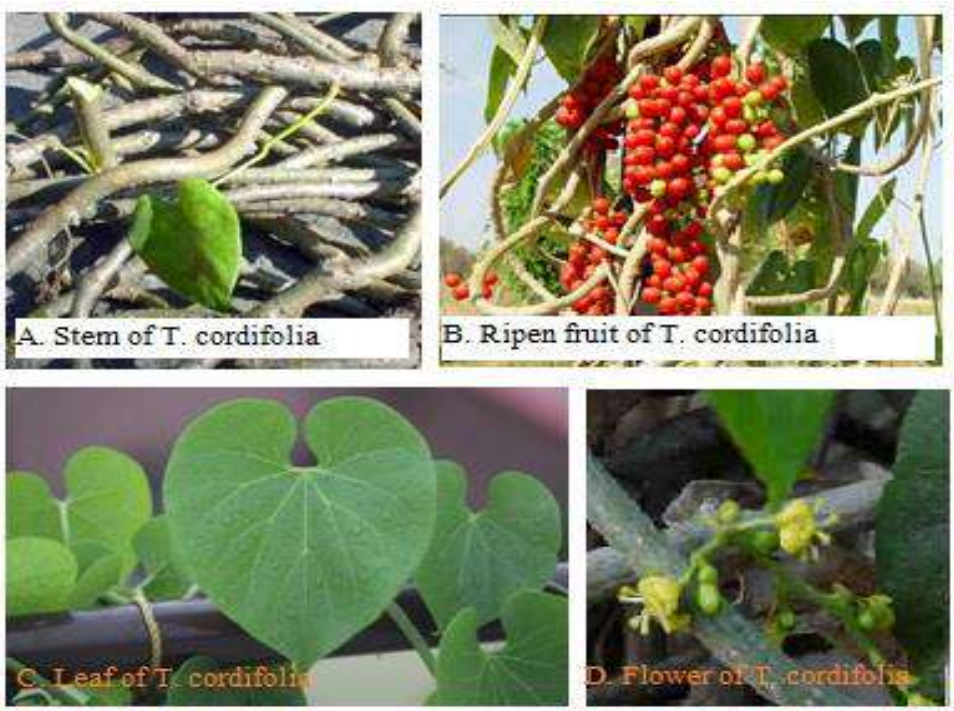


Figure-32: Stem of T. cordifolia (A), Ripen fruit of T. cordifolia (B), Leaf of T. cordifolia (C), and Flower of T. cordifolia (D)

climbing shrub with heart-shaped leaves; stem diameter 6 cm, light grey with papery bark; leaves: alternate, 7.5-14 cm long, 9-17 cm broad, broadly ovate or orbicular, deeply heart shaped at the base; Inflorescence: racemes 7-14 cm long; flower: tiny greenish yellow flowers; 3+3 sepals in 2 layers, the outer; small, the inner large; stamens 6; prominently protrude out; seed: lunar shape; many, flowering; summer and fruiting; winter.

Morphological characteristics of *T. cordifolia*

The morphological characters of the species were found as mentioned below:

Habit: - Perennial climber, upto 25 m in height found in tropical and sub tropical region.

Leaf: - Simple, petiolate, cordate, broad, deeply cut inside at base.

Inflorescence: -spike

Flowers: - Yellow in colour, axillary or in cluster

Fruit: - bright red, globose, berry like

Seeds: -Many, semilunar

Organoleptic parameters:

The stem and its powder characters were separately evaluated by organoleptic characters like surface, colour, odour, taste in table no. 35.

Table-36: Organoleptic parameters

Colour:	Greenish white or light grey bark, inner whitish and yellowish sappy wood
Odour:	Woody
Taste:	Bitter
Sound:	
Shape:	Elongated, cylindrical
Size:	Variable

The traditional Ayurvedic method for identification of drugs like *Nama, Rupa, Guna,* etc. was also studied separately.

Rupa (Structure): *Chakralakshanika* (Spike loike structure inner cut surface), Tantrika (Rope like structure)

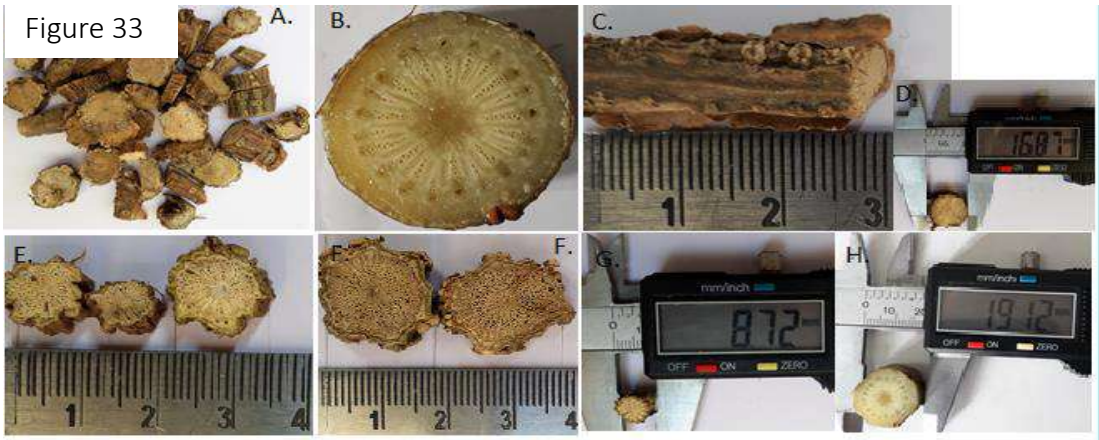
Rasa (Taste): Madhuparni (Viscid juice of leaf like honey)

Guna (Properties): Rasayani*, Visagni, Vayahstha, Jivanti*

B. Macroscopic characteristics

Drug occurs in pieces of varying thickness ranging from 0.6-5 cm in diameter, young

Figure 33



Tinospora cordifolia (Willd.) Miers: Small cut pieces of matured stem (A), Fresh matured stem (B), Dried stem length (C), Medium size diameter (D), Medium, small and large stem cut diameter (E), Cut piece of matured stem (F), Small (G) and Large (H) cut stem diameter (H)

stems green with smooth surfaces and swelling at nodes, older ones show a light brown surface marked with warty protuberances due to circular lenticels, transversely

smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues, taste bitter in figure 33.

PHYSICO-CHEMICAL EVALUATION

Determination of Foreign Matter

The foreign matter was found 2% in TC stem and presented in table no. 37. It isn't recommended in Ayurvedic Pharmacopiea of India.

Table No. 37: Determination of Foreign Mater in TC stem

Samples for	Percentage of Foreign Matter
	Dried Rhizomes of TC (%)
Foreign Matter	2

Determination of Loss on Drying (LoD)

The percentage of loss on drying was observed 1.34% in TC stem and shown in table no 38. It isn't recommended value in Ayurvedic Pharmacopiea of India.

Table No 38: Determination of Loss on Drying in TC stem

Samples for	Percentage Loss on Drying
	Dried rhizomes of TC (%)
Loss on Drying	1.34

Determination of Total Ash, Acid-insoluble Ash and Water-soluble Ash

The percentage total ash (0.215%), acid-insoluble (0.007%) and water-soluble ash (0.134%) values obtained from TC stem shown in Table No 39 below. It is lesser than the recommended values for total ash (<3%) and less than the recommended acid-insoluble (<2%) value; and water soluble (not mentioned) in Ayurvedic Pharmacopiea of India.

Table No. 39: Determination of Total Ash Values TC stem powder

Parameters	Values in Percentage (w/w)
	Dried Rhizomes of TC (%)
Total ash	0.215
Acid-insoluble ash	0.007
Water-soluble ash	0.134

Determination of PH Values

The pH values of 1% solution of TC stem powder was observed for 6.60 shown in the table No. 40 below. It isn't mentioned by Ayurvedic Pharmacopiea of India.

Table No. 40: Determination of PH Values of TC stem powder

Solution of Powder Drugs	PH expressed in Mean
	TC stem powder solution
1% Solution (w/v)	6.6

EXTRACTIVE VALUES

Cold Extractive Value: Extraction process of TC stem was carried out in distilled water (aqeous) and a mixture of water and ethyl alcohol.

Percolation Method: The extractive values for TC stem powder in the solvent such as ethanol and distilled water were found to be 1.69 % and 6.83% respectively shown in Table No. 41 below. Ayurvedic Pharmacopiea of India recommendes more than 3% and 11% in hydro-ethanolic and aqueous slovents respectively.

Table No. 41: Extractive values of TC stem

Solvents	Extractive Values (Percolation Method) % Yield
	TC stem powder solution
Ethanol	1.69
Aqueous	6.83

MICROBIAL LOADS:

Cold extract of TC stem powder was carried out evaluating total microbial and fungal loads and further was investigated in the sample by liquid froth agar method. Total count of microbial and fungal loads was 8265286 CFU/gm and 33858 CFU/gm respectively. E. coli and Salmonella spp., Clostridium spp., Enterobacteria, Pseudomonas aeruginosa, Shigella spp. and Staphylococcus aureus were not tested in the sample because of lesser than the limit recommended by WHO. The WHO limit of microbial load was recommended for total microbial and fugal load to be not exceeded Max. 10^7 CFU/gm and Max. 10^4 CFU/gm respectively. In the same way, API (2016) has recommended limit of total microbial load and total fungal count to be not exceeded Max. 10^7 CFU/gm and Max. 10^3 CFU/gm in raw materials respectively.

Table No. 42: Microbial load in NPS rhizomes powder

Parameters	Microbial load (CFU/gm)		
	Dried Kutaki rhizomes	Acceptable Limit	
		WHO (2007)	API (2016)
Total bacterial count	8265286	Max. 10^7	Max. 10^7
Total fungal count	33858	Max. 10^4	Max. 10^3
Clostridium spp.	NT	Absent	Not mentioned
Enterobacteria	NT	Max. 10^3	Not mentioned
Escherichia coli	NT	Max. 10^2	Absent
Pseudomonas aeruginosa	NT	Not mentioned	Absent
Salmonella spp.	NT	Absent	Absent
Shigella spp.	NT	Absent	Not mentioned
Staphylococcus aureus	NT	Not mentioned	Absent

The limit for E. coli in API should be absent and Max. 10^2 CFU/gm in WHO; Salmonella spp. should be absent in both API and WHO guidelines. API hasn't limit for Clostridium spp., Enterobacteria and Shigella spp.; and Pseudomonas aeruginosa and Staphylococcus aureus must be absent in the herbal raw materials. Furthermore, WHO hasn't determined limit for Pseudomonas aeruginosa and Staphylococcus aureus in its guidelines. However, other microbial of the table should be free from raw materials shown in the table 42 above.

HEAVY METALS, PESTICIDES AND AFLATOXINS: The heavy metals commonly recommended for evaluation for safety use are Mercury, Lead, Cadmium and Arsenic. Among these heavy metals, it was observed that 0.08 ppm, 0.40 ppm, 0.09 ppm and 0.08 ppm were mercury, lead, arsenic and cadmium respectively. Among the pesticides, 4,4, DDT and Parathion are most commonly recommended for evaluation of residues in raw materials. Aflatoxin is one of the most important toxin found in old raw materials. In the sample of BA root powder, pesticides such as 4,4 DDT and Parathion were found ansent. Total aflatoxins were also absent in the sample. These contaminants are accumulated in the various tissues of the body and ultimately causes damage to the organs. It indicates that fresh raw materials and collected from remote area may be lesser contaminated with heavy metals, pesticides and aflatoxins shown in the table 43 below.

Table No. 43: Heavy Metals, Pesticides and Aflatoxins in TC stem powder

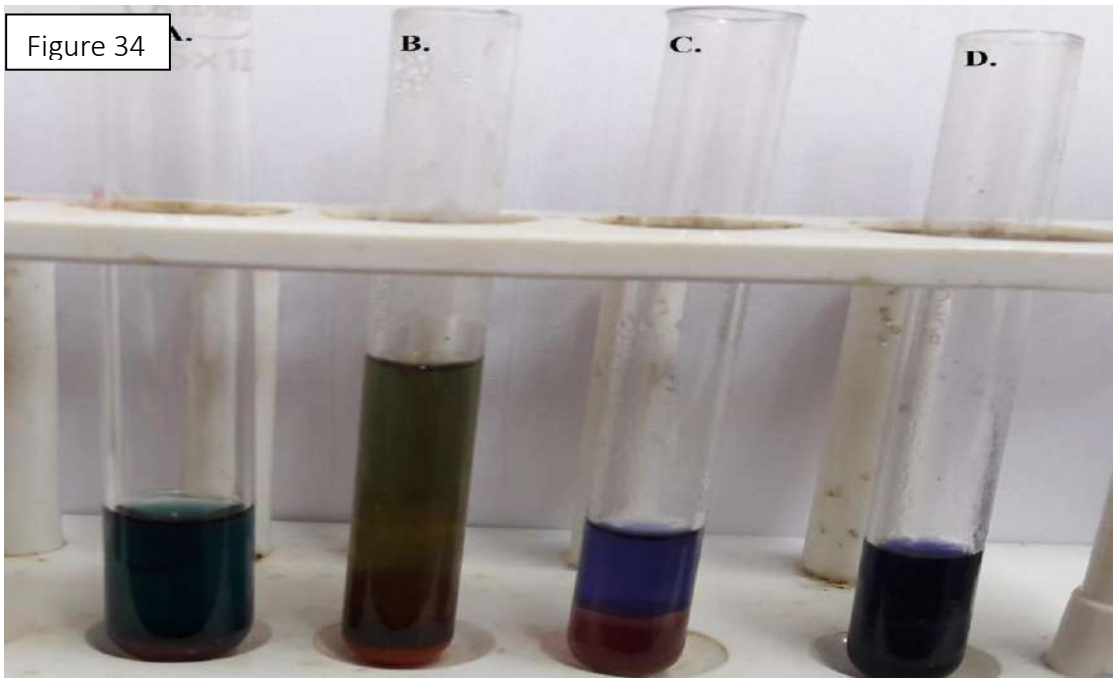
Parameters	Heavy Metals, Pesticides and Aflatoxins

	Heavy metals (ppm)	Pesticides (mg/kg)	Aflatoxins (ppb)	Acceptable Limit	
				WHO (2001)	API (2016)
Mercury (Hg)	0.08			0.03	1
Lead (Pb)	0.40			10	10
Arsenic (As)	0.09			3	3
Cadmium (Cd)	0.08			0.3	0.3
4,4, DDT		Absent		1	
Parathion		Absent		0.5 mg/kg	NA

PHYTOCHEMICAL STUDY

Preliminary Phytochemical screening (Qualitative test): Aqueous extracts of TC stem exhibited abundantly presence of glycosides and terpenoid whereas flavonoid, steroid and saponin were moderately present in the sample. Carbohydrates and phenol were present in trace in amount whereas alkaloid and protein were absent from the sample in Table 44.

Figure 34



TC is well known for glycoside and used as tridosha samaka.

Table No. 44: Phytochemical screening of TC stem extracts

Class of Compounds	Aqueous
Phenols	+ve
Flavanoids	++ve
Alkaloids	-ve
Carbohydrates	+ve
Sterol/steroid	++ve
Terpenoid	+++ve
Proteins	-ve
Glycoside	+++ve
Saponin	++ve

CHAPTER-I: DISCUSSION

Soil: However, it may be the required condition of soil for growth of the Berberis aristata at Dhading, Nepicrorhiza scrophulariiflora at Manang and Tinospora cordifolia at Parsa districts.

Carbon and nitrogen are major components of soil organic matter (Brady et al., 2008). Organic matter is important for many soil properties, including structure formation and maintenance, water holding capacity, cation exchange capacity, and for the supply of nutrients to the soil ecosystem (Brevik, 2009, Brevik and Burgess, 2013). Soils with an adequate amount of organic matter tend to be more productive than soils that are depleted in organic matter(Brevik, 2009), therefore, one of the biggest questions concerning climate change and its effects on soil processes and properties involve how potential changes in the C and N cycles will influence soils.

Early expectations were that increased atmospheric CO_2 would lead to increased plant productivity coupled with increased C sequestration by soil, meaning increased plant growth and the soil-plant system would help offset increasing atmospheric CO_2 levels (Coughenour and Chen, 1997, Hättenschwiler et al., 2002). This increase in plant growth is known as the CO_2 fertilization effect. However, recent studies indicate the CO_2 fertilization effect may not be as large as originally thought (Poorter and Navas, 2003, Zavaleta et al., 2003, Long et al., 2005, Körner, 2006, Zaehle and Friend, 2010). Increasing levels of ozone as the climate changes may actually counteract the CO_2 fertilization effect leading to reduced plant growth under elevated CO2(Long et al., 2005) and the negative effects of increased temperatures on plant growth may also cancel out any CO_2–fertilization effect that does take place. Nitrogen limitations may negatively affect plant growth(Hungate et al., 2003), and modeling of C dynamics as influenced by N indicates less C sequestration by soil than originally expected given CO_2 fertilization (Zaehle and Friend, 2010). A long-term elevated CO_2 experiment in a grasslands ecosystem indicated that N and P became limiting within two years, again limiting plant biomass response to elevated CO_2 (Niklaus and Körner, 2004). Niklaus and Körner(Niklaus and Körner, 2004) concluded that the increases in plant productivity they did see were due primarily to soil moisture status as opposed to a CO_2 fertilization effect. Experiments looking at the decomposition of plant tissues

grown under elevated atmospheric CO_2 also indicate that increased levels of CO_2 are emitted during that decomposition(Kirkham, 2016), and research by Carney *et al*. (Carney et al., 2007) observed soil organic C levels declining under increased atmospheric CO_2 levels due to increased microbial activity. Therefore, elevated CO_2 levels will not necessarily lead to increased soil C sequestration, but may instead result in more C turnover(Eglin et al., 2011).

Increased temperature is likely to have a negative effect on C allocation to the soil, leading to reductions in soil organic C and creating a positive-feedback in the global C cycle (increased temperatures lead to increased CO_2 release from soils to the atmosphere, which leads to more increases in temperature) as global temperatures rise(Gorissen et al., 2004, Wan et al., 2011). In a study of soils in a semi-arid steppe, Link *et al*.(Link et al., 2003) observed that soil warming and drying led to a 32% reduction in soil Carbon over a five year time period, a much more rapid reduction in soil Carbon than reductions that have been observed due to increased tillage. Modeling of Carbon responses to climate change in Canada predicted small increases in above ground biomass in forest and tundra ecosystems but larger decreases in soil and litter pools, for an overall increase in atmospheric Carbon(Price et al., 1999). Another modeling study predicted decreases in soil organic C of 2.0%–11.5% in the North Central United States by 2100 as compared to 1990 C values(Grace et al., 2006). It is important to keep in mind that in all these cases, the soil would only be a net source of Carbon to the atmosphere until a new equilibrium was reached.

When CO_2 enrichment increases the soil C:N ratio, decomposing organisms in the soil need more N(Nitrogen), which can reduce N mineralization(Gill et al., 2002, Reich et al., 2006a). Mineralization is an essential step in supplying N to plants(Brevik, 2013b). Therefore, if N mineralization is reduced, it would be expected that plant-available N levels in the soil would also be reduced and plant productivity would be negatively affected.

However, the stimulated C uptake is not enough to offset the N limitation and the net result is still an increase in atmospheric CO_2 and an overall reduction in soil C levels(Holland, 2011). Some researchers have reported that increasing temperatures increase N mineralization (Norby and Luo, 2004, Joshi et al., 2006, Reich et al., 2006b), which could have a positive effect on plant growth. However, a warming

study by An *et al*. (An et al., 2005) showed that N mineralization was stimulated in the first year but depressed afterward.

Livelihood Vulnerability Index: Previous studies have not been conducted to estimate livelihood vulnerability in these districts. At a first glance, the preceding suggests that vulnerability differences between the three districts were minimal except in some major components. However, upon closer inspection, the analysis reveals a number of subtle, yet important, differences between these districts. These are now discussed in greater detail. According to the findings, Dhading and Manang districts had a small range of agricultural livelihoods and more HHs with some dependence on nonagricultural livelihood income. Parsa district had more HHs with members working outside the community. In last decade, HHs in Manang district had continually moved in a direction of specializing in singular livelihoods (e.g. herbal farming and wild harvesting or forestry), usually one of the most income potential and/or relying on the available natural resources of area.

In contrast to Parsa, Dhading has experienced less economic change. The geographical condition of these community is fragmented and roads are in very poor condition that makes transportation very difficult, especially in the rainy season leading to geographical isolation. This is also a cause that hinders the seeking of help from the local government. Transportation is one of the indicators that increase the vulnerability of Dhading district. Although the implementation of the new rural program in Dhading has been implemented in the last few years, the effectiveness of the program in this area is still limited. Therefore, the government should continue to accelerate the new rural program, combining propaganda and mobilization of people in the construction of welfare facilities, donate land to make roads. The diversification of livelihood strategies is still low. Agricultural livelihood activities here are mainly cultivation. Other livelihood activities (e.g husbandry, vegetable gardening) are fragmented with the primary purpose for HH consumption. Unlike Dhading and Manang districts, Parsa district has the potential to develop a wide range of agricultural livelihoods i.e. cultivated tea, coffee, vegetable, fruit, husbandry, etc. Land tenure of farmers in Parsa is high. Many projects of governmental and nongovernmental organizations on livestock are implemented here. The economic structure transformation is urgent in Manang district to reduce food insecurity.

Government should focus on helping farmers here to make a transition from monoculture to economic diversification, with a focus on herbal farming and wild collection; that is the region's advantage. Although there is a significant reliance on non-timber forest products (NTFPs) for cash income, there is also widespread reporting of degradation of forest ecosystems due to illegal logging and overexploitation. Similar activities have been reported from Parsa district. This increases the vulnerability of natural resources and sensitivity to the climate change of Parsa and Manang communities. Percentage of those HHs with cultivation of the 3rd crop in Manang and Dhading had higher vulnerability than Parsa communities due to land with no access to irrigation (usually further from a water source). Farmers can only produce one crop a year as the production is highly dependent on the timing of the rains. The findings from Manang suggests higher vulnerability to health shocks than two districts, indicating that the average time of travel to health facilities is an important determinant of vulnerability. According to the data, it took thrice the time to travel to the nearest health facility in Manang as it did in Parsa, reflecting the lack of serviceable roadways connecting the Manang communities to the nearby towns. At night, the lack of street lighting makes the situation even more dire. Health vulnerability therefore reflects a lack of viable transportation options as opposed to a lack of healthcare facilities. In order to reduce the vulnerability of health, the government should develop policies for the development of health facilities, and strengthen the village health worker system.

Another factor was the access to clean drinking water. In this study area, access to safe water was nonexistent in Manang and limited in Dhading (20.1%) and Parsa (17.3%), although a clean water program has been widely implemented in the rural areas all over the country. Main water sources for crops and livestock are natural resources. Rainwater and wells in the dry season supplement this supply in Parsa district. Water vulnerability was therefore highly dependent on the national water resource authorities and infrastructure development plans for the area. By natural vulnerability and climate variation on HH vulnerability, the results suggest that levels of vulnerability in Parsa were the highest. The cause of the vulnerability is due to the extensive exploitation of natural resources and there was no plan for conservation of natural resources. The limited finance and cultural characteristics of ethnic minority

housing in the region that the material is mainly wood or bamboo. In addition, most residential houses in this area have been built without technical guidance or instruction from professionals in terms of disaster resistance. It was found similar to the previous study conducted in Vietnam (Tran et al. 2012). These results agreed that housing reinforcement for poor or low-income HHs is necessary to build their resilience, improve their living conditions, and help them escape from poverty (Huong et al., 2019, Panthi et al., 2016). Terms of land ownership is an important indicator of vulnerability(Hahn et al., 2009, Dechassa et al., 2017).

The cause of the similarity of knowledge and skills vulnerability in Dharapani rural municipality, Dhunebeshi municipality and Madhuwan Mithaul rural municipality communities is the percentage of HH head unlimited for learning behaviour. There is unlimited education policy in Nepal at the present time, which only concerns education universalization for school going children, university education and adult education for those not attended school or university. However, programs and policies support education, literacy education materials are not suitable with the reality of life and culture of ethnic minorities. This is also a factor leading to lowest percentage of HH where a family member has not taken any kind of vocational training. The government should undertake reforms and modifications of education policies for ethnic minorities. The immediate goal of educational modification is to eliminate illiteracy for local people, followed by the training of capacities and skills to improve their adaptive; and reduce vulnerability to climate change. The quality of labor in these communities has been constrained by low skills and inadequate experience. Women's skills in rural areas are an important contributor to rural development because of the vital role women play in farming activities and in running the HH. Unfortunately, particularly in ethnic minority communities, they often may not participate in vocational training courses because of the community's preconceived notions about gender roles. This result shows that interventions such as improvement of education level can reduce the vulnerability to climate variability. Higher vulnerability in Madhuwan Mithaul community is primarily a result of higher vulnerability on natural vulnerability and climatic variation, social networks and water capitals. Poverty and vulnerability are closely interlinked and poor HHs without potential to escape poverty are also characterized as vulnerable(Fischer, 2011). There

are several explanations to financial vulnerability. A complex administrative system and language difficulties are further constraints faced particularly by members of ethnic minority groups. Formal loans are almost informal credit entirely for the production and asset accumulation(Fischer, 2011). The main reasons why formal finance is rarely used to ease shocks due to climate change, however, is that it takes time to apply for a loan and HHs are locally screened; any income or consumption shocks may be reported to the relevant credit officer and the credit is consequently denied. Social networks as one part of the HH's social capital assets are able to provide basic support, so that these capitals should be taken into account for reducing livelihood vulnerability of these villages. Also, effects of climate change on flood, reduced access to roads, and the availability of aid were important.

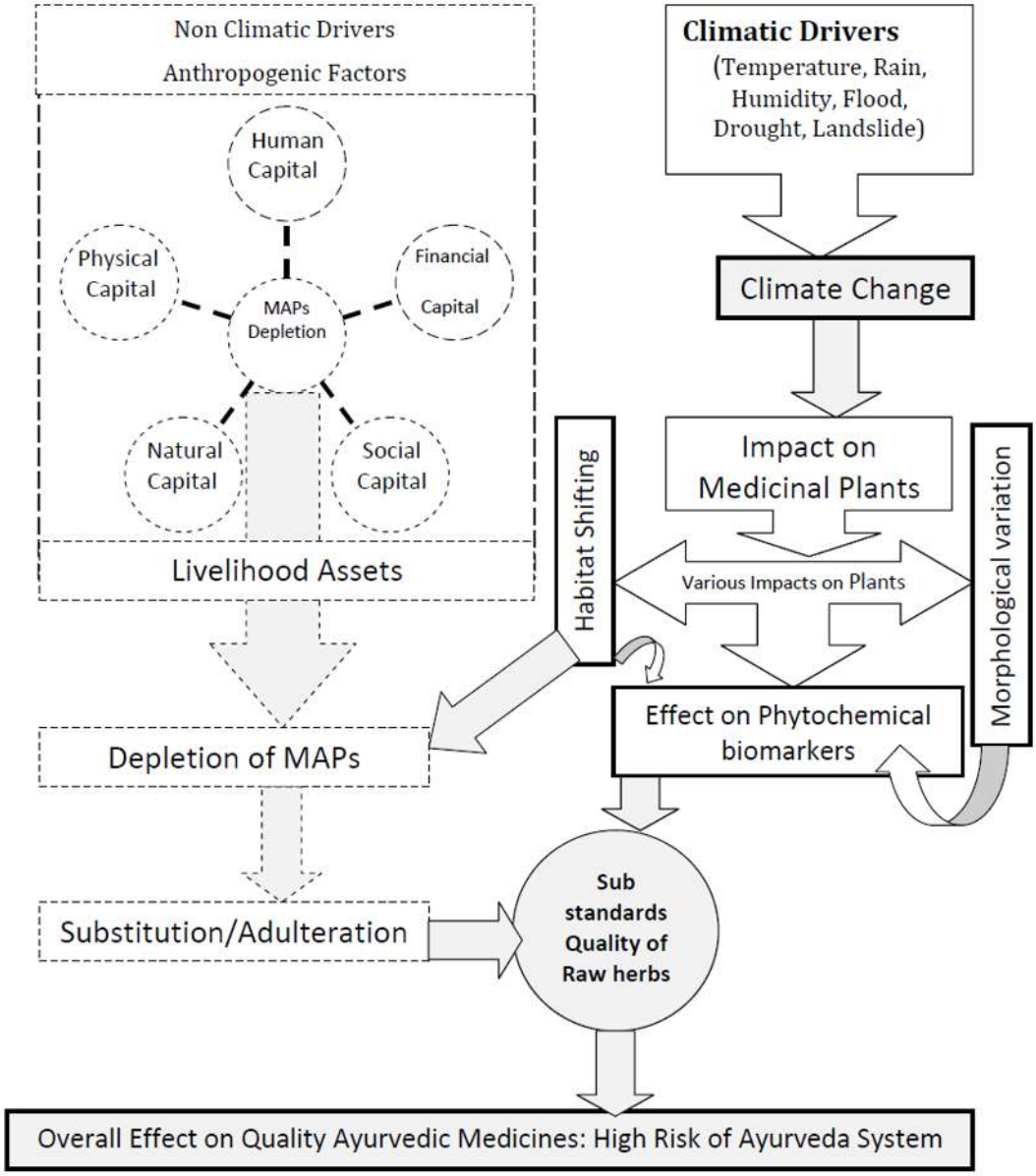
Rainfall and Temperature: Water resource has the prime concern for any future planning and development including flood control, flood protection and sustainable watershed management. The rainfall available in the watershed is key factor for determining the availability of water to fulfil the different demand mainly for agriculture, hydropower water supply, industry, etc. The timely availability of water influences the agriculture sector, food security and energy sector. Global climate changes affect the long-term rainfall pattern causes availability of water and may danger of occurrence of serious drought and flood. The high instantaneous rainfall in monsoon(June-August) may have shortage of water

The results shown in Table 12 indicated a rising trend of annual mean minimum temperature in the Bara district. An increasing trend with highly significant minimum temperature in the premonsoon and post-monsoon season will increase the duration of the summer season and enhance the global warming(Parthasarathy and Dhar, 1974), however, a lesser significant rising minimum temperature during monsoon and winter may decrease the length of crop period without reaching to its maturity hence reduce the yield. An increasing minimum temperature will support the global warming causing climate change(Kharmeshu, 2012). This suggests that temperature trend shows rising and due to this rising temperature, other climatic variables may experience affected in the hydrologic processes and surrounding environment of the district (Rao, 1993). The minimum temperature variability during the period 2009-

2019 is presented in Figure 18. It also indicated the linear trend line overlaid on the time series for different months and seasons.

The results shown in Figure 18. indicated a rising trend in the months of May, June and August, October and December, whereas in the months of January, March, July

Figure 36



Diagrammatic representation of Conceptual Framework of Impact of Climate Change on Medicinal Plants

Physico-chemical evaluation: The foreign matter in Kutaki was found lesser than recommended value in API; and the foreign matter in Daruharidra and Guduchi was observed below the limit. The percentage of loss on drying (LoD) was observed higher in *Kutaki* rhizomes than Guduchi stem and Daruharidra stem. The percentage total ash, acid-insoluble and water-soluble ash values obtained from Kutaki rhizomes, Guduchi stem and Daruharidra root was below the limit recommended in API. These findings also support that the study samples contain less inorganic foreign matters. The pH of three study samples was found slight acidity in nature which is good for internal consumption.

Phytochemical screening: Ethanolic and Aqueous extracts of the study samples were exhibited presence of carbohydrate, amino acid, glycosides, phenols, alkaloids and sterol in the extract.

Estimation of bioactive compounds: Quantification of berberine and picrosides I was found above the limit and the sample materials were considered standards for medicinal use.

Heavy metals and microbial contamination: Plant samples collected from urban and metropolitan city like Birgunj and Kathmandu showed lesser level of heavy metals than the sample collected from remote and high altitude. Microbial contamination was observed higher in the sample collected from rural area of central terai region which is because of poor hygiene and sanitation among them. Hence, plant materials collected from Manang district showed higher contamination with heavy metals.

CHAPTER-X: CONCLUSION & RECOMMENDATION

Impact of Environmental changes on medicinal plants observes in depletion of plants and shifting at higher altitudes. On the other hand, anthropogenic pollution is affecting plants diversity and soil by heavy metals and pesticides residues toxicity and is restricting medicinal plants for medical uses. A remote and rural area have been found safe for medicinal plants cultivation and wild collection where pollution has been recorded insignificant.

Overall, Environmental changes markedly affects on survival and quality of medicinal plants and ultimately a great threat to Ayurveda system of medicine in the future.

Recommendation:

- Greenery is major strategies to mitigate adervse effect of climate change.
- Medicinal plants must be collected or cultivated in unused land or far way from industrial area.
- Industrial area is considered life threating to biodiversity.
- Fresh raw materials are safe to human use.
- This data can be used for standardization of raw materials or pharmacopieal use.

CHAPTER-IX: REFERENCES

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CHAPTER-XII: ANNEX

Annex-1: Estimation of LVI of Dhunebeshi Municipality, Dhading

Major Components	SN	Sub-components	Unit	Observed value	Max.	Min.	VI
1. Socio-demographic	1	Dependency ratio	ratio	0.51	2	0	0.26
	2	Female headed HH	percent	6	100	0	0.06
	3	Age of house head	Average	44.77	78	24	0.27
	4	HH where head has not attended school	Percent	21	100	0	0.21
	5	HH size	Average	5.28	14	2	0.23
	6	HH farming experience	year/average	6.06	20	0	0.30
	7	HH herb collection experience	year/average	1.13	20	0	0.06
Socio demographic Vulnerability Index							0.20
2. Livelihood Strategies	1	HHs with members working in a different community or migrating	Percent	4.26	100	0	0.04
	2	HHs fully dependent on agriculture as a source of income	Percent	47.88	100	0	0.48
	3	Agricultural livelihood diversification index	1/#livelihood, average	0.34	0.50	0.14	0.40
	4	Herbs livelihood diversification index	1/#livelihood, average	0.4	0.50	0.16	0.48
	5	HHs who did not get the expected price for crops	Percent	70	100	0	0.70
	6	Semi pacca or kachha houses	Percent	65	100	0	0.65
	7	Total land cultivated	Acres, average	1.55	17	0	0.09
Livelihood Strategies Vulnerability Index							0.41
3. Social networks	1	Neighbours visiting in a month	Times	4.51	25	1	0.14
	2	Respondents feel can talk to others about private matters	Members	6.65	100	0	0.07
	3	Respondents reportedly willing to borrow money in need	Members	4.28	20	0	0.21
	4	Respondents reporting that other people can't be trusted	Percent	1.93	7	0	0.28
	5	Respondents are not willing to help you in case of necessity	Percent	1.72	5	0	0.34
	6	Respondents generally untrustfull in matters of lending or borrowing money	Percent	3.25	20	0	0.16
	7	Number of friends and relatives who would over	Members, average	9.76	100	0	0.10

		help during need					
	8	Times you have helped a friend or relative when in need	Times, average	12.24	100	0	0.12
	9	HHs where none is affiliated with any institution	Percent	6.05	100	0	0.06
Social Networks Vulnerability Index							0.16
4. Health	1	HHs where someone had to miss work or school due the illness in the past year	Percent	0.82	100	0	0.01
	2	HHs reducing food intake in bad times	Percent	0.85	100	0	0.01
	3	Life expectancy index	Years	0.62	1	0.18	0.41
Health Vulnerability Index							0.14
5. Food	1	HHs dependent on family farm for food	percent	11	100	0	0.11
	2	Number of months when the HHs trouble providing food	Months, average	3.72	12	0	0.31
	3	Crop diversity Index	1/# crops, average	0.2319	1	0.09	0.14
	4	Herb diversity Index	1/# crops, average	0.3892	1	0.14	0.25
	5	Number of times the HH needed to purchase food	Times, average	3.74	12	0	0.31
	6	Time to drinking water	Minutes, average	15	30	0	0.00
	7	Time to market	Minutes, average	145.95	4000	0	0.36
Food Vulnerability Index							0.21
6. Water	1	HHs with borrewell fail	percent	10	100	0	0.10
	2	HHs with access to water source	percent	10	100	0	0.10
	3	HHs without consistent water supply	percent	10	100	0	0.10
	4	HHs reporting depletion of their natural water source	percent	68	100	0	0.68
	5	HHs with risk investment	Percent	50	100	0	0.50
	6	HHs with vulnerability risk	Percent	7	100	0	0.07
Water Vulnerability Index							0.26
7. Natural Vulnerability and Climate Variation	1	HHs reporting less rain in the past 10 years	percent	5	100	0	0.05
	2	HHs reporting more droughts in the past 10 years	percent	10	100	0	0.10
	3	HHs reporting more floods in the past 10 years	percent	88	100	0	0.88
	4	HHs reporting unusual rains in the past 10 years	percent	91	100	0	0.91
	5	HHs reporting temperature increase in the past 10 years	percent	90	100	0	0.90
	6	HHs with the same crop	percent	33.3	100	0	0.33

		choice than in previous years					
	7	HHs reporting less rain this year than the average of rainy seasons	percent	4	100	0	0.04
	8	HHs with agricultural problems	percent	78	100	0	0.78
	9	HHs without adaptations to climate or weather problems	percent	96	100	0	0.96
	10	Previous herbs cultivation	percent	70	100	0	0.70
	11	Problems with herbs cultivation	percent	93	100	0	0.93
Natural Vulnerability and Climate Variation Vulnerability Index							0.60
							6.58
8. Knowledge and Skills	1	HHs without TV	percent	98	100	0	0.98
	2	HHs without Radio	percent	5	100	0	0.05
	3	HHs not participating in knowledge exchange with others	percent	99	100	0	0.99
	4	HHs perceiving lack of education	percent	9	100	0	0.09
	5	Year of schooling	Number, average	6.82	15	0	0.45
Knowledge and Skills Vulnerability Index							0.51
9. Finance	1	HHs with more expenditures than income	percent	49	100	0	0.49
	2	HHs member working outside (not farming)	percent	12	100	0	0.12
	3	HHs with money loans	percent	61	100	0	0.61
	4	HHs who had not repaid loans	percent	82	100	0	0.82
Finance Vulnerability Index							0.51
Livelihood Vulnerability Index (LVI)							0.35

Annex-2: Estimation of LVI of Dharapani Municipality, Manang

Major Components	SN	Sub-components	Unit	Observed value	Max.	Min.	VI
1. Socio-demographic	1	Dependency ratio	ratio	0.62	2	0	0.31
	2	Female headed HH	percent	24.00	100	0	0.24
	3	Age of house head	Average	48.33	79	17	0.40
	4	HH where head has not attended school	Percent	55.00	100	0	0.55
	5	HH size	Average	4.80	12	1	0.32
	6	HH farming experience	year/average	7.00	70	2	0.07
	7	HH herb collection experience	year/average	6.00	20	0	0.30
Socio demographic Vulnerability Index						0.31	2.18
2. Livelihood Strategies	1	HHs with members working in a different community or migrating	Percent	10.00	100	0	0.10
	2	HHs fully dependent on	Percent	8.00	100	0	0.08

		agriculture as a source of income					
	3	Agricultural livelihood diversification index	1/#livelihood, average	0.24	0.50	0.14	0.20
	4	Herbs livelihood diversification index	1/#livelihood, average	0.41	0.50	0.16	0.50
	5	HHs who did not get the expected price for crops	Percent	24.00	100	0	0.24
	6	HHs who did not get the expected price for herbs	Percent	6.00	100	0	0.06
	7	Semi pacca or kachha houses	Percent	90.00	100	0	0.90
	8	Total land cultivated	Acres, average	1.55	17	0	0.09
Livelihood Strategies Vulnerability Index						0.27	2.17
3. Social networks	1	Neighbours visiting in a month	Times	20.46	30	1	0.65
	2	Respondents feel can talk to others about private matters	Members	6.56	100	0	0.07
	3	Respondents reportedly willing to borrow money in need	Members	4.28	20	0	0.21
	4	Respondents reporting that other people can't be trusted	Percent	1.93	7	1	0.13
	5	Respondents are not willing to help you in case of necessity	Percent	1.72	5	1	0.14
	6	Respondents generally untrustfull in matters of lending or borrowing money	Percent	3.25	20	0	0.16
	7	Number of friends and relatives who would over help during need	Members, average	12.24	100	1	0.11
	8	Times you have helped a friend or relative when in need	Times, average	9.76	100	0	0.10
	9	HHs where none is affiliated with any institution	Percent	6.05	100	0	0.06
Social Networks Vulnerability Index						0.18	1.64
4. Health	1	HHs where someone had to miss work or school due the illness in the past year	Percent	24.00	100	0	0.24
	2	HHs reducing food intake in bad times	Percent	8.00	100	0	0.08
	3	Life expectancy index	Years	0.60	1	0	0.60
Health Vulnerability Index							0.92
5. Food	1	HHs dependent on family farm for food	percent	8.00	100	0	0.08
	2	Number of months when the HHs trouble providing food	Months, average	0.83	25	0	0.03
	3	Crop diversity Index	1/# crops,	0.19	0.50	0.09	0.24

			average				
	4	Herb diversity Index	1/# crops, average	0.24	0.50	0.08	0.38
	5	Number of times the HH needed to purchase food	Times, average	8.30	12	2	0.63
	6	Time to drinking water	Minutes, average	60.00	240	0	0.25
	7	Time to market	Minutes, average	400.00	500	100	0.75
Food Vulnerability Index						0.34	2.37
6. Water	1	HHs with borrewell fail	percent	90.00	100	0	0.90
	2	HHs with access to water source	percent	88.00	100	0	0.88
	3	HHs without consistent water supply	percent	87.00	100	0	0.87
	4	HHs reporting depletion of their natural water source	percent	46.00	100	0	0.46
	5	HHs with risk investment	Percent	6.00	100	0	0.06
	6	HHs with vulnerability risk	Percent	3.00	100	0	0.03
Water Vulnerability Index						0.53	3.20
7. Natural Vulnerability and Climate Variation	1	HHs reporting less rain in the past 10 years	percent	6.00	100	0	0.06
	2	HHs reporting more droughts in the past 10 years	percent	30.00	100	0	0.30
	3	HHs reporting more floods in the past 10 years	percent	33.00	100	0	0.33
	4	HHs reporting unusual rains in the past 10 years	percent	71.00	100	0	0.71
	5	HHs reporting temperature increase in the past 10 years	percent	60.00	100	0	0.60
	6	HHs with the same crop choice than in previous years	percent	80.00	100	0	0.80
	7	HHs reporting less rain this year than the average of rainy seasons	percent	19.00	100	0	0.19
	8	HHs with agricultural problems	percent	66.00	100	0	0.66
	9	HHs without adaptations to climate or weather problems	percent	76.00	100	0	0.76
	10	Previous herbs cultivation	percent	35.00	100	0	0.35
	11	Problems with herbs cultivation	percent	38.00	100	0	0.38
Natural Vulnerability and Climate Variation Vulnerability Index						0.47	5.14
8. Knowledge and Skills	1	HHs without TV	percent	25.00	100	0	0.25
	2	HHs without Radio	percent	73.00	100	0	0.73
	3	HHs not participating in knowledge exchange with others	percent	67.00	100	0	0.67
	4	HHs perceiving lack of education	percent	44.00	100	0	0.44
	5	Year of schooling	Number, average	5.71	14	1	0.34

Knowledge and Skills Vulnerability Index						0.49	2.43
9. Finance	1	HHs with more expenditures than income	percent	90.00	100	0	0.90
	2	HHs member working outside (not farming)	percent	10.00	100	0	0.10
	3	HHs with money loans	percent	25.00	100	0	0.25
	4	HHs who had not repaid loans	percent	42.00	100	0	0.42
Finance Vulnerability Index						0.42	1.67
Livelihood Vulnerability Index (LVI)							**0.36**

Annex-3: Estimation of LVI of Madhuwan Municipality, Parsa

Major Components	SN	Sub-components	Unit	Observed value	Max.	Min.	VI
1. Socio-demographic	1	Dependency ratio	ratio	0.73	3	0	0.24
	2	Female headed HH	percent	16	100	0	0.16
	3	Age of house head	Average	49	70	28	0.30
	4	HH where head has not attended school	Percent	42	100	0	0.42
	5	HH size	Average	8.69	20	3	0.28
	6	HH farming experience	year/average	17.5	40	2	0.39
	7	HH herb collection experience	year/average	21.77	50	14	0.16
Socio demographic Vulnerability Index							**0.28**
2. Livelihood Strategies	1	HHs with members working in a different community or migrating	Percent	19	100	0	0.19
	2	HHs fully dependent on agriculture as a source of income	Percent	74	100	0	0.74
	3	Agricultural livelihood diversification index	1/#livelihood, average	0.19	0.5	0.09	0.20
	4	Herbs livelihood diversification index	1/#livelihood, average	0.24	0.5	0.08	0.32
	5	HHs who did not get the expected price for crops	Percent	19	100	0	0.19
	6	HHs who did not get the expected price for herbs	Percent	22	100	0	0.22
	7	Semi pacca or kachha houses	Percent	46	100	0	0.46
	8	Total land cultivated	Acres, average	0.35	1.6	0	0.22
Livelihood Strategies Vulnerability Index							**0.32**
3. Social networks	1	Neighbours visiting in a month	Times	10	30	1	0.30
	2	Respondents feel can talk to others about private matters	Members	5.74	20	1	0.24
	3	Respondents reportedly willing to borrow money in need	Members	5	11	1	0.36
	4	Respondents reporting that other people can't be trusted	Percent	4.19	11	1	0.29

	5	Respondents are not willing to help you in case of necessity	Percent	4	10	1	0.30
	6	Respondents generally untrustfull in matters of lending or borrowing money	Percent	4	13	1	0.23
	7	Number of friends and relatives who would over help during need	Members, average	4.12	12	1	0.26
	8	Times you have helped a friend or relative when in need	Times, average	6.2	15	2	0.28
	9	HHs where none is affiliated with any institution	Percent	22	100	0	0.22
Social Networks Vulnerability Index							0.28
4. Health	1	HHs where someone had to miss work or school due the illness in the past year	Percent	24	100	0	0.24
	2	HHs reducing food intake in bad times	Percent	8	100	0	0.08
	3	Life expectancy index	Years	0.6	1	0	0.60
Health Vulnerability Index							0.31
5. Food	1	HHs dependent on family farm for food	percent	2	100	0	0.02
	2	Number of months when the HHs trouble providing food	Months, average	3.85	12	0	0.32
	3	Crop diversity Index	1/# crops, average	0.19	0.5	0.09	0.24
	4	Herb diversity Index	1/# crops, average	0.24	0.5	0.08	0.38
	5	Number of times the HH needed to purchase food	Times, average	3.7	12	0	0.31
	6	Time to drinking water	Minutes, average	11.65	60	0	0.19
	7	Time to market	Minutes, average	35	60	5	0.55
Food Vulnerability Index							0.29
6. Water	1	HHs with borrewell fail	percent	15	100	0	0.15
	2	HHs with access to water source	percent	82	100	0	0.82
	3	HHs without consistent water supply	percent	18	100	0	0.18
	4	HHs reporting depletion of their natural water source	percent	88	100	0	0.88
	5	HHs with risk investment	Percent	87	100	0	0.87
	6	HHs with vulnerability risk	Percent	84	100	0	0.84
Water Vulnerability Index							0.62
7. Natural Vulnerability and Climate Variation	1	HHs reporting less rain in the past 10 years	percent	88	100	0	0.88
	2	HHs reporting more droughts in the past 10 years	percent	87	100	0	0.87

	3	HHs reporting more floods in the past 10 years	percent	90	100	0	0.90
	4	HHs reporting unusual rains in the past 10 years	percent	91	100	0	0.91
	5	HHs reporting temperature increase in the past 10 years	percent	91	100	0	0.91
	6	HHs with the same crop choice than in previous years	percent	65	100	0	0.65
	7	HHs reporting less rain this year than the average of rainy seasons	percent	90	100	0	0.90
	8	HHs with agricultural problems	percent	87	100	0	0.87
	9	HHs without adaptations to climate or weather problems	percent	89	100	0	0.89
	10	Previous herbs cultivation	percent	76	100	0	0.76
	11	Problems with herbs cultivation	percent	83	100	0	0.83
Natural Vulnerability and Climate Variation Vulnerability Index							0.85
8. Knowledge and Skills	1	HHs without TV	percent	25	100	0	0.25
	2	HHs without Radio	percent	55	100	0	0.55
	3	HHs not participating in knowledge exchange with others	percent	67	100	0	0.67
	4	HHs perceiving lack of education	percent	79	100	0	0.79
	5	Year of schooling	Number, average	5.33	17	1	0.25
Knowledge and Skills Vulnerability Index							0.85
9. Finance	1	HHs with more expenditures than income	percent				
	2	HHs member working outside (not farming)	percent				
	3	HHs with money loans	percent				
	4	HHs who had not repaid loans	percent				
Finance Vulnerability Index							
Livelihood Vulnerability Index (LVI)							

Annex-4: Herbarium of Kutaki (Neopicrorhiza scrophulariiflora (Pennell) D. Y. Hang)

- Stem:
  - Dry 7.5 x 2.16 cm
    - Small: 10 x 0.4cm
    - Medium: 19.2 x 0.6 cm
    - Large: 24.6 x1.2 cm
- Leaves:
    - Small: 2.5 x 1.5 cm
    - Large: 6 x 1.9 cm
- Petiole
    - sessile:

Annex-5: Herbarium of Daruharidra (Berberis aristata DC.)

- **Stem:**
  - Dry 7.5 x 2.16 cm
- **Leaves:**
  - Small: 5 x 2.5 cm
  - Large: 5.4 x 3.7 cm

- Petiole
  - Sub-sessile: 0.6 cm

Annex-6: Herbarium of Guduchi (Tinospora cordifolia Wild.)

- Stem:
  - Fresh: 1.9 cm
  - Dry
    - Small: 0.10 cm
    - Medium: 1.1 cm
    - Large: 1.3 cm
- Leaves:
    - Small: 10 x 8.2 cm
    - Medium: 13.7 x 14 cm
    - Large: 18.1 x15.1 cm
- Petioles
    - Small: 5 cm
    - Medium: 6 cm
    - Large: 7.1 cm