ECOLOGICAL STUDY OF *Swertia chirayita* (Roxb. ex Fleming) H. Karst IN LAPRAK AREA, GORKHA, NEPAL

A THESIS

SUBMITTED FOR THE
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE MASTER'S DEGREE IN BOTANY

BY
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"FEBRUARY, 2021"

DECLARATION

I, Mr. Bishal Subedi, hereby declare that the work enclosed here is entirely my own, except where states otherwise by reference or acknowledgement, and has not been published or submitted elsewhere, in whole or in part, for the requirement for any other degree or professional qualification. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and listed in the reference section.

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RECOMMENDATION

This is to recommend that the Master's thesis entitled "**Ecological Study of *Swertia chirayita* (Roxb. ex Fleming) H. Karst in Laprak area, Gorkha, Nepal**" is carried out by Mr. Bishal Subedi under my supervision. The entire work is based on original scientific investigations and has not been submitted for any other degree in any institutions. I therefore, recommend this thesis work to be accepted for the partial fulfilment of M.Sc. Degree in Botany.

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APPROVAL

The thesis work submitted by Mr. Bishal Subedi (TU Registration no. 5-2-37-664-2012) entitled "**Ecological Study of *Swertia chirayita* (Roxb. ex Fleming) H. Karst in Laprak Area, Gorkha, Nepal**" to Department of Botany, Amrit Campus, Tribhuvan University has been accepted for the partial fulfilment of the requirement for Master's Degree in Botany.

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Bishal Subedi

ACRONYMS AND ABBREVIATIONS

%	Percentage
>	Greater than
^0C	Degree Celsius
ANOVA	Analysis of Variance
AD	Anno Domini (After the death of Christ)
CBS	Central Bureau of Statistics
df	Difference
eds.	Editors
Fig.	Figure
G	Grams
IUCN	International Union for the Conservation of Nature and Natural Resources
kg	Kilogram
m^2	Square meter
masl	Meter above sea level
ml	Milliliter
no.	Number
PCA	Principal component analysis
pH	Potential of Hydrogen
Pop^n	Population
S.D.	Standard deviation
S.E.	Standard error
TU	Tribhuvan University
TUCH	Tribhuvan University Central Herbarium
WHO	World Health Organization

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ABSTRACT

Swertia chirayita (Roxb.ex Flem) Karst is one of the highly traded medicinal plants from Nepal. Systematic sampling was done in three different places of the Gorkha district (Laprak-2360-2825masl, Barpak-2080-2790masl and Kashigaun-1860-2036masl) to understand the existing ecological status of *S. chirayita*. The soil pH in these sampling sites ranged from 3.2 to 5.9. The soil total nitrogen content (0.01%-0.14%) and total soil organic carbon (0.3%-4.65%) in the sampling sites was low. The phosphorous content of the soil in the sampling sites ranged from 47.84kg/hectors to 85.9kg/hector. The slope angle of the sampling plots ranged from 19.7^0 to 37.4^0. The density of *S. chirayita* had significant positive correlation with total nitrogen content of soil (0.57), total organic carbon (0.61) which means it grows well in the soil rich in nitrogen and organic carbon and significant negative correlation with phosphorous content of the soil (-0.61), which means the plant doesn't favors the high amount of phosphorous. The distribution of *S. chirayita* was not uniform, and was either contagious or random in the study area. The most common associated species were *Anaphalis contorta, Osbeckia stellata, Boehmeria platyphylla, Miscanthus nepalensis Artemisia indicia,* etc. Invasive plant species like A*geratina adenophora, Bidens pilosa* and *Oxalis latifolia* were also found associated with *S. chirayita* in the study site.

Key words: *Association, Density, Soil relation*

1. INTRODUCTION

1.1 Background

Nepal contains 118 ecosystems that harbor over 2% of flowering plants, 3% of the pteridophytes, and 6% of the bryophytes in the world's flora (Gon/MoFSC, 2014). Official Pharmacopeias mentions that more than 28,000 plant species have medicinal uses worldwide (Willis, 2017), while only 2,331 species of plants from Nepal have been recorded to have medicinal uses (Rokaya *et al.*, 2012). Many of these plants are in high demand in national and international markets. Among them one of the most important medicinal plant is *Swertia chirayita* (Roxb.ex Fleming) Karst (Ghimire, 1999). Humankind has explored the importance of this plant from ancient time. "Vaidyas" (Traditional healers) have been extensively using it in various treatments. *S. chirayita* is mentioned in Classical Ayurvedic books as Kirata, Kirataka, Bhnimba, Kiratiktata (Kiratatakta, 2001). *S. chirayita* is commonly known Chiraito and locally known as Tite or Lekhtite, Pothi chiraito, Kalo chiraito, Dakhle chiraito in various parts of Nepal.

Nepal comprises around 30 species of *Swertia* including varieties and nine species of *Swertia* are being traded in Nepal among them *Swertia chirayita* is the most valuable and high demanding medicinal plant of Nepal. These are *Swertia chirayita, S. angustifolia, S. tetragona, S. racemosa, S. ciliata, S. multicaulis, S. alata, S. nervosa*. Among these *Swertia chirayita* plays a dominant role in trade covering about 80% of the traded volume of *Swertia* sp. The demand is adversely triggering the illegal exploitation of *S.chirayita* and its related species which has been a national problem of Nepal. *Swertia chirayita* falls under IUCN threatened category "Vulnerable plant" (Press *et al.*, 2000).

S. chirayita has high commercial demand (Badola and Pal, 2002) which has led to unsustainable harvesting in different parts of the Himalaya (Anonymous, 2008; Phoboo and Jha, 2010). This has caused tremendous pressure on its natural population. Unscientific collection and over-harvesting of *S. chirayita* from its natural habitat are taking place in Nepal which has contributed to rapid depletion of *S. chirayita* from its natural habitats. Due to its high price, collectors have high competition for its collection and it is collected before maturation.

Unsustainable harvesting of *S. chirayita* traditionally from the natural population for domestic use and commercial benefits in recent decades from different parts of the Himalaya (Badola and Pal, 2002; Dutta, 2004; Olsen, 2005) is leading to high pressure on its natural populations. So far, no initiative for systematic evaluation of the species in its natural habitats has been conducted, except for central and north-western Himalaya (Bhatt *et al.*, 2006). There is hardly any literature available on population structure, status, habitat characteristics of *S. chirayita* hindering the suitable conservation framework.

The successful conservation and effective management planning of the threatened plant species largely depend on an understanding of the effects of the natural and/or man-made changes on habitats (Hegland *et al*., 2001), their ecological requirements, and the demography of the existing populations (Kalliovirta *et al.*, 2006), and their response to disturbances (Ticktin, 2004). The habitat characters of the site play a significant role in the successful regeneration of the plant species (Tilman, 1993; Jensen and Meyer, 2001; Overbeck *et al.*, 2003) and its identification is very important for the species conservation (Sypros *et al.*, 2008). Insufficient knowledge regarding the disturbances on the plant communities and plant populations are the main constraints in designing appropriate management plants, which makes the conservation initiatives more complicate (Fidelis *et al.*, 2008).

1.2 Justification of the study

Swertia chirayita populations from the wild are being declined with their high market demand beyond its regeneration capacity (Shrestha *et al.*, 2013).

Studies on quantitative assessment of population help in determining the performance of populations under different sets of conditions and provide desired information about the specialized ecological requirements of a taxon (Kaul, 1997). Therefore, the present study was undertaken in the Upper part (Barpak, Laprak and Kashigaun) of Gorkha, to document its population status in different soil conditions at different altitudinal ranges of the temperate belt of the Himalaya. This will help in the management in wild as well as will provide insight for the cultivation in private and/or community forests.

1.3 Research Questions

a) Is the habitat character of *S. chirayita* same in all the three study sites?
b) What is the population status of *S. chirayita* in the Laprak area of Gorkha district?
c) Which soil parameters affect the *Swertia chirayita* density?

1.4 Objectives

The overall aim is to assess the population size of *Swertia chirayita* along the environmental gradient. The specific objectives are to:

1. Study population size of *S. chirayita* in different ecological habitat
2. Evaluate soil parameters associated with density of *S. chirayita*

1.5 Limitations

a) The details of the life stages of *S. chirayita* was not studied separately.

2. LITERATURE REVIEW

2.1 Taxonomic position of *Swertia chirayita*

Plantae

Tracheophyta

Magnoliopsida

Gentianaceae

Swertia L

Swertia chirayita (Roxb. ex Fleming) H. Karst

2.2 Distribution of *Swertia chirayita*

Swertia is widely distributed globally with more than 135 species from temperate areas (Shrestha *et al.*, 2013). Nepal possesses high diversity with more than 30 species due to varying climatic and physiographic composition (Shrestha *et al.*, 2013). *S. chirayita* grows in the temperate Himalayas from Kashmir to Bhutan and Khasia hills of Meghalaya (Chanda, 1976). It is also known as Cheraito in Nepal and is reported from fifty-four districts of Nepal (Joshi, 2012) including Gorkha. *Swertia chirayita* does not have uniform distribution and its distribution depends upon the altitudes and slopes (Khanal *et al.*, 2014). It is distributed within the altitude of 1500-3000 masl throughout Nepal. However, the 2000 masl is the most preferable range (Bhattarai, 1996).

2.3 Ecological study of *Swertia chirayita* and its allied species

Swertia chirayita is an annual herb (Singh, 2008) which prefers to grow in north-facing slopes (Joshi and Dhawan, 2005; Phoboo and Jha, 2010; Sharma *et al.*, 2011). It prefers to grow in acidic soil conditions with a pH of 4.7 to 5.5 (Bhattarai and Shrestha, 1996). Its population mainly comprises of rosettes followed by the rosette stage and adults in wild (Pyakurel, 2008).

Some of the major associated species of *S. chirayita* are *Anaphalis* sp., *Desmodium* sp., *Anemone obtusiloba*, *Elsholtzia* sp., *Fragaria* sp., *Oxalis corniculata*, etc. (Ghimire *et al.*, 2008; Pyakurel, 2008).

2.4 Ethnobotanical use of *Swertia chirayita*

Swertia chirayita is a traditional medicinal plant used by different communities in different ways for curing various diseases. The whole plant is used by traditional and indigenous system of medicine such as Unani, Ayurveda and Siddha (Kirtikar and Basu, 1984; Joshi and Dhawan, 2005). It is also used in local level for the treatment of hepatitis, inflammation, and digestive diseases (Bhatt *et al.*, 2006). It has a wide range of medicinal uses which includes the treatment of chronic fever, malaria, anemia, bronchial asthma, hepatotoxic disorders, liver disorders, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, ulcers, scanty urine, hypertension, melancholia, and certain types of mental disorders, secretion of bile, blood purification, and diabetes (Karan *et al.*, 1999; Banerjee *et al*., 2000; Rai *et al.*, 2000; Saha *et al.*, 2004; Joshi, K.,2008; Chen *et al.*, 2011; Bhattarai K.R., 2018), as cited by Kumar and Staden (2016).

2.5 Trade on *Swertia chirayita*

Swertia species constitute the major proportions in the trade of the medicinal and aromatic plants from Nepal (Joshi, 2012). From different trading centers of Nepal, about nine species of *Swertia* (*S. chirayita*. *S. angustifolia, S. ciliate, S. dilatata, S. multicaulis, S. racemosa, S. tetragona, S. alata*, *S. nervosa*) are traded (Barakoti, 2002) with the common name "Chiraito" except for S. multicaulis i.e. Sarmaguru. *S. chirayita* plays a dominant role in trade and is considered superior quality among the traded species of Swertia.

Swertia chirayita is one of the largest exported medicinal plants of Nepal due to its high ethnomedicinal values (Joshi and Joshi, 2008; Phoboo and Jha, 2010; Shrestha *et al.*, 2010; Khanal *et al.*, 2014). Yearly, tones of *S. chirayita* are exported to the neighboring countries. Most of the species are traded to India while some are exported to China, Malaysia, Singapore, Germany, Italy, France, Switzerland, Sri Lanka, Bangladesh, Pakistan, and the USA (Phoboo *et al.*., 2011). The demand for *S. chirayita* is very high due to its multi-purpose medicinal values in the different medical systems such as Ayurveda, herbal, allopathy, etc. Phoboo and Jha (2010) reported that *S. chirayita* is treated in 61 of the 75 districts of Nepal and constituted about 3% in the total medicinal plants traded in the fiscal year 2008-2009. Nepal is reported to trade more than 45% of the world's total volume of *S. chirayita* (Barakoti, 2004).

2.6 Status of *Swertia chirayita* in Nepal

Due to the high medicinal value of *S. chirayita*, and high demand in foreign countries, most of the collectors collect it from the wild for export which has led to the depleting population of the plants and sequential danger of extinction (Joshi, 2008). As the government of India has prioritized the plant beside its conservation (Joshi and Dhawan, 2005), it is highly exported from Nepal illegally. Large scale export of the whole plant has made this plant vulnerable in Nepal and its immediate conservation is necessary (Phoboo and Jha, 2010). For the sustainable conservation of *S. chirayita*, sustainable harvesting training to the local harvesters, raising awareness, in situ strict management with community participation, and government approach should be done (Ghimire *et al.*, 2008; Phoboo and Jha, 2010; Pyakurel and Baniya, 2011).

Extensive and unmanaged collection of the whole plants from the wild by the local traders has led to the depleting population of the plant and sequential danger of extinction (Joshi, 2008). The Government of Nepal has enforced proper harvesting protocols, forbidding the collection and trade from May to September and the cultivation of sustainable methods within the local communities.

3. METHOD AND METHODOLOGY

3.1 Study Area

Gorkha district is located in Gandaki Province of Nepal. Geographically it extends from $28^0$28‘35.0220 “N latitude to $84^0$41‘23.1036 ’’E longitude. The district is surrounded by Dhading district from the east, Manang, Lamjung, and Tanahu district from the west, Chitwan district from the south, and Tibet from the north. The district covers an area of 3610 km2 with the elevation ranging from 228 to 8156 m. above sea level which includes low and high forest-covered mountains and many snow-covered Himalayan peaks like Mt. Manaslu. The climate varies from lower tropical to upper tropical, sub-tropical, temperate, subalpine, alpine, trans-Himalayan to naval zone (Barnekow *et al.*., 2005). The present study was conducted on Dharche and Sulikot Rural Municipality of the Gorkha district.

Dharche Rural Municipality is surrounded by Dhading District and China on the East, Sulikot Rural Municipality on the West, Chumnubri Rural Municipality on the North, and Aarughat Rural Municipality and Dhading District on the South. The total area of this Municipality is 651.52.52 km^2. The total population of the municipality is according to the 2011 (2068 BS) Nepal census is 13,229. Here, the population density of this rural municipality is 24/km^2.

Sulikot Rural Municipality was established in 2017 (2073 BS). This rural municipality is surrounded by Dharche and Arughat rural municipality on the East, Ajirkot, and Siranchok rural municipality on the West, Chumnubri Rural Municipality on the North, and Bhimsen rural municipality on the South. The total area of this Municipality is 200.63 km^2. The total population of the municipality is 25,389 according to the 2011 (2068 BS) Nepal census. The density of this rural municipality is 130/km^2.

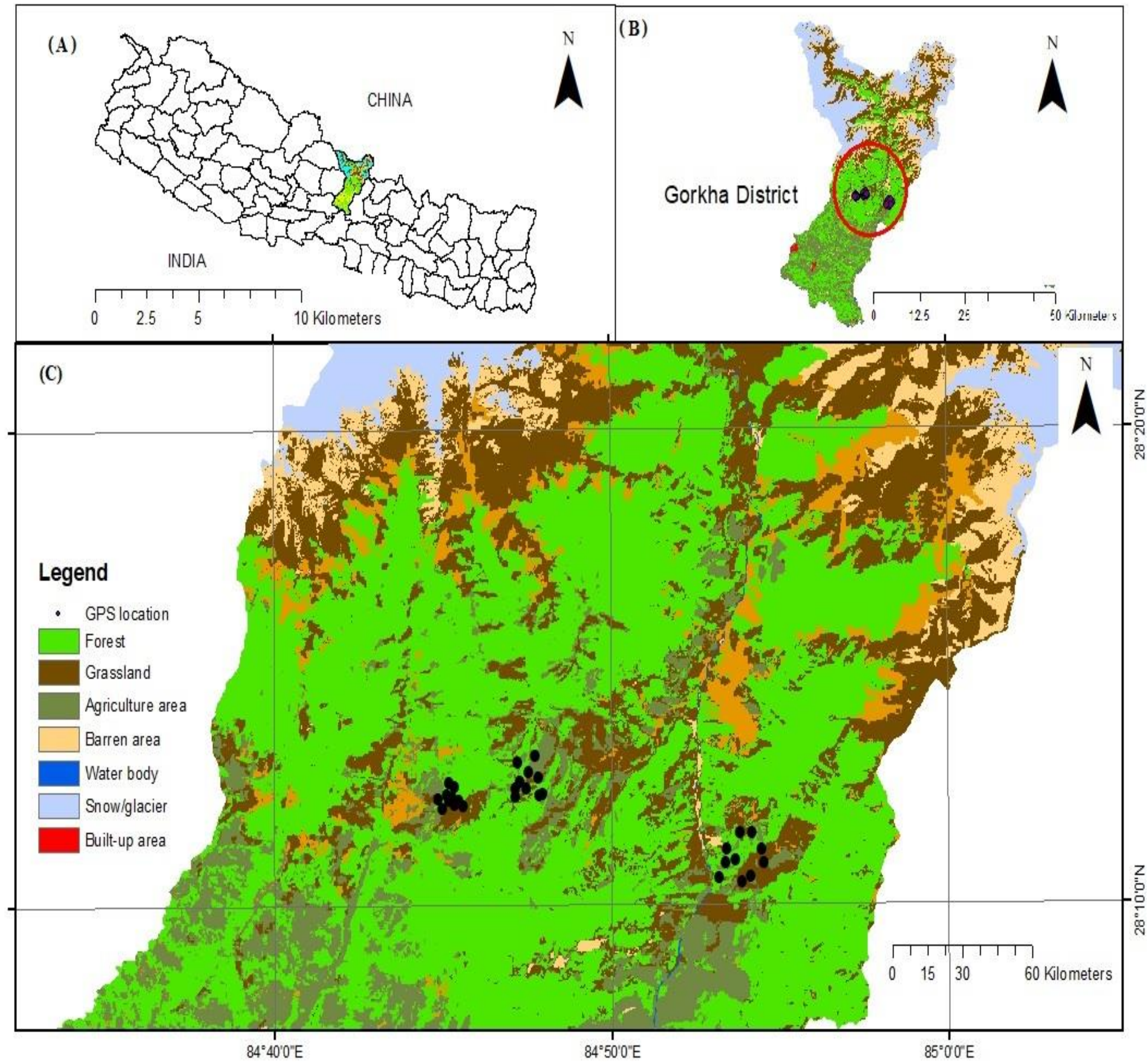

Fig. 3.1: Map of the study area (A=Map of Nepal showing Gorkha district, B= Map of Gorkha district with the vegetation and coverage, C= Zoom out the map of the study area showing study plots)

Gorkha district consists of six different climatic zones i.e. tropical (<1000m), subtropical (1000-2000m), temperate (2000-3000m), subalpine (3000-4000m), alpine (4000-5000m) and nival zone (>5000m) (Negi, 1994).

The temperature in the Gorkha district is highest during June. The maximum temperature ranges between 30^{0}C to 35^{0}C during the months' March, April, May, June, July, August, and September.

The lowest maximum temperature ranged between 20^0C to 25^0C during December, January, and February. The highest minimum temperature ranged between 20^0C to 25^0C during May, June, July, August, and September. The lowest minimum temperature ranged from 5^0C to 10^0C during December and January (Fig.3.2).

The highest precipitation was recorded in July and followed by August, June, September, May, April, respectively. Very low precipitation occurred during December, January, and February (Fig.3.2).

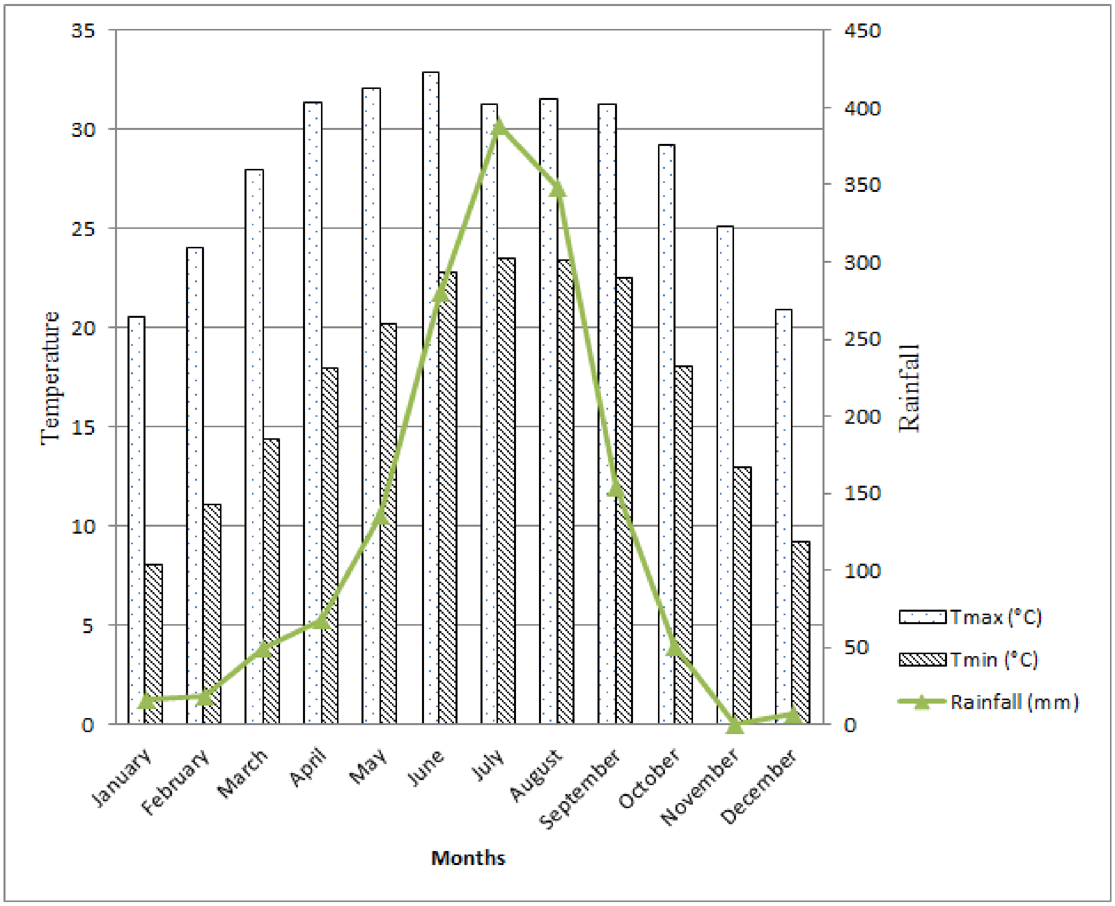

Fig. 3.2: Graphical representation of average maximum, minimum temperature and rainfall data from 2014-2018 of Gorkha district (Source: Department of Hydrology and Meteorology, Kathmandu, Nepal)

3.2 Vegetation and Flora of the study area

Two types of vegetation (Eastern Himalayan humid vegetation and Western Himalayan drier vegetation are found in the Gorkha district (Sapkota *et al*.., 2017). On one hand, eastern Himalayan vegetation consists of Green oaks, Rhododendron and laurels dominated area while on the other hand western vegetation is characterized by *Pinus wallichiana, Picea smithiana, and Tsuga dumosa* (Sapkota *et al.*., 2017). Gorkha district consists of six climatic zones (Negi, 1994). There is the presence of dominant species like *Abies spectabilis, Pinus roxburghii, P. wallichiana, Juniperus indica, J. recurva, Ephedra gerardiana, Rhododendron arboreum* in each zone (Sapkota *et al.*., 2017).

Both the rural municipalities (i.e. Sulikot and Dharche rural municipalities) have vegetation ranging from subtropical to alpine.

3.3 People, Population, and Socio-economic condition

In Sulikot rural municipality, a total of 25,389 populations (44% male and 56% female) were present in 5,829 households (CBS, 2011). The sex ratio of females to males was 70.104. Here, 97.478% of the household use wood/firewood (CBS, 2011). According to the census of 2011, the highest ethnic group inhabiting here is Gurung (38.97%) followed by Brahmu/Baramo (10.32%), Brahmin (9.74%), Chhetri (8.30%), Ghale (8.09%), Kami (5.53%), Magar (5.35%), Sharki (5.01%), Tamang (3.37%), Newar (2.48%) respectively.

In Dharche rural municipality, a total of 13,229 populations (45% male and 55% female) were present in 3,102 households (CBS, 2011). The sex ratio of females to males was 77.80. Here, 99% of the household use wood/firewood (CBS, 2011). According to the census of 2011, the highest ethnic group inhabiting here is Gurung (82.75%) followed by Ghale (11.32%), Kami (4.98%), Brahmin (0.212%), Chhetree (0.136%) respectively.

In both rural municipalities, Gurung is the dominant ethnic group. Ghale, Kami, Brahmin, Chhetri are the common ethnic group in both places. Every ethnic group of people has its own tradition and culture. There is a close relationship between their culture and plants.

3.4 Sampling Design and Data Collection

A preliminary study was carried out in June 2019 to access the distribution of *Swertia chirayita* in the Gorkha district. The field was conducted during August 2019. Vegetation sampling was carried out in Barpak, Laprak, and Kashigaun. Systematic sampling was done after resource selection to identify the different population of *S. chirayita,* 30 sampling plot of 10×10 m^2 were made by maintaining a distance of about 200m between them, and to calculate population status the 10×10

m^2 were further divided into subplots of 1×1 m^2 which were prepared by maintaining the distance of 2m on each side of the plot.

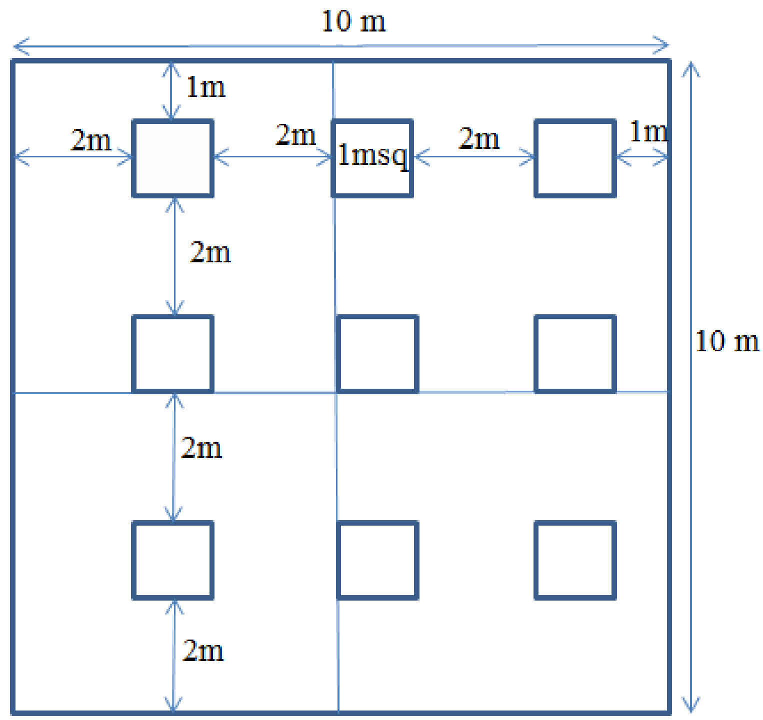

Fig. 3.3: Diagrammatic representation of the 10m x 10m plots showing nine 1m x 1m quadrats

Individual of *Swertia chirayita* of different life stages in each quadrat were recorded for a population study. The life stages were defined into 3 categories i.e. rosette, Vegetative aerials, and Reproductive aerials (Lyngdoh, 2011). Individuals after the emergence of seedling were considered rosette. The larger ones without the inflorescence were considered vegetative aerials and the ones with the inflorescence were considered the reproductive aerials. The number of associated species (either herbs or shrubs or trees) in each quadrat was also noted.

3.5 Specimen collection and identification

Collection of the associated species either flowering or fruiting or both presents in the sampling quadrats was done, as far as possible for proper identification. The herbarium was prepared for the collected specimens. Identification of the collected voucher specimens was carried out by following the standard literature (Polunin and Stainton, 1984; Stainton, 1988; Zheng-Yi and Raven, 1996-2003; Ohba *et al.*., 2008), expert consultation, visit the herbaria (TUCH and KATH), as well as by comparing with the high-resolution herbarium images of University Herbarium, University of Tokyo (TI).

3.6 Measurement of Slope angle

The angle of the slope was measured by using Clinometer application of the cell phone.

3.7 Soil Collection

The soil sample up to the depth of 15-20cm was collected from the four corners and center of the 10m×10m plot. It was then mixed thoroughly and kept on the plastic bag making it airtight.

3.8 Laboratory analysis of soil

Air-dried soil samples were analyzed for, total nitrogen (%), total organic matter (OM) %, phosphorous (P_2O_5), and potassium (K_2O) at the Soil and Fertilizer testing laboratory of Nepal Government in Hetauda Nepal.

3.8.1 Potential of Hydrogen (pH)

The soil pH was measured by using digital pH meter in the field.

3.8.2 Soil moisture content

The soil moisture was measured using the Oven drying method. The weight of the wet soil sample on the polythene bag was recorded in the field with the help of digital balance. Then in the lab, the soil sample was dried within a week from the date of collection in a hot air oven at 105^0C until a constant weight was obtained and the dry weight of the sample was recorded. The moisture content was calculated by using the following formula-

$$Moisture\ Content(\%) = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Dry weight}} \times 100\%$$

3.8.3 Chemical parameters

a) Soil Organic Carbon (SOC)

Soil organic matter present in soil was determined by using Walkey and Black wet soil oxidation method (Walky and Black, 1934). The method is based on the principle that carbon is oxidized by the dichromate ion and excess dichromate ion is then back titrated with ferrous ion.

In this process, first of all, 0.5 g of soil sample was taken and transferred to a dried 500ml of a conical flask and 10 ml of 1N $K_2Cr_2O_7$ was added into it. Then 20 ml. of H_2SO_4 was added and mixed by gentle swirling. The mixture was allowed to react for 30 minutes. The suspension was diluted with 200ml of distilled water and then 20 ml of o-phosphoric acid was added followed by 1ml of diphenylamine indicator. The sample was then titrated with 0.5N Ferrous Ammonium Sulphate (FAS) till the color changed to brilliant green at the endpoint. The process was repeated for blank with the same quantity of the chemicals but without soil.

The following relation was used to determine the soil organic matter:

$OM(\%)=(B-S)\times N\times 3\times 100\times 100\times 100/(W\times 1000\times 77\times 58)$

Where,

OM (%) = Percentage of Soil Organic Matter

B = Volume of Ammonium Ferrous Sulphate (AFS) consumed by Blank

S = Volume of Ammonium Ferrous Sulphate (AFS) consumed by Sample

N = Normality of AFS used

W = Weight of sample taken

This method assumes that soil Organic Matter (OM) contains 58 % of Carbon and the recovery factor of OM is 77 % by Walkey and Black Method. Again, the conversion of Organic Matter (%) to Organic Carbon (%) was determined according to Nelson and Sommers (1996).

$$Organic\ Carbon(\%) = \frac{58 \times \text{OM}\%}{100}$$

b) Total Nitrogen

Total nitrogen was measured using the Kjeldahl method (Kjeldahl, 1883). This method is based on the principle of digestion of the sample with sulphuric acid and conversion of nitrogen into ammonia, then ammonia trapping and quantification with standard acid.

In this method, 1 g of fine soil was transferred to a Kjeldahl digestion flask and 10 ml. of distilled water was added and left for 30 minutes. Then 2 g of catalyst digestion mixture followed by 10 ml. of concentrated H_2SO_4 was added and the content was refluxed for one and half hours and cooled. Then the mixture was made 100 ml. by adding distilled water. 20 ml. of the mixture was taken followed by 20 ml. of 40% NaOH, 4% of boric acid, and 2 drops were added and refluxed. The color change of Boric acid into blue color was the indicator that the solution was ready for titration. Then, it was titrated against a 0.01 HCL solution. When the color was changed in brownish the endpoint was noted.

The following relation was used to determine total nitrogen:

Total Nitrogen (%N) = 7 × n × (T-B) S

Where,

n = Normality of acid

T = Volume of acid used in titration

B = Volume of acid used in Blank

S = Sample weight

c) Available Phosphorus (P)

Available Phosphorus was measured by Olsen's method or sodium bicarbonate extractable P, given by Olsen *et al.* (1954) in which Sodium bicarbonate ($NaHCO_3$) of pH 8.5 is used as an extractant.

In this method, a 2.5g sample of air-dried soil was taken in a 125 mL Erlenmeyer flask. Then little phosphorous free Darco-G-60 and 50 mL of $NaHCO_3$ solution were added respectively at 25^0C. It was then shaken for 30 min on a reciprocating shaker at 120 strokes per minute. A similar process was done with and without using soil. Then the extract was filtered using Whatman No. 40 filter paper. On finding the filtrate cloudy, it was re-filtered if required. 10 mL aliquot of the extract was pipetted in a 50 mL volumetric flask and 10 mL of distilled water and one drop of the p-nitrophenol indicator were added. Then the content was acidified to pH 5.0 by adding 2.5M H_2SO_4 dropwise till the color disappears. 8 mL of the Murphy-Riley solution was added and the volume was brought up to 50 mL with distilled water. After waiting for 15 minutes, the intensity of the blue color was read on a spectrophotometer at 730 nm (as in the case of standard).

Available Phosphorous (Kg ha^{-1}) = $\frac{C \times Volume\ of\ extractant \times 2.24}{Volume\ of\ aliquot \times Weight\ of\ soil}$

Where,

C= µg P in the aliquot

3.9 Data Analysis

3.9.1 Quantitative Analysis

For each study area, data were analyzed to know the compositional features such as frequency, density, abundance, abundance frequency ratio (A/F), and important value index (IVI) as stated by Zobel *et al.* (1987).

Frequency and Relative frequency

The frequency and relative frequency was calculated by using the following formula-

Frequency (F) = $\frac{Number\ of\ quadrats\ in\ which\ the\ species\ occurred}{a\ total\ number\ of\ quadrats}$

And

Relative Frequency (RF) = $\frac{Frequency\ of\ the\ individual\ species}{total\ frequency\ of\ all\ species} \times 100$

Density and Relative density

The density and relative density was calculated by using the following formula-

Density (D) = $\frac{Number\ of\ individuals\ of\ species\ \text{A}\ in\ all\ quadrats}{Total\ number\ of\ quadrats\ \times Size\ of\ the\ quadrats}$

And

Relative Density (RD) = $\frac{Density\ of\ species\ A}{Total\ density\ of\ all\ species} \times 100$

Abundance and Relative abundance

The abundance and relative abundance was calculated by using the following formula-

Abundance (A) = $\frac{Total\ Number\ of\ individuals\ of\ species\ \text{A}\ in\ all\ quadrats}{Total\ number\ of\ quadrats\ in\ which\ species\ A\ occurred}$

And

Relative Abundance (RA) = $\frac{Abundance\ of\ species\ A}{Total\ abundance\ of\ all\ species} \times 100$

Important value index (IVI)

The IVI was calculated using the following formula-

IVI = RF + RD + RA

Spatial distribution of *S. chirayita*

The spatial distribution of *S. chirayita* was determined following Whitford (1949).

Similarity index

The similarity index of the associated species in the different study sites was calculated by the Sorenson similarity index (S_{SD}) given by Thorvald Sorensen in 1948.

$S_{SD} = \frac{2a}{2a+b+c}$

Where, a= number of species on both sites, b= number of species in the first site only, and c= number of species on the second site only

3.9.2 Statistical Analysis

Both the parametric and non-parametric statistical tests were performed to analyze the data. General relationships between population parameters and soil factors were identified using non-parametric correlation analysis and then further statistical analysis was done using statistical software: Statistical Package for Social Sciences (SPSS) version 25, R-software, and Ms-Excel based on the requirements.

Non-parametric tests, analysis of variance (ANOVA), and associated pairwise comparisons were done to detect differences among the populations. One way ANOVA was used to access the difference in plant population density and structure. The abundance and frequency ratio was used to analyze the distribution pattern of the species.

4. RESULT

4.1 Population Size and Structure of *S. chirayita* in Gorkha

The population of *S. chirayita* was found to be highest in Laprak followed by Barpak and Kashigaun respectively. A significantly high population was observed in between Laprak than in Kashigaun, while the *S. chirayita* population in Barpak didn't differ significantly with both Laprak and Kashigaun (Table 4.1).

The density of the rosette form of the Laprak and Kashigaun varied significantly, while the density of Barpak didn't vary significantly with either of the place (Table 4.1). The vegetative aerials' and reproductive aerials' density varied significantly within the study area (i.e. Laprak, Barpak, and Kashigaun) (Table 4.1).

The abundance frequency ratio (A/F ratio) was highest in Laprak (0.066) followed by Barpak (0.059) and Kashigaun (0.049) respectively (Table 4.1). The A/F ratio suggests that Kashigaun has random (A/F<0.050), while Barpak and Laprak have the contagious (A/F>0.050) distribution.

Table 4.1: Comparisons of Rosette, vegetative aerials, reproductive aerials, and the total population in three different places of Gorkha district along with an abundance frequency ratio.

Place	Rosette (Mean $Pop^n \pm$ S.D.)	Vegetative aerials (Mean $Pop^n \pm$ S.D.)	Reproductive aerials (Mean $Pop^n \pm$ S.D.)	Total Pop^n (Mean $Pop^n \pm$ S.D.)	A/F ratio
Laprak	2.55 ± 1.58^c	2.95 ± 2.14^{ac}	2.09 ± 1.09^{ac}	6.07 ± 4.05^c	0.066
Barpak	2.78 ± 2.05	2.37 ± 1.66^{bc}	1.75 ± 0.82^{bc}	5.55 ± 3.91	0.059
Kashigaun	2.05 ± 0.87^a	2.07 ± 0.74^{ab}	1.39 ± 0.92^{ab}	4.59 ± 1.48^a	0.049
P value	0.017	0.003	0	0.015	
F value	4.178	5.963	8.29	4.244	

^{a}= mean difference significant with Barpak, ^{b}= mean difference significant with Laprak and ^{c}= mean difference significant with Kashigaun, at p=0.05 at df=2 and n=90 in one way ANOVA followed by L.S.D.

4.2 Physiographic and soil characteristics of *S. chirayita*

Swertia chirayita was recorded from open, grassy slopes and old landslide area from the altitude ranging from 1860 masl to 2825 masl (Larpak (2360-2825 masl), Barpak (2080-2790 masl), and

Kashigaun (1860-2036 masl) in Gorkha district. As *S. chirayita* prefers to grow on acid soil, the acidity of the soil ranged from 3.2 to 5.9. The total soil nitrogen concentration was very low (0.01% to 0.14%). The phosphorous concentration ranged from 47.86.1 Kg/hector to 85.9 Kg/hector, while the soil total organic carbon ranged from 0.3% to 4.65%. The slope ranged from 19.7^o to 37.4^o.

On analyzing the different soil and environmental factors, it was found that the total soil nitrogen content and slope degree remained almost the same (Table 4.2) throughout the habitat of *S. chirayita* (i.e. Laprak, Barpak, and Kashigaun). It means that *S. chirayita* is found on the similar condition of soil nitrogen and slope of the land. On the other hand, soil and environmental factors like soil pH, total organic carbon content, phosphorous content, moisture content, and altitude showed significance (Table 4.2) difference in the habitat of *S. chirayita*.

Table 4.2: Relationship of different factors determining the habitat of *S. chirayita*

Variables	Laprak (Mean±S.E.)	Barpak (Mean±S.E.)	Kashigaun (Mean±S.E.)	F value	P-value
pH^2	4.29±0.126	4.72±0.305	5.1±0.159		0.034
Total org. C^1 (%)	2.49±0.342^{ac}	1.48±0.387^b	1.154±0.112^b	5.227	0.012
N^1 (%)	0.040±0.0096	0.061±0.0156	0.032±0.0071	1.748	0.193
P^2 (kg/hector)	66.522±4.098	62.68±3.044	76.02±2.499		0.039
Moisture content^1 (%)	41.59±5.005^c	41.35±10.274^c	20.53±2.85^{ab}	3.160	0.012
Altitude^1 (masl)	2496±56.49^{ac}	2278.7±75.35^{bc}	1956.3±20.015^{ab}	23.867	0.000
Slope^2 (^{0})	29.3±1.73	28.53±1.33	26.27±1.65		0.309

^{1}= One way ANOVA analysis followed by L.S.D., ^{2}= Kruskal Wallis test, and ^{a}= mean difference significant with Barpak, ^{b}= mean difference significant with Laprak and ^{c}= mean difference significant with Kashigaun, at p=0.05 at df=2 and n=10.

4.3 Relation of *S. chirayita* density with different soil and environmental factors

The relation between different soil and environmental variables were determined by using the Spearman correlation (Table 4.3). On one hand, the test showed that the density of *Swertia chirayita* in the rosette, vegetative aerials, and reproductive aerial stage showed a significant positive correlation with total density and soil parameters like total organic carbon, total nitrogen content, and total phosphorous content. On the other hand, the density of *S. chirayita* showed a significant negative correlation with the soil phosphorous content. But the density of the plant didn't show any significant correlation with soil pH, soil moisture, and altitude.

The correlation of *S. chirayita* density with phosphorous content was significantly (p=0.001) negative (-0.613) but it was significantly (p=0.001) positive with total organic carbon and total nitrogen content in the soil. The density of the plant had the least insignificant correlation with slope (0.006) followed by soil pH (0.076), altitude (0.136), and soil moisture content (0.224) respectively. The density of the vegetative (-0.011) and reproductive aerials (-0.004) of the plant had a negative insignificant correlation with the soil pH, while the density of rosette and the total density of the plant has an insignificant positive correlation with it.

The phosphorous content of the soil has a significant negative correlation (0.619) with the total nitrogen content. The altitude had a significant positive correlation (0.530) with the soil moisture content and the angle of the slope (0.372) and had a significant negative correlation with the soil pH (-0.367).

Table 4.3: Spearman Correlation between population density of *Swertia chirayita* and different soil parameters (n=30).

Variables	1	2	3	4	5	6	7	8	9	10	11
1. Rosette plant density (m^{-2})	1.00										
2. Vegetative aerial density (m^{-2})	.60**	1.00									
3. Reproductive aerial density (m^{-2})	.49**	.40*	1.00								
4. Total density (m^{-2})	.83**	.84**	.68**	1.00							

5. Total nitrogen (%)	$.58^{**}$	$.42^{*}$	$.60^{**}$	$.57^{**}$	1.00						
6. Phosphorous content (kg/hector)	$-.510^{**}$	$-.463^{*}$	$-.52^{**}$	$-.61^{**}$	$-.62^{**}$	1.00					
7. Soil pH	0.12	-0.01	-0.01	0.08	-0.20	-0.04	1.00				
8. Total organic carbon (%)	$.37^{*}$	$.63^{**}$	$.53^{**}$	$.61^{**}$	0.28	-0.30	-0.223	1.00			
9. Altitude (masl)	0.02	-0.02	0.21	0.14	0.21	-0.29	$-.37^{*}$	0.18	1.00		
10. Moisture content (%)	0.143	0.157	0.163	0.196	0.267	-0.18	-0.29	0.33	$.53^{**}$	1.00	
11. Slope Angle (^{0})	-0.36	-0.03	-0.15	-0.16	0.01	0.02	-0.12	-0.10	$.37^{*}$	0.194	1.00

Note: **= Correlation is significant at the 0.01 level and *= Correlation is significant at the 0.05 level

The relationship between the population density of *S. chirayita* with altitude and different soil properties is shown by using the scatter plot of PCA in fig. 4.1. The PCA biplot clearly shows that the density of *S. chirayita* has a close relation with total soil organic carbon and soil total Nitrogen. But the density of *S. chirayita* didn't have a close relation with altitude and other soil parameters.

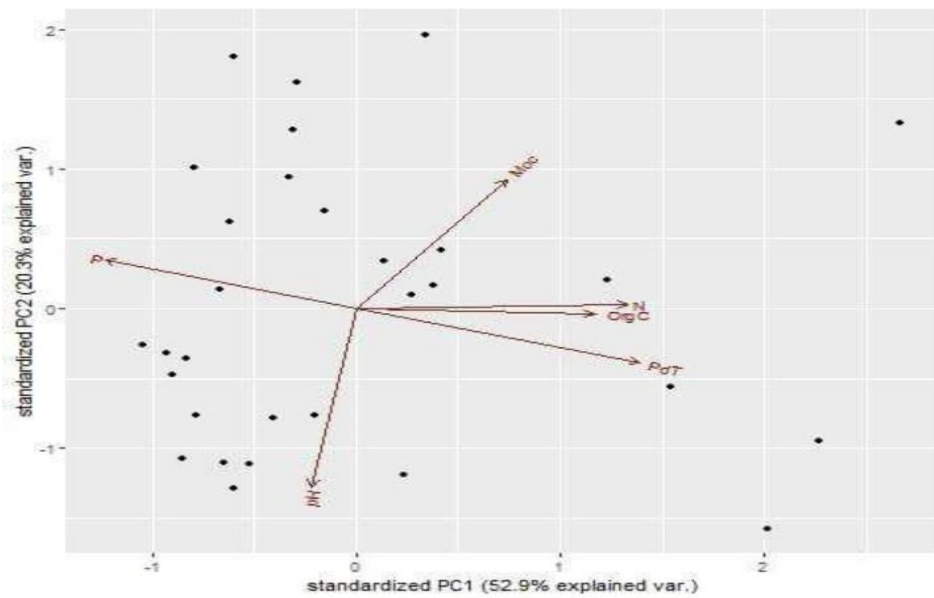

Fig. 4.1: PCA of the density of *Swertia chirayita* with different soil properties (Moc= Moisture Content, N= Total nitrogen content of the soil, OrgC= total carbon content of the soil, PdT= Population density of *S. chirayita*. pH= Soil pH, P= Soil phosphorous content

4.4 Associated species with *S. chirayita*

Altogether 50 species of 35 families were recorded in Laprak (Appendix 1), 61 species of 39 families in Barpak (Appendix 2), and 60 species of 47 families in Kashigaun (Appendix 3). Rosaceae is the family having the highest number of species in all three study sites (Barpak, Laprak, and Kashigaun).

In Barpak, *S. chirayita* had the highest importance value index (20.10) followed by *Setaria* sp. (14.69), *Miscanthus nepalensis* (Trin.) Hack (13.33), *Oxalis corniculata* L. (12.13), *Potentilla fulgens* Wall. ex Sims. (10.80), *Anaphalis contorta* (D. Don) Hook.f (9.79) respectively (Fig. 4.2).

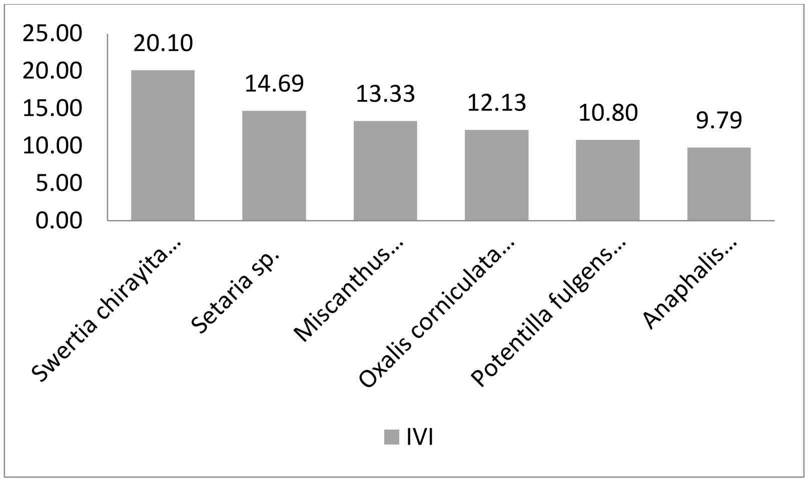

Fig. 4.2: IVI value of *S. chirayita* and its associated species (having high IVI value) at Barpak

In Laprak, *S. chirayita* had the highest importance value index (24.03) followed by *Osbeckia stellata* Buch.-Ham. ex D. Don (15.45), *Potentilla fulgens* Wall. ex Sims. (14.03), *Bidens pilosa* L. (13.56), *Anaphalis contorta* (D. Don) Hook.f (13.17), and *Oxalis corniculata* L. (11.97) respectively (Fig. 4.3).

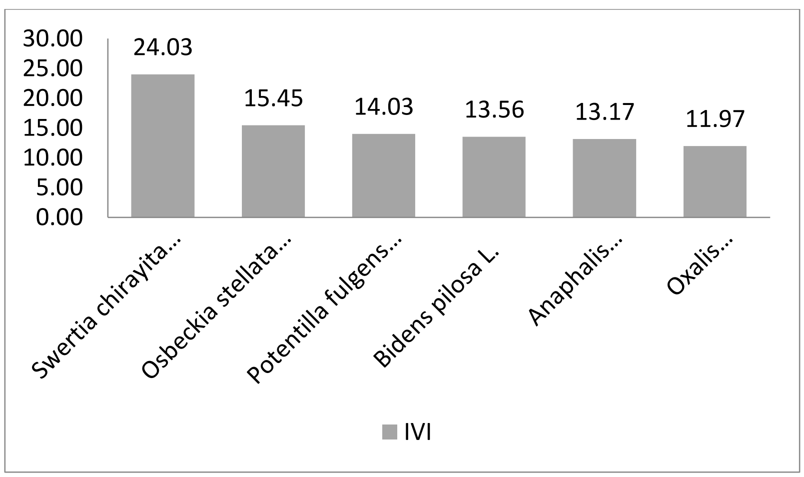

Fig. 4.3: IVI value of *S. chirayita* and its associated species (having high IVI value) at Kashigaun

In Kashigaun, *Oxalis corniculata* L. had the highest importance value index (30.03) followed by *Fragaria nilgerrensis* Schltdl. ex J. Gay (19.94), *Ageratina adenophora* (Spreng.) R. King and H. Rob. (19.88), *Artemisia indica* Willd. (18.04), *Swertia chirayita* (Roxb. Ex Fleming) H. Karst (18.04) and *Impatiens scabrida* DC (14.73) respectively (Fig. 4.4).

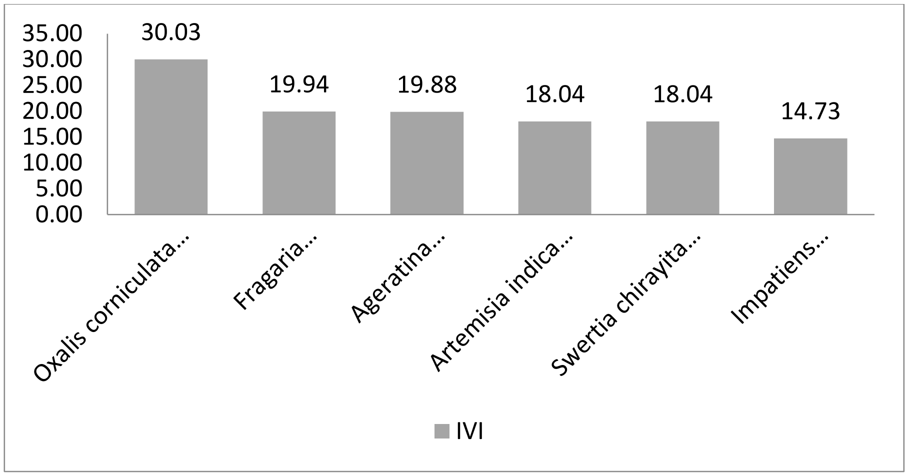

Fig. 4.4: IVI value of *S. chirayita* and its associated species (having high IVI value) at Barpak

Using the Sorenson (1948) similarity index, the similarity coefficient was found high between Larpak and Barpak (0.366) followed by Barpak and Kashigaun (0.229) and Larpak and Kashigaun (0.225).

5. DISCUSSION

5.1 Population Size and structure of *S. chirayita* in Gorkha

In the present study, quadrats were laid based on resource selection, hence the high frequency (>90%) of *Swertia chirayita* occurred. The high frequency of *Swertia chirayita* indicates that the species tends to cover the wide species range in suitable habitat. At the same time, low mean density (4.59-6.07 individuals/m^2) of *Swertia chirayita* in the present study shows that the species is unable to grow densely, which resembles with the findings of Bhatt *et al.* (2006) in the west and north-west Himalaya in India. The low population density of *S. chirayita* may be due to extensive harvesting from the wild population. As the whole plant has a high medicinal value (Kartikar and Basu, 1984; Nadkarni, 1998; Joshi and Dhawan, 2005), individuals are uprooted when needed; as a result, low densities might have occurred. The removal of the entire plant before seed maturation reduces the possibility of seed development for future regeneration (Sheldon *et al.*., 1997). In this study, the highest density of *S. chirayita* was found at Laprak (higher altitude than Barpak and Kashigaun). The higher densities of *S. chirayita* at higher altitudes suggest that it performs better at a higher elevation, which also supports the observation of Bhatt *et al.* (2006).

The natural population is usually a mixture of individuals of different ages or cohorts. Early cohorts are often large in size and show high fecundity and exhibit high survival rate (Zimmerman and Weiss, 1984; Kalisz, 1986; Miller, 1987), but some studies have reported the lower survival rate of early emerging cohorts (Baskin and Baskin, 1972; Van der Toorn and Ten Hove, 1982). This study showed the density of the rosette plant to be less than that of vegetative and reproductive aerials.

The distribution of *S. chirayita* is not uniform as it depends on altitude and slopes degree (Joshi and Dhawan, 2005; Phoboo and Jha, 2010; Sharma *et al.*, 2011). In this study, *S. chirayita* showed either contagious or random distribution, comparable to the findings of Bhatt *et al.* (2006) and Pradhan and Kumar (2015). Due to the significant variations in the environmental conditions, this (contagious or randon distribution) is the most common type of distribution in natural conditions (Odum, 1971). As this research was also conducted on the wild population, due to which this distribution might have occurred.

5.2 Physiographic and soil characteristics of *S. chirayita*

Swertia chirayita grows in the temperate Himalayas from Kashmir to Bhutan and the Khasia hills of Meghalaya (Chanda, 1976). It is distributed within the altitude of 1500-3000 masl throughout

Nepal, but 2000 masl is the most preferable range (Bhattarai and Shrestha, 1996). But in the present study, the highest density of *S. chirayita* were recorded from the altitude 2360-2825 masl in Laprak than in Baprak and Kashigaun. Bhatt *et al.* (2006) also reported the performance of the *S. chirayita* to be better at a higher altitude than the lower altitude as it has greater densities at higher altitudes. It prefers to grow in acidic soil conditions (Bhattarai and Shrestha, 1996; Barakoti, 2004). The total soil organic carbon, nitrogen content and phosphorous content of the *S. chirayita* growing region in the present study are also similar to Barakoti (2002). In the present study also, *S. chirayita* was found on open grassy slopes, which resembles with the findings of Bhatt *et al.* (2006). *Swertia chirayita* grows and performs well in open habitats such as open grassy slope, old landslide debris, and open moss-covered slope may be due to low interspecific competition for sunlight, moisture, and nutrients.

The population of *S. chirayita* occurred in varied habitats (different slopes and altitude) with varied environmental conditions (different total soil organic carbon and moisture conthent) was probably due to its broad range of tolerance to light and moisture and also to different soil types, which is also supported by Joshi and Dhawan (2005).

5.3 Relation of *S. chirayita* density with different soil and environmental factors

The density of *Swertia chirayita* had a significant relation with total nitrogen content, phosphorous content, and total organic soil carbon at all rosette, vegetative aerials, and reproductive aerials forms. The significance and non-significance of the density of *S. chirayita* with soil phosphorous content, total nitrogen content of the soil, soil pH, total soil organic carbon, altitude, soil moisture content and slope angle in the correlation in all life stages are similar. This may be due to the collection of the soil sample from the same plot where all life stages were present and no seasonal collection of the data was done separately for separate life stages. Optimum phosphorous is required along with soil pH to stimulate flowering (Erel, 2008). This may be the reason for the significant correlation of phosphorous with plant density as the study was conducted during the flowering season. The positive significant correlation was observed between total nitrogen content of soil and density of *S. chirayita,* which indicates that the nitrogen rich soil favors the growth of *Swertia chirayita*.

The population density of *S. chirayita* in all stages of its life cycle didn't show any correlation with soil pH, altitude, moisture content, and slope. This may be the possible explanation for the fact that the plant can establish in a wider range of habitat than other forest plant species.

The significant positive correlation was observed between soil moisture content and altitude in the present altitude. Smith (1979) reported an increase in precipitation with increase in altitude in mountain region, which might be the reason for the significant correlation between soil moisture content and altitude the present study. Similarly, significant negative correlation was observed between total soil organic carbon and altitude in the present study, which might be due to increase in precipitation with elevation (Smith, 1979) as the rain washes up the above ground organic matter of the soil and lowers the soil microbial activities. The decrease in soil microbial activities with increase in altitude was also reported by Bangroo *et al.* (2017).

5.4 Associated species with *S. chirayita*

Swertia chirayita grows and performs well in open habitats such as open grassy slope, old landslide debris, and open moss-covered slope may be due to low interspecific competition for sunlight, moisture, and nutrients (Kalliovirta *et al.*, 2006). In the study area, only a few tree species and more herbs species were recorded. Most of the associated species were common in three study sites (Laprak, Barpak, and Kashigaun). As the plant is found between the specific altitude (1500-3000masl), the associated species found at that altitude level are also somewhat similar. The associated species *Ageratina adenophora*, *Artemisia vulgaris*, *Anaphalis triplinervis, Anemone obtusiloba, Stachys sp., Desmodium sp., Anemone sp., Elsholtzia sp., Fragaria sp., Oxalis corniculata*, etc. recorded in this study were also observed by Kunwar (2003), Ghimire *et al.* (2008), Pyakurel (2008) and Pradhan and Kumar (2015). Invasive plant species like A*geratina adenophora, Bidens pilosa* and *Oxalis latifolia* were also found associated with *S. chirayita* in the study site.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study concludes that the Gorkha district offers a high potential and greater availability of *S. chirayita*. The population of *S. chirayita* occurred in varied habitats with varied environmental conditions was probably due to its broad range of tolerance to light and moisture and also to different soil types. It was recorded from the places having acidic soil and high organic matter content.

Although the density of the species was recorded low, its frequency was high (i.e.>90%). It may be due to the rare occurrence of the plant which again may be due to over-collection of the plant for medicinal purposes. The highest density of the plant was recorded from the Laprak, where the study was carried out at higher altitudes as compared to Barpak and Kashigaun. It may be due to the preferable climatic conditions, all soil conditions, and favorable microhabitat conditions in that site.

The density of the reproductive aerials was found comparatively low with that of the rosette and vegetative aerials at Barpak and Kashigaun which might be due to harvesting of the whole plant before reaching the reproductive stage.

The *Swertia chirayita* was found to grow densely on soil rich in nitrogen, it means it favors the nitrogen rich soil.

In Barpak and Kashigaun, invasive species like A*geratina adenophora, Bidens pilosa* and *Oxalis latifolia* were also found associated with *S. chirayita* in the study site. These invasive species may change the habitat of *S. chirayita* and may decline its population in the future.

6.2 Recommendations

i) On the basis of highest densities of *Swertia chirayita* at higher altitude (Laprak) than lower altitudes (Barpak and Kashigaun), cultivation of the species is recommended at higher altitudes.

ii) Relation of the phenology of the plant with different soil factors is recommended.

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APPENDIX - I: IVI of the associated species in Laprak

S.N.	Botanical name	Family	F	RF	D	RD	A	RA	IVI
1	*Achyranthes aspera* L.	Amaranthaceae	0.27	2.19	0.84	2.04	3.17	2.21	6.44
2	*Adiantum tibeticum* Ching and Y.X.Lin	Pteridaceae	0.16	1.28	0.29	0.7	1.86	1.29	3.27
3	*Ageratum conyzoides* L.	Asteraceae	0.21	1.74	0.37	0.88	1.74	1.21	3.83
4	*Alnus nepalensis* D. Don.	Betulaceae	0.11	0.91	0.13	0.32	1.2	0.84	2.07
5	*Anaphalis contorta* (D. Don) Hook.f	Asteraceae	0.54	4.48	2.36	5.68	4.33	3.01	13.17
6	*Anaphalis triplinervis* Sims. ex C.B. Clarke.	Asteraceae	0.34	2.83	1.08	2.6	3.13	2.18	7.61
7	*Anemone rivularis* Buch.-Ham. ex DC.	Ranunculaceae	0.06	0.46	0.12	0.29	2.2	1.53	2.28
8	*Arisaema concinnum* Schott	Araceae	0.17	1.37	0.26	0.62	1.53	1.07	3.06
9	*Arisaema tortuosum* (Wall.) Schott	Araceae	0.09	0.73	0.14	0.35	1.63	1.13	2.21
10	*Artemisia dubia* Will.	Compositae	0.19	1.55	0.84	2.04	4.47	3.11	6.7
11	*Berberis aristata* DC	Berberidaceae	0.14	1.19	0.36	0.86	2.46	1.71	3.76
12	*Bidens pilosa* L.	Compositae	0.46	3.75	2.49	6	5.46	3.81	13.56
13	*Boehmeria platyphylla* D. Don	Urticaceae	0.07	0.55	0.18	0.43	2.67	1.86	2.83
14	*Campanula pallida* Wall.	Campanulaceae	0.08	0.64	0.13	0.32	1.71	1.19	2.16
15	*Cautleya spicata* (Sm.) Baker	Zingiberaceae	0.16	1.28	0.19	0.46	1.21	0.85	2.58
16	*Centella asiatica* (L.) Urban.	Umbelliferae	0.37	3.02	1.23	2.97	3.36	2.34	8.33
17	*Cotoneaster microphyllus* Wall. ex Lindl.	Rosaceae	0.08	0.64	0.16	0.38	2	1.39	2.41
18	*Curculigo orchioides* Gaertn	Hypoxidaceae	0.43	3.56	1.62	3.91	3.74	2.61	10.08
19	*Cyperus* sp.	Cyperaceae	0.08	0.64	0.19	0.46	2.43	1.69	2.79
20	*Daphne bholua* Buch.-Ham.ex D. Don	Thymelaeaceae	0.36	2.92	1.08	2.6	3.03	2.11	7.64
21	*Digitaria ciliaris* (Retz.) Koeler	Gramineae	0.37	3.02	1.24	3	3.39	2.36	8.38
22	*Erigeron cuneifolius* DC.	Asteraceae	0.09	0.73	0.36	0.86	4	2.79	4.38
23	*Erigeron sublyratus* DC.	Compositae	0.23	1.92	0.74	1.8	3.19	2.22	5.94
24	*Euphorbia hirta* L.	Euphorbiaceae	0.08	0.64	0.13	0.32	1.71	1.19	2.16
25	*Galium aparine* L.	Rubiaceae	0.37	3.02	1.24	3	3.39	2.36	8.38
26	*Geranium nepalense* Sweet	Geraniaceae	0.18	1.46	0.84	2.04	4.75	3.31	6.81

27	*Hemiphragma heterophyllum* Wall.	Plantaginaceae	0.12	1.01	0.28	0.67	2.27	1.58	3.26
28	*Ilex dipyrena* Wall.	Aquifoliaceae	0.3	2.47	1.01	2.44	3.37	2.35	7.25
29	*Justicia adhatoda* L.	Acanthaceae	0.3	2.47	0.74	1.8	2.48	1.73	5.99
30	*Lilium nepalense* D. Don	Liliaceae	0.06	0.46	0.12	0.29	2.2	1.53	2.28
31	*Lycopodium clavatum* L.	Lycopodiaceae	0.33	2.74	0.66	1.58	1.97	1.37	5.69
32	*Lyonia ovalifolia* (Wall.) Drude	Ericaceae	0.22	1.83	0.26	0.62	1.15	0.8	3.25
33	*Osbeckia stellata* Buch.-Ham. ex D. Don	Melastomataceae	0.48	3.93	2.98	7.18	6.23	4.34	15.45
34	*Oxalis corniculata* L.	Oxalidaceae	0.37	3.02	2.08	5.01	5.67	3.95	11.97
35	*Pinus roxburghii* Sarg.	Pinaceae	0.23	1.92	0.27	0.64	1.14	0.8	3.36
36	*Poa stapfiana* Bor	Poaceae	0.38	3.11	1.34	3.24	3.56	2.48	8.83
37	*Potentilla fulgens* Wall. ex Sims.	Rosaceae	0.52	4.3	2.6	6.27	4.98	3.47	14.03
38	*Prunella vulgaris* L.	Lamiaceae	0.12	1.01	0.16	0.38	1.27	0.89	2.27
39	*Pteridium* sp.	Pteridaceae	0.18	1.46	0.26	0.62	1.44	1	3.08
40	*Rhododendron arboretum* Sm.	Ericaceae	0.12	1.01	0.14	0.35	1.18	0.82	2.18
41	*Roscoea alpina* Royle	Zingiberaceae	0.18	1.46	0.42	1.02	2.38	1.65	4.14
42	*Rubus acuminatus* Sm.	Rosaceae	0.13	1.1	0.22	0.54	1.67	1.16	2.79
43	*Rubus ellipticus* Sm.	Rosaceae	0.43	3.56	1.26	3.03	2.9	2.02	8.61
44	*Selaginella* sp.	Selaginellaceae	0.13	1.1	0.28	0.67	2.08	1.45	3.22
45	*Stellaria latifolia* Edgew. & Hook. f.	Caryophyllaceae	0.16	1.28	0.4	0.96	2.57	1.79	4.04
46	*Swertia chirayita* (Roxb. ex Fleming) H. Karst	Gentianaceae	0.93	7.68	5.18	12.49	5.55	3.86	24.03
47	Unidentified 1	Lamiaceae	0.46	3.75	0.82	1.98	1.8	1.26	6.99
48	Unidentified 3	Polygonaceae	0.03	0.27	0.08	0.19	2.33	1.63	2.09
49	Unidentified 4	Rosaceae	0.08	0.64	0.47	1.13	6	4.18	5.95
50	*Viola biflora* L.	Violaceae	0.23	1.92	0.83	2.01	3.57	2.49	6.42
	Total		12.16		41.47		143.54		299.99

APPENDIX - II: IVI of the associated species in Barpak

S.N.	Botanical name	Family	F	RF	D	RD	A	RA	IVI
1	*Adiantum tibeticum* Ching and Y.X.Lin	Pteridaceae	0.16	1.73	0.79	1.18	5.07	1.25	4.16
2	*Ageratina adenophora* (Spreng.) R. King and H. Rob.	Asteraceae	0.21	2.35	1.83	2.73	8.68	2.15	7.23
3	*Ageratum conyzoides* L.	Asteraceae	0.03	0.37	0.07	0.10	2.00	0.49	0.96
4	*Ainsliaea latifolia* (D. Don) Sch. Bip.	Asteraceae	0.09	0.99	0.38	0.56	4.25	1.05	2.60
5	*Anaphalis contorta* (D. Don) Hook.f	Asteraceae	0.22	2.47	2.81	4.19	12.65	3.13	9.79
6	*Anaphalis triplinervis* Sims. ex C.B. Clarke.	Asteraceae	0.20	2.22	2.32	3.46	11.61	2.87	8.55
7	*Anemone rivularis* Buch.-Ham. ex DC.	Ranunculaceae	0.19	2.10	1.26	1.87	6.65	1.64	5.61
8	*Arisaema tortuosum* (Wall.) Schott	Araceae	0.17	1.85	0.51	0.76	3.07	0.76	3.37
9	*Artemisia dubia* Will.	Compositae	0.19	2.10	1.93	2.88	10.24	2.53	7.51
10	*Arundinella nepalensis* Trin.	Poaceae	0.09	0.99	0.67	0.99	7.50	1.85	3.83
11	*Berberis aristata* DC	Berberidaceae	0.10	1.11	0.29	0.43	2.89	0.71	2.26
12	*Boehmeria platyphylla* D. Don	Urticaceae	0.26	2.84	2.78	4.14	10.87	2.69	9.67
13	*Bupleurum longicaule* Wall.	Apiaceae	0.03	0.37	0.21	0.31	6.33	1.56	2.25
14	*Butomopsis latifolia* (D.Don)Kanth.	Butomaceae	0.09	0.99	0.22	0.33	2.50	0.62	1.94
15	*Catharanthus roseus* (L.) G. Don	Apocynaceae	0.03	0.37	0.18	0.27	5.33	1.32	1.95
16	*Centella asiatica* (L.) Urban.	Umbelliferae	0.13	1.48	0.37	0.55	2.75	0.68	2.71
17	*Coccinia grandia* (L.) Viogct.	Cucurbitaceae	0.06	0.62	0.08	0.12	1.40	0.35	1.08
18	*Cotoneaster microphyllus* Wall. ex Lindl.	Rosaceae	0.10	1.11	0.23	0.35	2.33	0.58	2.04
19	*Cynodon dactylon* (L.) Pers.	Gramineae	0.07	0.74	0.39	0.58	5.83	1.44	2.76
20	*Cyperus* sp.	Cyperaceae	0.19	2.10	1.07	1.59	5.65	1.40	5.08
21	*Desmodium elegans* DC.	Leguminosae	0.21	2.35	0.33	0.50	1.58	0.39	3.23
22	*Digitaria ciliaris* (Retz.) Koeler	Gramineae	0.13	1.48	1.07	1.59	8.00	1.98	5.05
23	*Elsholtzia* sp.	Lamiaceae	0.01	0.12	0.01	0.02	1.00	0.25	0.39
24	*Erigeron sublyratus* DC.	Compositae	0.23	2.59	1.44	2.15	6.19	1.53	6.28
25	*Eriocapitella vitifolia* (Buch.-Ham.ex DC.) Nakai	Ranunculaceae	0.27	2.96	2.71	4.04	10.17	2.51	9.52
26	*Fragaria nilgerrensis* Schltdl. ex J. Gay	Rosaceae	0.12	1.36	0.88	1.31	7.18	1.77	4.44
27	*Galium aparine* L.	Rubiaceae	0.11	1.23	0.60	0.89	5.40	1.33	3.46

28	*Globba clarkei* Baker	Zingiberaceae	0.13	1.48	0.92	1.38	6.92	1.71	4.57
29	*Hemiphragma heterophyllum* Wall.	Plantaginaceae	0.10	1.11	1.00	1.49	10.00	2.47	5.07
30	*Impatiens scabrida* DC	Balsaminaceae	0.03	0.37	0.61	0.91	18.33	4.53	5.81
31	*Indigofera cassioides* Rottler ex DC.	Fabaceae	0.07	0.74	0.31	0.46	4.67	1.15	2.36
32	*Justicia adhatoda* L.	Acanthaceae	0.09	0.99	0.54	0.81	6.13	1.51	3.31
33	*Lilium nepalense* D. Don	Liliaceae	0.08	0.86	0.18	0.27	2.29	0.56	1.69
34	*Lindera pulcherrima* (Nees) Benth. ex Hook.f	Lauraceae	0.10	1.11	0.17	0.25	1.67	0.41	1.77
35	*Lyonia ovalifolia* (Wall.) Drude	Ericaceae	0.06	0.62	0.18	0.27	3.20	0.79	1.67
36	*Miscanthus nepalensis* (Trin.) Hack	Poaceae	0.19	2.10	4.01	5.98	21.24	5.25	13.33
37	*Osbeckia stellata* Buch.-Ham. ex D. Don	Melastomataceae	0.18	1.98	1.54	2.30	8.69	2.15	6.42
38	*Oxalis acetosella* L.	Oxalidaceae	0.10	1.11	0.58	0.86	5.78	1.43	3.40
39	*Oxalis corniculata* L.	Oxalidaceae	0.17	1.85	3.46	5.15	20.73	5.12	12.13
40	*Potentilla fulgens* Wall. ex Sims.	Rosaceae	0.22	2.47	3.20	4.77	14.40	3.56	10.80
41	*Potentilla monanthes* Wall. ex Lehm.	Rosaceae	0.21	2.35	1.08	1.61	5.11	1.26	5.21
42	*Prunella vulgaris* L.	Lamiaceae	0.09	0.99	0.20	0.30	2.25	0.56	1.84
43	*Pteridium revolutum* (Bl.) Nakai	Dennstaedtiaceae	0.12	1.36	0.96	1.43	7.82	1.93	4.71
44	*Pteridium* sp.	Pteridaceae	0.18	1.98	0.74	1.11	4.19	1.03	4.12
45	*Roscoea alpina* Royle	Zingiberaceae	0.10	1.11	0.26	0.38	2.56	0.63	2.12
46	*Rubia manjith* Roxb.	Rubiaceae	0.27	2.96	1.44	2.15	5.42	1.34	6.46
47	*Rubus acuminatus* Sm.	Rosaceae	0.08	0.86	0.52	0.78	6.71	1.66	3.30
48	*Rubus ellipticus* Sm.	Rosaceae	0.23	2.59	1.28	1.91	5.48	1.35	5.85
49	*Rumex hastatus* D. Don	Polygonaceae	0.13	1.48	1.48	2.20	11.08	2.74	6.42
50	*Satyrium nepalense* D.Don	Orchidaceae	0.10	1.11	0.26	0.38	2.56	0.63	2.12
51	*Selaginella* sp.	Selaginellaceae	0.11	1.23	0.77	1.14	6.90	1.70	4.08
52	*Setaria* sp.	Poaceae	0.26	2.84	4.82	7.19	18.87	4.66	14.69
53	*Stellaria latifolia* Edgew. & Hook. f.	Caryophyllaceae	0.10	1.11	0.36	0.53	3.56	0.88	2.52
54	*Stephania japonica* (Thunb.) Miers	Menispermaceae	0.04	0.49	0.16	0.23	3.50	0.86	1.59
55	*Swertia chirayita* (Roxb. ex Fleming) H. Karst	Gentianaceae	0.92	10.25	5.60	8.35	6.07	1.50	20.10
56	*Thalictrum foliolosum* DC	Ranunculaceae	0.20	2.22	2.58	3.84	12.89	3.18	9.25

57	Unidentified 3	Polygonaceae	0.34	3.83	0.99	1.47	2.87	0.71	6.01
58	Unidentified 5	Scrophalariaceae	0.04	0.49	0.12	0.18	2.75	0.68	1.36
59	*Veronica cana* Wall. ex Benth	Plantaginaceae	0.03	0.37	0.06	0.08	1.67	0.41	0.87
60	*Viburnum cylindricum* Buch.-Ham. ex D.Don	Caprifoliaceae	0.16	1.73	0.79	1.18	5.07	1.25	4.16
61	*Viola biflora* L.	Violaceae	0.08	0.86	0.49	0.73	6.29	1.55	3.15
	Total		9.00		67.06		404.74		300.00

APPENDIX - III: IVI of the associated species in Kashigaun

S.N.	**Botanical name**	**Family**	**F**	**RF**	**D**	RD	A	RA	IVI
1	*Ageratina adenophora* (Spreng.) R. King and H. Rob.	Asteraceae	0.49	5.68	7.44	9.89	15.23	4.30	19.88
2	*Alocasia* sp.	Araceae	0.08	0.90	0.36	0.47	4.57	1.29	2.67
3	*Anaphalis contorta* (D. Don) Hook.f	Asteraceae	0.27	3.10	2.11	2.81	7.92	2.24	8.14
4	*Arisaema concinnum* Schott	Araceae	0.08	0.90	0.21	0.28	2.71	0.77	1.95
5	*Arisaema jacquemontii* Blume	Araceae	0.04	0.52	0.07	0.09	1.50	0.42	1.03
6	*Artemisia indica* Willd.	Compositae	0.50	5.81	6.46	8.58	12.91	3.65	18.04
7	*Asparagus racemosus* Willd.	Liliaceae	0.08	0.90	0.16	0.21	2.00	0.57	1.68
8	*Asplenium* sp.	Aspleniaceae	0.13	1.55	0.62	0.83	4.67	1.32	3.70
9	*Bauhinia purpurea* L.	Leguminosae	0.04	0.52	0.12	0.16	2.75	0.78	1.46
10	*Berberis aristata* DC	Berberidaceae	0.06	0.65	0.22	0.30	4.00	1.13	2.07
11	*Bergenia ciliata* (Haw.) Stemb.	Saxifragaceae	0.27	3.10	1.70	2.26	6.38	1.80	7.16
12	*Butomopsis latifolia* (D.Don)Kanth.	Butomaceae	0.07	0.78	0.19	0.25	2.83	0.80	1.83
13	*Carex cruciata* Wahlenb.	Cyperaceae	0.07	0.78	0.39	0.52	5.83	1.65	2.94
14	*Cassia mimosoides* L.	Leguminosae	0.04	0.52	0.40	0.53	9.00	2.54	3.59
15	*Centella asiatica* (L.) Urban.	Umbelliferae	0.06	0.65	0.23	0.31	4.20	1.19	2.14
16	*Cinnamomum tamala* (Buch.-Ham.) T. Nees and C.H. Eberm	Lauraceae	0.12	1.42	0.72	0.96	5.91	1.67	4.05
17	*Crotalaria kanaii* H. Ohashi	Fabaceae	0.04	0.52	0.21	0.28	4.75	1.34	2.14
18	*Cuscuta reflexa* Roxb.	Convolvulaceae	0.19	2.20	1.08	1.43	5.71	1.61	5.24
19	*Dendrobium heterocarpum* Wall. ex Lindl	Orchidaceae	0.03	0.39	0.08	0.10	2.33	0.66	1.15
20	*Desmodium elegans* DC.	Leguminosae	0.14	1.68	0.60	0.80	4.15	1.17	3.65
21	*Dicranopteris linearis* (Burm.) Underw.	Gleicheniaceae	0.07	0.78	0.32	0.43	4.83	1.37	2.57
22	*Dryopteris cochleata* (D.Don.)C. Chr.	Aspidiaceae	0.01	0.13	0.03	0.04	3.00	0.85	1.02
23	*Elymus* sp.	Poaceae	0.10	1.16	1.97	2.61	19.67	5.56	9.33
24	*Euphorbia hirta* L.	Euphorbiaceae	0.07	0.78	0.10	0.13	1.50	0.42	1.33
25	*Ficus lacor* Buch-Ham	Moraceae	0.08	0.90	0.10	0.13	1.29	0.36	1.40
26	*Fragaria nilgerrensis* Schltdl. ex J. Gay	Rosaceae	0.48	5.56	7.49	9.95	15.67	4.43	19.94
27	*Hedychium coronarium* J. Koenig	Zingiberaceae	0.18	2.07	1.01	1.34	5.69	1.61	5.02

28	*Herpetospermum pedunculosum* (Ser.) Baill.	Cucurbitaceae	0.03	0.39	0.04	0.06	1.33	0.38	0.82
29	*Hypericum* sp.	Hyperiaceae	0.03	0.39	0.07	0.09	2.00	0.57	1.04
30	*Impatiens falcifer* Hook. F	Balsaminaceae	0.06	0.65	0.46	0.61	8.20	2.32	3.57
31	*Impatiens scabrida* DC	Balsaminaceae	0.10	1.16	3.27	4.34	32.67	9.23	14.73
32	*Litsea monopelata* (Roxb.) Pers.	Lauraceae	0.04	0.52	0.06	0.07	1.25	0.35	0.94
33	*Melastoma melabathricum* L.	Melastomaceae	0.13	1.55	0.63	0.84	4.75	1.34	3.73
34	*Myrica esculenta* Buch.-Ham. ex D.Don	Myricaceae	0.04	0.52	0.06	0.07	1.25	0.35	0.94
35	*Origanum vulgare* L.	Labiatae	0.04	0.52	0.06	0.07	1.25	0.35	0.94
36	*Osbeckia stellata* Buch.-Ham. ex D. Don	Melastomataceae	0.10	1.16	1.59	2.11	15.89	4.49	7.76
37	*Oxalis corniculata* L.	Oxalidaceae	0.70	8.14	12.63	16.79	18.05	5.10	30.03
38	*Phyllanthus parvifolius* Buch.-Ham. ex D. Don	Euphorbiaceae	0.02	0.26	0.03	0.04	1.50	0.42	0.73
39	*Polystichum* sp.	Dryopteridaceae	0.01	0.13	0.02	0.03	2.00	0.57	0.72
40	*Potentilla fulgens* Wall. ex Sims.	Rosaceae	0.27	3.10	1.58	2.10	5.92	1.67	6.87
41	*Pyracantha crenulata* (D.Don.) Roem.	Rosaceae	0.04	0.52	0.38	0.50	8.50	2.40	3.42
42	*Pyrus pashia* Buch.-Ham. ex D.Don	Rosaceae	0.04	0.52	0.10	0.13	2.25	0.64	1.29
43	*Quercus semecarpifolia* J.E. Smith.	Fagaceae	0.02	0.26	0.02	0.03	1.00	0.28	0.57
44	*Rhododendron arboretum* Sm.	Ericaceae	0.06	0.65	0.06	0.07	1.00	0.28	1.00
45	*Roscoea purpurea* Sm.	Zingiberaceae	0.09	1.03	0.47	0.62	5.25	1.48	3.14
46	*Rubia manjith* Roxb.	Rubiaceae	0.10	1.16	0.40	0.53	4.00	1.13	2.82
47	*Rubus ellipticus* Sm.	Rosaceae	0.11	1.29	0.21	0.28	1.90	0.54	2.11
48	*Schima wallichii* (DC.) Korth.	Theaceae	0.02	0.26	0.03	0.04	1.50	0.42	0.73
49	*Selaginella* sp.	Selaginellaceae	0.17	1.94	0.88	1.17	5.27	1.49	4.59
50	*Solanum torvum* Swartz.	Solanaceae	0.02	0.26	0.04	0.06	2.00	0.57	0.88
51	*Spiranthes sinensis* (Pers.) Ames.	Orchidaceae	0.23	2.71	1.12	1.49	4.81	1.36	5.56
52	*Stachys melissaefolia* Benth.	Lamiaceae	0.06	0.65	0.19	0.25	3.40	0.96	1.86
53	*Swertia chirayita* (Roxb. ex Fleming) H. Karst	Gentianaceae	0.94	10.98	4.33	5.76	4.59	1.30	18.04
54	*Thalictrum foliolosum* DC	Ranunculaceae	0.30	3.49	4.36	5.79	14.52	4.10	13.38
55	Unidentified 1	Lamiaceae	0.03	0.39	0.20	0.27	6.00	1.70	2.35

56	Unidentified 2	Compositae	0.11	1.29	0.60	0.80	5.40	1.53	3.62
57	Unidentified 3	Polygonaceae	0.03	0.39	0.11	0.15	3.33	0.94	1.48
58	Unidentified 4	Rosaceae	0.08	0.90	0.38	0.50	4.86	1.37	2.78
59	Unidentified 6	Compositae	0.34	4.01	3.44	4.58	10.00	2.83	11.41
60	*Viola biflora* L.	Violaceae	0.42	4.91	3.06	4.06	7.24	2.05	11.01

APPENDIX - IV: PHOTOPLATE

1. Searching for S. chirayita with local people

2. Selecting plot for data collection

3. Making plots

4. $10\times10m^2$ plots and $1\times1m^2$ quadrats inside it

5. Sheep and Goats on the study site

6. Cattle grazing on the study site

7. Rosette of *S. chirayita*

8. Vegetative aerials of *S. chirayita*

9. Reproductive aerial *S. chirayita* with its length

10. My data collecting team with the president of Bhume Mantuli Community forest, Laprak