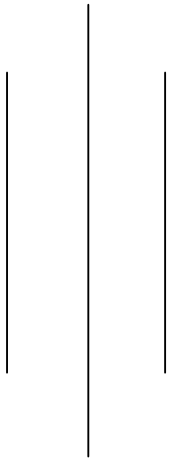
ECOLOGICAL STUDY OF MEDICINAL PLANT PARIS POLYPHYLLA IN GHANDRUK VDC, KASKI, NEPAL

A dissertation submitted as a partial fulfillment for the requirement of Master's Degree of Science in Botany (Ecology)



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CENTRAL DEPARTMENT OF BOTANY

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KIRTIPUR, KATHMANDU

NEPAL

RECOMMENDATION

This is to certify that the dissertation work entitled "**Ecological Study of Medicinal Plant- *Paris Polyphylla* In Ghandruk VDC, Kaski, Nepal"** submitted by **Ms. Madhu K.C.** has been carried out under our joint supervision. The entire work is primarily based on the results of her research work and has not been submitted for any other degree. We recommend this dissertation work to be accepted for the partial fulfillment of Master of Science in Botany (Ecology) from Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

This dissertation paper entitled **"Ecological Study of Medicinal Plant- *Paris Polyphylla* In Ghandruk VDC, Kaski, Nepal"** submitted at the Central Department of Botany, Tribhuvan University by **Ms. Madhu K.C.** has been accepted for the partial fulfillment of requirements for Master of Science in Botany (Ecology).

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ABSTRACT

Medicinal plants are the local heritage of global importance as they contribute towards quality health care. While demand for medicinal plants is increasing, their survival in natural habitat is under growing threat due to man made and natural calamities. *Paris polyphylla* Sm. (Satuwa) is one of the medicinal plants, listed as vulnerable under IUCN threat category and found in altitude ranging from 2100 m to 2900m altitude. Local people use this plant for headache, fever, burns and wounds and to counteract poison. Ecological information on this medicinal plant is lacking. Therefore, the present study was undertaken to document the ecological status, distribution pattern and the reproductive biology of this plant in Ghandruk VDC of Central Nepal.

The research was done in four different localities of Ghandruk VDC viz, Ghandruk village, Komrong Danda, Chhomrong and Tadapani. Five transects were laid out at 20 – 50m distance and six quadrats of 1m x 1m was laid out in an interval of 5m. Plant's number, coverage, associated species, litter coverage and thickness were noted. Soil test, seed length, breadth, weight, output, viability and germination, dry biomass of rhizome, antibacterial activity of seed and rhizome were also studied. The average population density of the plant in study area was found to be low in Ghandruk VDC (1.78 ind./m^2). The plant was found growing in moist, acidic soil with high nutrient content. No commercial collection was found to be undertaken in the study area but the collection for domestic use was found to be done in an unsustainable manner. Seed viability was found to be low and the seeds did not germinate in laboratory conditions even under different chemical treatments. The plant was found to reproduce mainly by vegetative propagation in the field. There seems to be a need for raising awareness among the local people about the sustainable use of the rhizome of this plant and its cultivation practice for the conservation of this plant for the future.

ACRONYMS

ACA	Annapurna Conservation Area
ACAP	Annapurna Conservation Area Project
CAMP	Conservation Assessment and Management Plan
Conc.	Concentration
DPR	Department of Plant Resources
GoN	Government of Nepal
ha	hectare
ind	individual
IUCN	International Union for Conservation of Nature
K	Potassium
KATH	National Herbarium and Plant Laboratory
km^2	square kilometer
LRMP	Land Resource Mapping Project
m^2	square meter
MAP	Medicinal and Aromatic Plant
MIC	Minimum Inhibitory Concentration
MoFSC	Ministry of Forest and Soil Conservation
N	Nitrogen
No.	Number
NTFP	Non Timber Forest Product
NW	North West
OM	Organic Matter
P	Phosphorus
pl	plant
ppm	parts per million
RAPD	Random Amplified Polypmorphic DNA
sp.	species
TTC	Triphenyltetrazolium chloride
TUCH	Tribhuvan Univeristy Central Herbarium
VDC	Village Development Committee

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Chapter 1

INTRODUCTION

1.1 Background

Medicinal plants are globally important natural plant resource, as they contribute towards quality health care and have potentiality to augment income and employment for rural communities. Billions of people in the world rely chiefly on herbal medicines. Medicinal plants play a vital role for the development of new drugs. Medicinal plants are viewed as a possible bridge between sustainable economic development, affordable health care and conserving vital biodiversity (Dhar *et al.* 2002)

At present wild and useful medicinal plants are highly threatened. Present rapid socio-economic changes/damages and several years of unregulated collection of these medicinal plant species have resulted in the depletion of their populations. Many of them have become rare and endangered and some species are on the verge of extinction (Kala, 2000). Among the many factors involved in the depletion of a species from the wild, the most important is the small population size (Oostermeijer, 1999) and restricted distribution of plant species (Semwall *et al*. 2007). In some cases the population size of a species can inherently be low or at times anthropogenic pressure in the form of grazing, trampling and extraction result in the decline in population (Uniyal *et al*. 2002).

All the institutions involved in medicinal plant research, policy makers and various stakeholders should, prepare a state level action plan for conservation and sustainable utilization of medicinal plants. Initially this would require detailed inventory of populations related to distribution, uses, status, cultivation practices and traditional knowledge (Dhar *et al*. 2002). Studies on quantitative assessment 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 and Handa, 2001). Variations in response to environmental stresses are species specific and therefore must be considered while developing strategies for sustainable harvest and conservation (Airi *et al*. 2000).

Even if a particular variety of a plant is put under several millions hectares of active cultivation, the species can still go extinct in the wild if its wild populations with all their inherent intra-specific diversity is not conserved. So the only way to decrease over exploitation is to encourage sustainable and discrete collection of medicinal plants from the wild. For this purpose, knowledge about distribution and ecological features of the plants are necessary (Bhattacharya and Sharma, 2008). But the availability and population status of the important medicinal plants in their wild habitats is limited. Total of 138 native vascular plants taxa are threatened in Nepal, including over 50 species of medicinal plants only (CAMP 2001, Tandon *et al.* 2001). One of them is *Paris polyphylla* Sm. which is listed under vulnerable category (V) by IUCN and CAMP. The present study was undertaken in Ghandruk VDC to document the ecological status of the plant *Paris polyphylla*, one of the important medicinal plants found in temperate belt.

1.2 Plant Description

Paris polyphylla Smith, Synonym - *Daiswa polyphylla* (Sm.) Raf., Family: Liliaceae is an important perennial medicinal plant growing under the canopy of moist temperate forest in Nepal.

Photo: Post flowering stage of *Paris polyphylla*

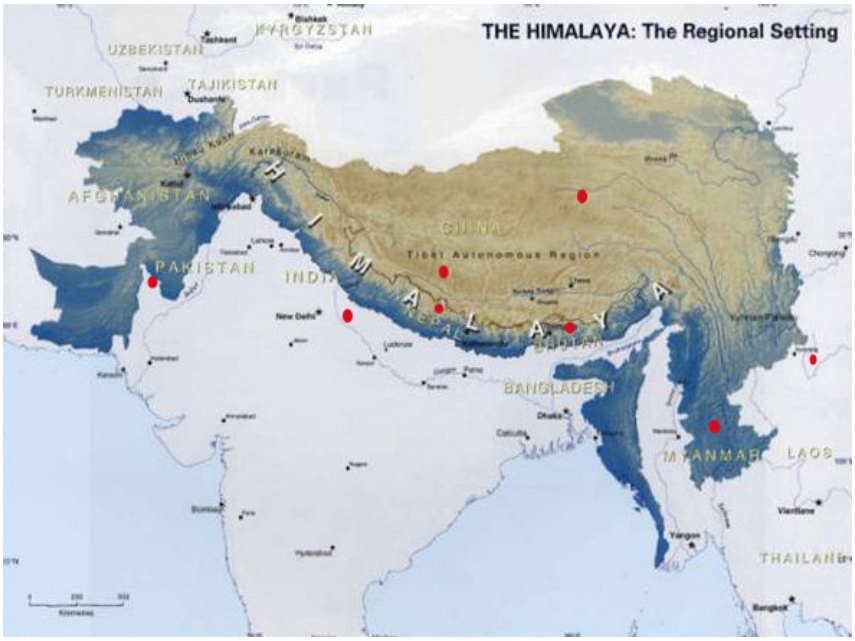
It is a perennial herb, upto 1m tall. Stem is erect with whorl of 6 -9 (13) leaves in upper part. Leaves are oblong – oblanceolate, finely acuminate with truncate base and glaborous surface. The size of leaf varies from (7 - 13.7) cm x (3.5 - 6) cm while petioles ranges 1.2 – 4 cm in length. Flower is single, terminal and pedicellate with 0.9 - 5.5 cm (upto 7.4 cm in fruit) long pedicel. Sepals are persistent, lanceolate, acuminate, usually 4 (sometimes 3 or 6) with size ranging in between (3.6 – 6.5) cm x (0.8 – 1.6) cm. Petals are of same number as that of sepals but (1/2 – 2/3) longer than sepals. They are filiform, yellowish or greenish in color. Stamens are $\pm$ twice number of sepals. Anthers with two lateral locules. Ovary is superior, oblong to globose, angled, triuncate with thickened rim at apex. Style is thick, divide into stigmatic lobes at apex. Stigma lobes are usually 4, 2 – 3 mm in length recurved at tips. Ovules are many with parietal placentation. Fruit is a fleshy loculicidal capsule with fleshy, scarlet and sarcotesta seeds (Noltie, 1994).

Its growth form and cultivation method have been described by Bhattarai and Ghimire, 2006; Shrestha and Shrestha, 2064. It is known by different names in different languages: love apple in English; Satuwa / Kalchun / Tintale banko in Nepali; Khadirah / Satuwa / Haimavati in Sanskrit; Satuwa in Gurung; Dhumbi Mando in Sherpa; and Natar Dhap in Tamang language.

1.3 Distribution range

1.3.1 Distribution in World

Paris polyphylla is globally distributed in the Himalayan range across Pakistan, India, Nepal, Bhutan, Burma, South Tibet, Myanmar, Taiwan and China (Ghimire *et al.* 2008; Envis.htm)



Map adopted from ICIMOD, 2005

Fig.1: World Wide Distribution of *Paris polyphylla*

1.3.4 Distribution in Nepal

In Nepal, *Paris polyphylla* is found to be reported from 34 districts. According to herbarium deposited in National Herbarium Center, Godawari (KATH), it is found in altitudes ranging from 1800m – 3400m altitudinal range in Nepal. It distribution pattern in different districts of Nepal is shown in map of Nepal in Figure 2.

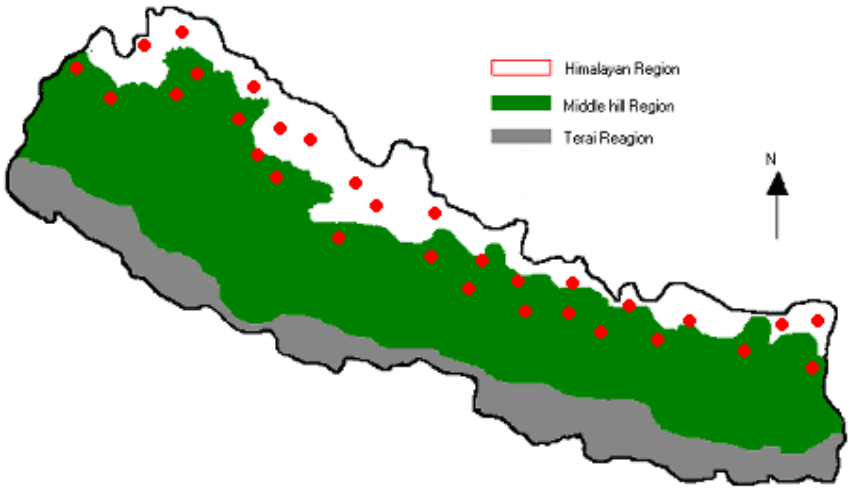


Fig. 2: Distribution of *Paris polyphylla* in Nepal.

1.4 Uses

Rhizome of *Paris polyphylla* is widely used as antihelmintic. It is also used as antispasmodic, digestive stomachic, expectorant and vermifuge (Bhattarai and Ghimire, 2006; IUCN, 2004). Powder from rhizome is used for fever and food poisoning. Root paste is applied as an antidote to snake bites and poisonous insect bites and also to alleviate narcotic effects. Chewing a piece of the root is believed to heal internal wounds below the throat. Root paste mixed with honey is prescribed with warm milk to the postnatal women for its alleged galactagogue property and as a vital tonic in Makwanpur district of Nepal (Rajbhandari, 2001; Dutta, 2007). Sugars and glycosides present in *Paris polyphylla* have a depressant action on carotid pressure, myocardium and respiratory movements. It produces vasoconstriction in kidney but vasodilation in spleen and limbs and also stimulates the isolated intestine (Dutta, 2007; Baral and Kurmi, 2006). Pieces of the root are fed to cattle with diarrhoea and dysentery. Root paste is directly applied to wounds in Central Nepal (Manandhar, 2002) while in South West Dolpa district, root extract mixed with *Dactylorhiza hatagirea* is applied on fresh cuts to induce healing of wounds (Ghimire *et al.* 2001).

It is an important folk medicinal herb of China. Its rhizomes are used for injuries from falls, fractures, convulsions and strains (Liang, 2000). Root is also used as medicine for rheumatism. Whole plant can be used as febrifuge, while roots can be used as analgesic, antiphlogistic (removes heat), antipyretic, antitussive and depurative (Yung, 1985; Duke and Ayensu, 1985). A decoction of root is used in the treatment of ulcers, diphtheria, epidemic Japanese B encephalitis, appendicitis, lymphadenopathy, tonsillitis, parotitis and mastitis. It causes the subsidence of swelling, alleviates pain and relieves boils, carbuncles, sore throat and traumatic pain. *Paris polyphylla* is used as a primary herb in the treatment of liver, stomach, nose and throat cancer in traditional Chinese medicine. It is being used in hospitals in China along with other herbs in conjunction with conventional drugs for the treatment of lung and breast cancers. The department of Biochemistry in Hongkong found that polyphyllin D found in *Paris polyphylla* is a potent anticancer agent that can overcome drug resistance in hepatocellular carcinoma cells and elicit programmed cell death via mitochondrial dysfunction. Chinese scientists in Pharmaceuticals and Biotechnology through in vitro research have proven that all compounds isolated from the rhizome of

Paris polyphylla possess medium to significant inhibition against tumor (Vassilopoulos, 2009).

1.5 Chemical compositions

The major components isolated from the rhizome of *Paris polyphylla* are:

Polyphyllin A, Polyphyllin B, Polyphyllin C, Polyphyllin D, saponoides/ saponins, polysaccharides, sugars and two glucosides viz. α – paristyphnin, and α – paridin, dioscin, diosgenin, pariphyllin, steroid glycosides, kaempferol 3 – gentiobioside, prewalskinone B, and stigmasterol (Devkota, 2005; Dutta, 2007; Bhattarai and Ghimire 2006; Watanabe *et al.* 2005).

1.6 Rationale of the study

The population of *Paris polyphylla* is in dwindling condition due to unwise and unscientific harvesting thereby raising the question of its very existence. No quantitative information on availability / population of *Paris polyphylla* is available in Nepal and there has been no research regarding the ecology of this species. Ecological studies of a few medicinal plant of Nepal were undertaken especially of higher altitude (Ghimire *et al.* 1999; Gahire, 2003; Shrestha *et al.* 2007 (b); Baral, 2008). The research regarding this species at present is confined only to biochemical work. Most of these researches are done by Chinese scientists. In context to Nepal, the information on this plant is limited only to ethnobotanical work and listing of the plant name with its description and uses in different books of medicinal plants and non timber forest products. Understanding the ecology of individual species is important for conservation and for cultivation purposes. The present study focuses on the detailed study of the plant, *Paris polyphylla* regarding its ecological status, biomass production, seed output and regeneration. The rationale for ecological research of *Paris polyphylla* has come from its medicinal importance and increasing popularity of this plant in the world. The present study will provide ecological information on *Paris polyphylla*'s status in Ghandruk VDC and will help in formulating conservation plan and cultivation practices in future. It will also help in carrying out further related research.

1.7 Objectives

The present research work aims to explore how different environmental factors influence growth, distribution and production of *Paris polyphylla*. Considering the importance of the plant in the study area with respect to conservation the present study was undertaken with following objectives:

- To find out the distribution pattern and altitudinal range of the plant in Ghandruk VDC.
- To find out the ecological status and biomass production of the plant *Paris polyphylla*.
- To study the regeneration potentiality of *Paris polyphylla*.
- To find out the antibacterial effect of the plant extract as it is being used for diseases of parasitic origin.

1.8 Limitation of the study

Following limitations caused the research work to be limited causing some objectives of this study to be unfulfilled.

- More locality and elevation higher than 3000 m or whole VDC could not be surveyed due to time constraints (as it requires many days to walk to these sites).
- Entire phenology of the plant could not be included in this study due to inaccessibility of monthly field visit.
- Seed germination at field could not be assessed as it might takes more than two years.
- Root biomass estimation was limited to few samples due to its threatened status.

Chapter 2
LITERATURE REVIEW

2.1 Literature review on medicinal plants related to ecological study

Very few works have been done regarding ecological study and population study of medicinal plants. Some relevant ones are summarized below.

Shrestha *et al.* (1998) studied the ecological distribution and status of MAPs in Shey – Phoksundo National Park (SPNP) and its surrounding areas. They recorded a total 205 species of MAP among which 40 species occurred in lower temperate (2500 – 3000m), 54 species in lower sub alpine (3000 – 3500m), 63 species in upper sub alpine (3500 – 4000m) and 20 species in alpine (above 4000m) ecozones. They studied the density and dominance of medicinal plants and their associates in the different sites of SPNP and reported that medicinal plants are dominant over their associates in most of the sampling sites.

Ghimire *et al.* (1999) assessed the distribution pattern, population density, regeneration status, and biomass production of five most important high altitude medicinal and aromatic plants (*Aconitum orochryseum*, *Dactylorhiza hatagirea*, *Nardostachys grandiflora, Picrorhiza scrophulariiflora and Rheum australe*) in Gyasumdo valley of Manang district. They found *D. hatagirea* mostly concentrated on relatively dry open southeast facing slopes, *R. australe* on steep dry slopes, *N. grandiflora* and *P. scrophulariiflora* in moist habitats. The lowest frequency and density was noted for *D. hatagirea* while the highest frequency was noted for *R. australe*. Similarly, highest density was that of *P. scrophulariiflora*. It was observed that regeneration of *N. grandiflora* and *P. scrophulariiflora* was much higher compared to other medicinal plants. The underground biomass (kg/ha) and the productivity potential (tons/yr) was found higher for *N. grandiflora* while the underground biomass and productivity potential was found lowest for *D. hatagirea*. The extent of human disturbances was attributed as the major factor influencing the population of medicinal plants in the study sites.

Kala (2000) studied the distribution pattern, population structure and conservation status of rare and endangered medicinal plant species of Spiti in Indian Trans – Himalaya. He found 23 rare and endangered medicinal plants in Spiti distributed over ten major habitat types. The highest mean density was estimated for *Picrorhiza kurrooa* followed by *Saussurea gnaphaloides*.

Airi *et al.* (2000) studied about the availability and habitat preference of Jatamansi of west Himalaya, India and found the dripping moss-laden rocks and moist boulders as the most preferred habitats of the plant. Density was found higher on west facing slopes than east facing slopes. Density and frequency had significant positive relationship with altitude. Biological parameters like plant density, plant height and above ground biomass and environmental features like moisture content and soil nitrogen were found positively correlated with below ground biomass. The substrate of the plant was invariably acidic with high mean organic carbon and nitrogen.

Gurung (2001) carried out the ecological studies on two species of Seabuckthorn (*Hippophae salicifolia* and *Hippophae tibetana* in Mustang and Manang districts. He found that *Hippophae sp.* occurred between 2000 – 3600m. Soil analysis indicates that nutrients contents in the soil in this plant occurred sites were more than that of barren mountain lands.

Larsen (2002) focuses her research in one plant species, *Nardostachys grandiflora* out of 82 plant species recorded in Chaudabise valley, Jumla district. She found that the abundance of *N. grandiflora* of Chaudabise valley, Jumla district was greater in areas not collected than in areas subject to annual collection. Length of roots and volumes of root and biomass were also significantly larger in uncollected than collected areas, indicating smaller root sizes as a consequence of the high frequency of collection.

Gahire (2003) studied the ecology and distribution of Kutki (*Neopicrorhiza scrophulariflora*) in Chame, Tachi and Bagarchap VDC in Manang district. He found Kutki in stony slopes, cliffy mountains, grassy slopes, on rock scars and the density was highest at the middle belt (4000-4300m). The nutrient content was high in the Kutki growing areas.

Kurumbang, (2003) studied the ecology, harvest and trade of five important medicinal plants viz, *Nardostacys grandiflora*, P*icrorhiza scrophularaiifolia*, *Rheum australe*, *Jurinea dolomiaea* and *Valeriana jatamansii* of Shey – Phoksundo National Park and its bufferzone. He found the density of *N. grandiflora* at Mukraman pasture to be highest while the density of *R. australe* at Kotakhola pastures to be lowest. Although the harvesting of MAP occurs throughout the year; peak season was found to be from September to November. 14 medicinal and aromatic plants were mostly collected and traded from Phada VDC alone.

Kala (2004) did the assessment of species rarity and found that the total annual production of *Dactylorhiza hatagirea, Picrorhiza kurrooa and Rheum moorcorftianum* was approximately 19,250, 32,560 and 3,165,980 tonnes respectively in North West Himalaya of India. In total, Jammu-Kashmir obtained the highest quantity of these species, followed by Himachal Pradesh and Uttaranchal. The available biomass of these plant species in nature shows that proportionately critically endangered species obtain lesser quantity than the rest of the two categories – endangered and vulnerable. According to his findings, he suggests that although the demand of the species *D. hatagirea* and *P. kurrooa* is high but the availability in the wild does not reflect that *D. hatagirea* should be ranked as critically endangered and *P. kurrooa* as endangered.

Ghimire *et al.* (2005) studied the effect of different harvesting patterns on the population ecology of two threatened species, *Nardostachys grandiflora* and *Neopicrorhiza scrophularriiflora* in Shey-Phoksundo National Park and its buffer zone. Their experiments revealed a positive effect of low harvesting levels on plant density but recruitment and survival rates decreased with increasing harvesting levels. Recruitment and survival rates were higher in *N. scrophulariiflora* than in *N. grandiflora*, the latter species more vulnerable to harvesting than the former.

Kala (2005) monitored the population density of threatened medicinal plant species in seven protected areas in the Indian Himalayas. He found 60 threatened medicinal plant species of which 54 species were found to occur in the sampling plots. 22% of

threatened medicinal plant species were critically endemic to the Himalayan region. The density of threatened medicinal plant species varied with protected areas. The moist habitat type was found richest in these species among 10 habitat types sampled. *Arnebia euchroma* and *Ephedra gerardiana* were the most common threatened medicinal plant species.

Pai (2005) studied the population ecology of *Acorus calamus* in Southeast Ohio, U.S.A. She found significant effects of light, nutrient and moisture on rhizome growth. Seed germination occurred only in light and varied significantly across temperatures with seeds germinating maximally in spring and summer in submerged conditions. There was no significant difference in seed germination after storage for 24 months.

Xie and Zhendong (2005) did some ecological research to determine the habitat preference and germination characteristics of *Meconopsis* species in the NW Yunnan, China. They found that these plants have a highly narrow ecological niche growing only in marginal areas of poor rocky soils in cold alpine and sub-alpine climates. Plants highest relative cover was found to be 5 % and usually occurring in small disjunct patches. Seed germination rates in the field were relatively low.

Obratov-Petkovic *et al.* (2006) carried out the ecobiological study of medicinal plants in some regions of Siberia and found the low values of ecological indexes: Nitrogen, moisture and soil acidity. Medicinal plant species with high abundance and potential preference for alkaline soils were found to be *Calamintha vulgaris, Galium verum, Cichorium intybus, Eupatorium cannabinum, Agrimonia eupatoria, Campanula glomerata, Calamintha officinalis, Teucrium chamaedrys, Primula veris, Anthylis vulneraria, Sanguisorba minor, Ranunculus bulbosus* etc.

Uniyal *et al.* (2006) studied the current status of eight highly traded and locally used medicinal plants of Chhota Bhangal of Himalchal Pradesh, India in terms of density, frequency and biomass and also document the indigenous use of these plants for traditional healthcare. They found that steep slopes of Chhota Bhangal had the highest species richness and diversity while rocky areas had the least. However, illegal

extraction of plants for commercial purposes seems to have affected their population in nature.

Vashistha *et al.* (2006) surveyed the natural population of two species of medicinal plant *Angelica* viz. *A. glauca*, and *A. archangelica* in subalpine and alpine regions of Garhwal Himalaya, Uttaranchal, India. They found that frequency of both species was more than 50 % in nature but density of individuals and area occupied were low compared to other species of alpine and subalpine region. Based on species occurrence in selected areas, both species were identified as critically endangered to endanger in different areas.

Bhatt *et al.* (2007) studied the population assessment and biomass variation in selected populations of *Swertia anustifolia* in Kumaon Himalaya, India. They found the low population density across the surveyed population. Species showed random distribution and higher frequency of occurrence in most of the population. Density was found to positively correlate with biomass. The biomass showed peak value in senescence phase.

Semwal *et al.* (2007) found out the population structure of rare and endangered medicinal plants on the basis of density, distribution and diversity dominance pattern in Kedarnath Wildlife Sanctuary, Uttarkhand, India. Out of ten habitats identified, distribution of most of the species (out of 10 species selected) was found to be restricted in 2 – 3 habitats. *Picrorhiza kurrooa* showed wide distribution in 6 habitats while *Swertia chirayita* was restricted to a single habitat. *Aconitum balfourii, A. heterophyllum, Dactyloriza hatagirea, P. kurrooa and S. chirayita* had lower average density in different habitats. Likewise, *Allium stracheyi, Bergina stracheyi, Berberis osmastonii* and *Nardostachys jatamansi* had the highest density in the respective habitats. It was observed that even in their favored habitat almost every species had patchy distribution and was restricted to a small area.

Shrestha *et al.* (2007a) studied the distribution and population status of *Aconitum naviculare* in Upper Manang. They found the plant was distributed only on dry south facing slope between 4000 and 4700m a.s.l. Small population of *A. naviculare* was found to be restricted to a specific pocket habitat in Ice Lake area, Manang hill and

Upper Khangsar whereas in Ledar the population of the plant was relatively large. The cause for declining population density was analyzed to be collection of plant before seed dispersal.

Shrestha *et al.* (2007b) studied the ecology of *Neopicrorrhiza scrophulariiflora* in Manang, trans Himalayan dry valley. They found the highest ramet density at post fire site than pastureland and cliff. Only ¼ th of the total ramets i.e. on average 46 % were found to bore flowering spikes, a spike having 12.8 flowers and 8.93 fruits. The average soil nitrogen and organic carbon content was found to lie near the upper limit of the value reported for soil of high mountain region of eastern Nepal. They concluded that morphological variation among the populations was relatively weak.

Baral (2008) studied the ecology of *Valeriana jatamansi* and its role in the economy of Dolpa, Western Nepal. He found better growth and abundance of the plant in slightly acidic soil with higher percentage of nitrogen content and organic matter. Out of 3 Village Development Committee studied Phada and Tripurakot (other being Phoksundo) were found better for resource yield through resource estimation. The market and value adding processes of this resource was found to be very poor within the study area. Majority of the income from this resource went to the stakeholders other than those in the local level.

Bhattacharya and Sharma (2008) studied the ecological feature, habitat preference and availability of medicinal herb *Hauttuynia cordata* of Assam, India and found that the clay loam soil with average soil pH of 5.9 and 78% soil moisture were the favorable soil characteristics for the better growth of the herb. The frequency and density of the plant was higher in the moist habitats with higher organic carbon. They found significant positive relationship of density, biomass production and growth with the soil physicochemical properties. Soil moisture was found to be the most dependent factor for the plant growth.

Chauhan *et al.* (2008) analyzed the ecological features of medicinal orchid *Malaxis muscifera* of Himalayan region, India and found that moss-laden moist slopes are the preferred microhabitat for the plant. Low percentage frequency and plant density found showed the species as both rare in distribution and adapted for specific microhabitats. Exploitation for medicinal purpose, poor regeneration, low seed

germination and seedling establishment, habitat loss, grazing, forest fire, competition with other dominant species of community were identified as the major factors for the low density of the plant species among studied populations.

Giri *et al.* (2008) studied the declining population of an endangered orchid *Dactylorhiza hatagirea* in Tungnath alpine meadows of Garhwal Himalaya, India. Out of six study sites, only two sites showed the presence of *D. hatagirea* and its density was found minimum among other associated species.

Shrestha and Jha (2008) studied the habitat range of two alpine medicinal plants, *Aconitum naviculare* and *Neopicrorhiza scrophulariiflora* in trans-Himalayan dry valley of Manang district. They found that *A. naviculare* occurs on warm and dry south facing slopes between 4090 – 4650m asl along with sclerophyllous and thorny alpine scrubs, while *N. scrophulariiflora* on cool and moist north facing slope between 4000 - 4400m asl where adequate water was available from snow melt. The populations of both the plants were found fragmented and small. Accumulation of soil organic carbon was higher in *N. sscrophularriflora* but soil nitrogen content was lower. Warm and sunny site with nitrogen rich soil was found suitable for *A. naviculare*. Due to collection by human and trampling damage by livestock, the population of *A. naviculare* was found absent in open areas in five of the six sampling sites and it was confined only within the bushes of alpine scrubs.

Singh *et al.* (2008) explored the species diversity and population status of five threatened medicinal plants in different landscape of the Rohtang Pass, Himalchal Pradesh, India. They recorded the largest population of threatened medicinal plants on northeast and northwest facing slopes where population density of *Bergenia stracheyi*, *Picrorhiza kurrooa* and *Rhododendron anthopogon* was highest while *Aconitum heterophyllum* was recorded for the lowest density. Distribution of medicinal plants was found to be very habitat specific.

2.2 Researches related to *Paris polyphylla*

There has been many researches done on *Paris polyphylla* in the last few years but there are no reports regarding its ecology. Most of the researches till to date are related to biochemical work like isolation of saponin from the rhizome (Zhao *et al.*, 2005; Yinan *et al.*, 2005; Yin *et al.*, 2008; Zhang *et al.*, 2007a; Huang *et al.*, 2007;

Deng *et al.*, 2008), isolation of glycosides (Yan *et al.*, 2008; Wang *et al.*, 2007), isolation of oligosaccharides (Zhou *et al.*, 2003), isolation of phytoecdysone (Singh and Thakur, 1982) and the different biological activity of these compounds (Devkota, 2005; Zhou *et al.*, 2007; Devkota, *et al.* 2007; Lee *et al.*, 2005; Zhang *et al.* 2007b; Matsuda *et al.*, 2003).

Other works include isolation of endophytic fungi from rhizome of *Paris polyphylla* and their screening for antitumour and antifungal activities (Li *et al.*, 2008; Li *et al.*, 2005; Wang *et al.*, 1999), genetic diversity of the plant (He *et al.*, 2007), RAPD variation among four population of *Paris polyphylla* (Zhang *et al.*, 2004a)

Some relevant literatures related to present study are summarize here:-
Zhang *et al.* (2004b) studied on domestication and propagation technology of *Paris polyphylla* var. *yunnnanensis* and *P. polyphylla* var. *chinensis.* They found that there were more differences in growth among the six populations, the aboveground part of the plants from tropical populations had a longer growth duration and a more rapid increase rate of underground stem weight. They concluded that as a short culture cycle and a simple operation, asexual cutting reproduction had more economic benefit than sexual reproduction.

Li *et al.* (2005) tested 130 endophytic fungi isolated from 12 Chinese traditional medicinal plants for antitumour and antifungal activities on human gastric tumour cell line and the growth inhibition test against seven phytopathogenic fungi. Though *Paris polyphylla* failed to show antitumour activity, it showed 33 % antifungal activity.

Shengji *et al.* (2006) found that resources declining occurred with habitat change in eight medicinal plant species in Dian Bai village in NW Yunan, China, *Paris polyphylla* being one of it. Large scale cultivation of *Paris polyphylla* var. *yunnanensis* are being tested in Lameirong village and Dian village of NW Yunan, China. Contribution of medicinal plants to family economy varies from 10 – 70 % and 30 % family's livelihood solely depends on cultivation of medicinal plants in these villages.

Chen *et al.* (2007) evaluated the potential of *Paris polyphylla* to control oral yeast in biofilm. They found that the minimum inhibitory concentration (MIC) of *P. polyphylla* extract against yeast, *Candida albicans* was 320 times higher than Amphteriane B. They concluded *P. polyphylla* as a prominent herb to treat opportunistic oral candidiasis and other yeast infections.

Deng *et al.* (2008) isolated three steroidal saponins from the rhizomes of *Paris polyphylla* and evaluated them for their antifungal activity against *Cladosporium cladosporioides* and *Candida* species and showed comparable activity to chemicals used in some commercial products.

Chapter 3

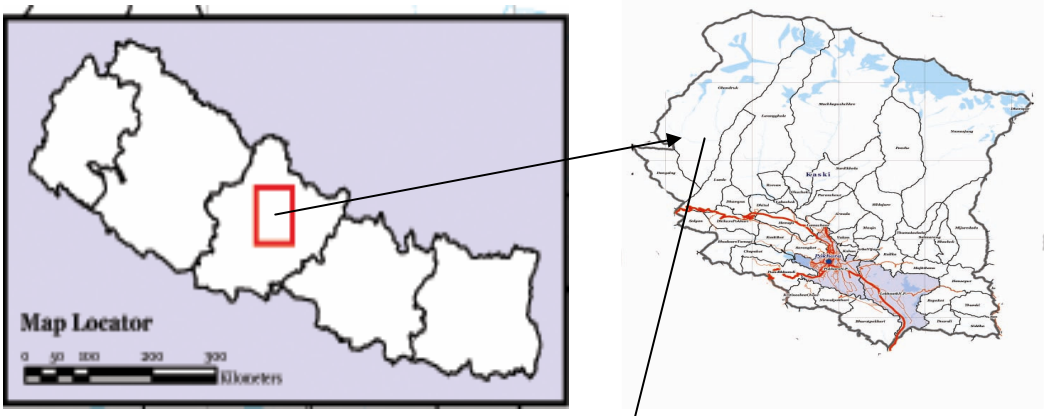
STUDY AREA

3.1 Background

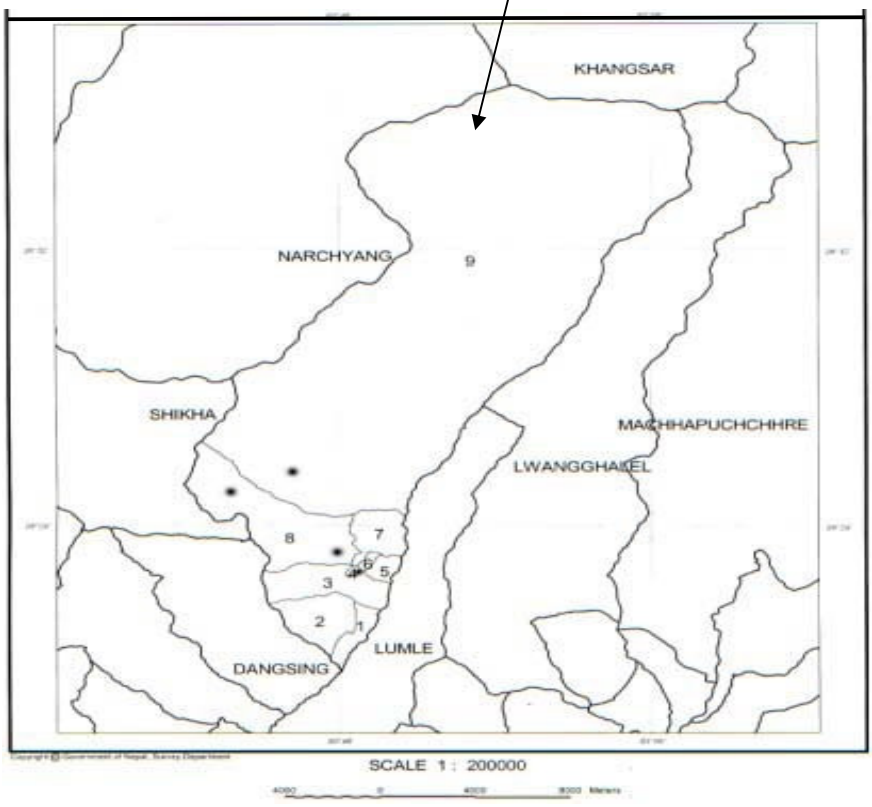
The present study area (28º22.64' - 28º25.23'N latitude and 83º45.87' - 83º49.98'E longitude) lies within Ghandruk Village Development Committee (VDC) of Kaski district, Central Nepal. This VDC falls under the Annapurna Conservation Area (ACA), the first conservation area of Nepal and the largest protected area of Nepal (7,629 km^2). Ghandruk VDC is surrounded by Annapurna range to the north, Dansing VDC to the south, Modi river adjoining Lumle VDC and Lwang – Ghalel VDC to the east and Myagdi district to the west. The total area of this VDC is estimated as 282 km^2. It is located above Modi river at 1100 m above sea level and goes as high as upto Annapurna Himal (8091m). The VDC is famous for the Annapurna Sanctuary along with Annapurna Base Camp (ABC) and Machhapuchhre Base Camp (MBC) which are the one of the most popular trekking destinations of trekkers in the Annapurna region.

3.2 Climate

The climate is subtropical to temperate monsoonal (LRMP, 1984). The climatic data were taken from the Meteorological station Ghandruk and Lumle as data of temperature was unavailable for Ghandruk VDC. The rainfall is maximum in July and August (i.e. 762.36 and 755.72mm respectively) and minimum in December (i.e.7.4mm) as shown in Figure 4. The average monthly maximum temperature reaches upto 24.64ºC in August and minimum temperature falls upto 5.32ºC in January (according to Lumle station, the nearest station from the study area (28º 18' N, 83º 48'E, 1740 masl).

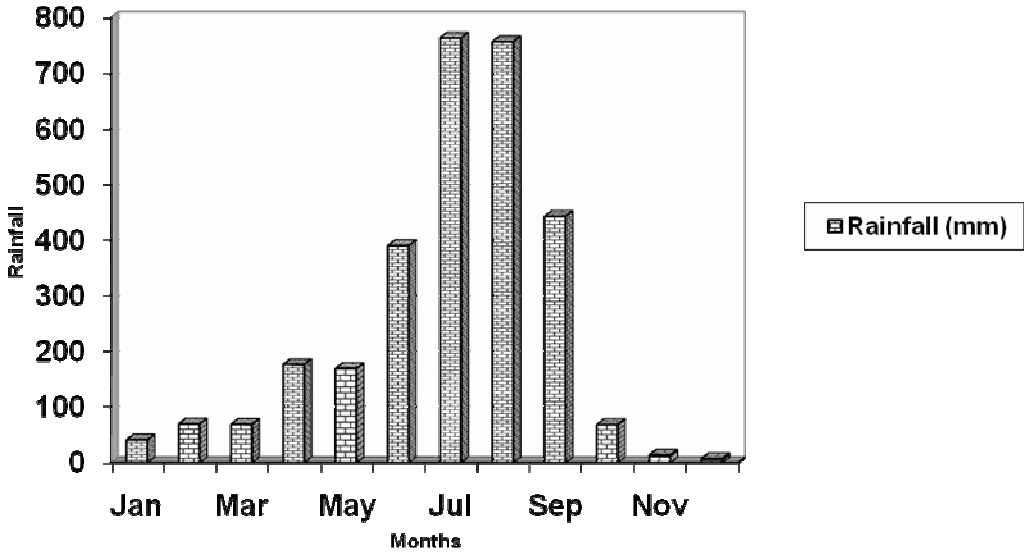


Kaski district

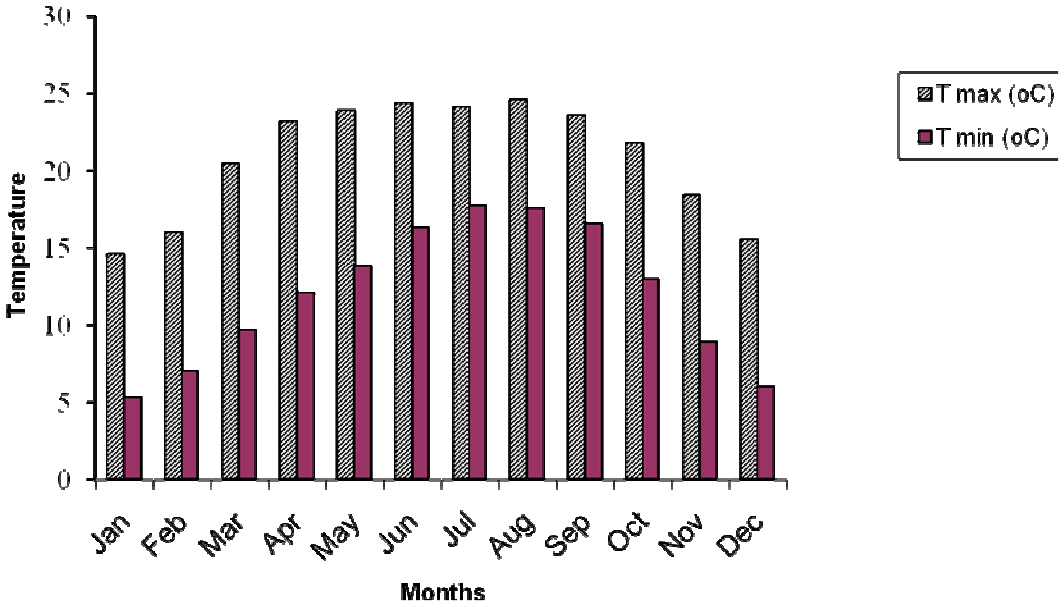


Ghandruk VDC

Fig. 3: Study Area Map



(a)



(b)

Fig. 4: Climatic data of Narayani Basin (Station – Ghandruk for rainfall and Lumle for temperature, 2003 – 2007).

a) Monthly average Rainfall

b) Monthly average maximum and minimum temperature.

3.3 Vegetation

The general vegetation of Ghandruk VDC is described by Kayastha (1989) while Stainton (1972) dealt in general for the Annapurna region. The forest type is related to elevation (Stainton, 1972), as the elevation increases there is a gradual change in vegetation. Forest pattern in Ghandruk VDC is shown in Table 1.

Table 1. Forest pattern in Ghandruk VDC.

Altitude	**Eco-climatic zone**	**Forest type**
2500 – 3500 m	Upper temperate	Birch forest Rhododendron forest Mixed broadleaved forest
1500 – 2500 m	Lower temperate	Quercus lamellosa forest Mixed broadleaved forest
1000 – 2000 m	Sub – tropical	Semi – evergreen hill forest Schima – Castanopsis forest Riverine forest.

Source : Adopted from Stainton, 1972 and Kayastha, 1989.

3.4 Demography

Ghandruk VDC has a total population of 5, 138 people comprising 2,497 male and 2,641 female distributed over nine wards. About 1,142 households exist with an average household size of 4.5 (National Population Census, 2001). The population density of VDC is 18.22 persons per km^2. The literacy rate is 51 %. Gurungs are the major ethnic groups and the earliest settlers (Sherpa *et al*. 1989). Others are Kami, Damai, Magar, Brahamin, Chhetri, etc. The percentage of different ethnic groups inhabiting Ghadruk VDC is shown in Figure 5. Gurung constitute the highest percentage (i.e. 46.40 %) followed by Dalit comprising mostly Damai, Kami (i.e. 30.23 %).

3.5 Economy

Agriculture is the dominant activity for livelihood. Fifty one percent of total households depend directly on agriculture. Remittance in the form of overseas employment /pensions and tourism related business is another important source of income of 35% and 14% of the total households respectively (Kraijo, 1997).

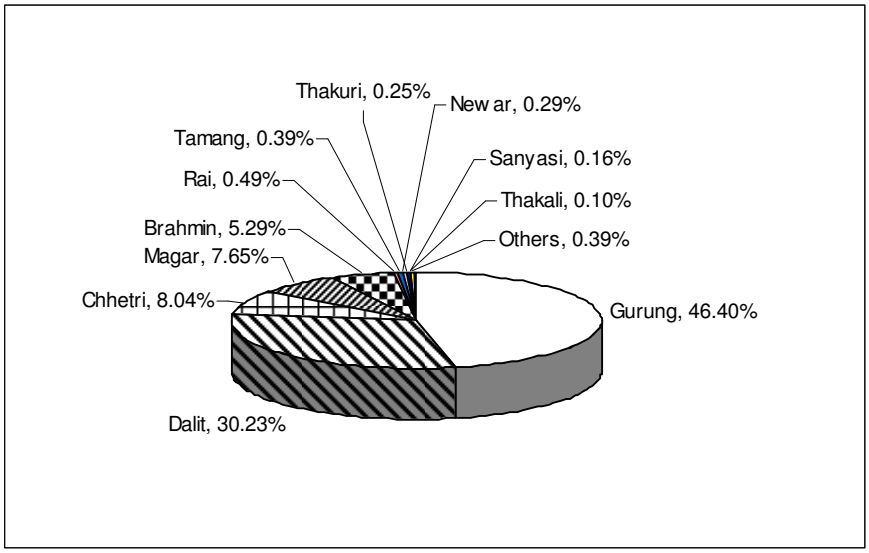


Fig. 5: Ethnic composition of Ghandruk VDC.

3.6 Land use

The total land area for the VDC is 5490.5 hectares. The land use pattern within Ghandruk VDC is shown in Table 2.

Table 2. Land use pattern of Ghandruk VDC

S.N.	Land Use	Area (in hectare)	Area (%)
1.	Forest cover	1251.9	22.5
2.	Snow covered	1000	18.5
3.	Agriculturally productive land	1500	27.5
4.	Land use for Agriculture	1218.6	22
5.	Irrigated land	50	0.8
6.	Uncultivated land, grazing land, fallow	150	2.6
7.	Rock, barren land, steep slope	300	5.6
8.	Settlement	20	0.3
Total		5490.5	

Source:- VDC Secretariat

In the monsoon, irrigated lowland terraces are cultivated with rice while in the uplands generally, maize or millet with legume are cultivated. In the dry season legumes, cereals like wheat, barley and potato are cultivated or the fields are left fallow.

Chapter 4

MATERIALS AND METHODS

Present study is focused on *Paris polyphylla*, one of the important medicinal plant species of Ghandruk VDC. The study is mainly based on primary information. For data collection, field visits were carried out three times in 2008. The first field visit was conducted in March / April 2008 at the time of germination period of the plant. Second field visit was on June 2008 at post flowering stage when the ecological status of the plant in the study area was studied. The last field visit was carried out in October 2008 for seed biological study. Soil, air dried seeds, and some rhizomes collected from the study area were brought to the laboratory for different experimental observations.

4.1 Ecological study

4.1.1 Selection of Sampling Sites

A technique of random systematic design was applied in which stands are selected by either a random or stratified random plan then locating the starting point and direction of a transect within the stand, samples are taken to some systematic plan (Barbour *et al.*1999). The sampling sites were selected in such a way that a comparative study can be made in its status on the basis of disturbance factors, altitudinal difference, dryness, etc. With the help of local people's information the sampling sites were selected in different localities so as to cover the whole VDC.

4.1.2 Transect studies

Transects were laid in different habitats within Ghandruk VDC to assess variation in distribution, density, dominance and other plant associations. Four different localities were selected for the study namely Ghandruk (Sikuwa), Komrong, Chhomrong and Tadapani based on the information given by local people and ACAP's staffs.

In each locality, five transect lines were set up at an interval of 30 – 50m in *Paris polyphylla* available site. In each transect line about six quadrats of 1m x 1m were laid down at an interval of 5m. Hence, altogether 30 quadrats were studied in each locality by making a grand total of 120 such quadrats within whole VDC. In each small quadrat, total number of *Paris polyphylla* plant, its coverage percentage and list of

associated species were recorded. 100g of soil samples were collected from each subplot from the depth of 25 cm. Herbarium specimen were prepared for unidentified plant specimens found within each quadrat.

Tallest one individual of *Paris polyphylla* from each quadrat was selected to measure the height of the aerial part at the matured stage and their average heights were determined in different localities. Along with that, disturbance factors like grazing, distance from nearby village, collection of the plant, litter thickness, litter coverage etc. were observed. The field notes regarding longitude, latitude, altitude, aspect, slope and forest type were also recorded. For recording altitudes, latitudes, and longitudes, Global Positioning System (GPS) was used where as slope and aspect of the study area was taken by using clinometer.

4.1.3 Quantitative Analysis

For each locality, data were analyzed to assess frequency, density and coverage of the plant. (Zobel *et al.* 1987).

4.1.3.1 Frequency

The frequency of occurrence of *Paris polyphylla* was determined to assess the distribution pattern. The frequency of the species was obtained by using the following formula (Zobel *et al.* 1987).

$$\text{Frequency (F)} = \frac{\text{No. of quadrats in which the plant occurs}}{\text{Total no. of quadrats}} \times 100$$

4.1.3.2 Density

Denstiy is the number of individuals of a species per unit area which gives the numerical strength of a species. In general, density is the total number of individuals of a species relative to total area studied. It is calculated by the following formula. (Zobel *et al.*1987).

$$\text{Density } (\text{Pl}/\text{m}^2) = \frac{\text{No. of individuals of } \textit{Paris polyphylla} \text{ in all quadrats}}{\text{Total no. of quadrats sampled} \times \text{size of quadrat}}$$

4.1.3.3 Coverage

Cover (also called coverage) is the percentage of quadrat area covered by a given species. Coverage is estimated by visual assumption method with the help of following scale (Zobel *et al.* 1987).

Scale value	Range of coverage (%)	Mid point value
1	0-5	2.5
2	5-25	15.0
3	25-30	37.5
4	50-75	62.5
5	75-90	85.0
6	95-100	97.5

Average coverage of a species is calculated by taking average of the values for all quadrats.

4.1.3.4 Identification of associated plant species

Unidentified associated plant species of *Paris polyphylla* were collected and herbarium specimens were prepared. Those herbarium species were identified consulting experts, comparing specimens deposited at Tribhuvan University Central Herbarium (TUCH), Kirtipur and National Herbarium and Plant Laboratoreis (KATH), Godawari.

4.1.4 Estimation of dry biomass and moisture percentage

To determine the moisture content and dry biomass of the species, underground parts were harvested from the sample units. Since the plants is cited as vulnerable and low population found on the field site, only one individual plant from one site / transect line (i.e. 5 from one locality) was taken by a pointed digger that dug out whole part of the rhizome. After removing the soil particles, each individual plant's rhizome was packed separately in airtight polythene bag for weighing. After taking the fresh weight the samples were kept on open air for 24 hours and again weighed to take air dried weight. Then the samples were packed in paper bag separately and kept inside the hot air oven for 48 hours maintaining the temperature at 65^0 C. Then oven dry weight was measured. The oven dry weight was taken as dry biomass.

The percentage of moisture were calculated by using the following formula (Zobel *et al.* 1987)

$$\text{Moisture}(\%) = \frac{\text{Air dry weight} - \text{oven dry weight}}{\text{Air dried weight}} x100$$

4.1.5 Laboratory analysis of Soil

Soil samples were air dried in shade and analyzed for pH, total nitrogen (%), total organic matter (OM)%, phosphorus (P_2O_5 kg/ha) and potassium (K_2O kg/ha) at Regional Soil Testing Laboratory, Pokhara, Kaski.

4.1.5.1 pH

Soil pH was determined by the potentiometric method (Gupta 2000) using a pH meter. 10g of air dried fine soil was mixed with 10 ml of distilled water and was stirred with the help of magnetic stirrer. The mixture of soil and water was left for decantation for about half an hour. Buffer solutions of pH 7 and 9.2 / 4 were prepared freshly. The pH meter was warmed up for 15 minutes before starting pH measurement. pH meter was calibrated with the help of buffer solution of pH 7.0 and 9.2. Then, pH measurement was taken for each solution of soil sample. Electrode of pH meter was flushed by distilled water and wiped by cotton each and every time before dipping it from one solution to next either buffer or of soil sample.

4.1.5.2 Total Organic Matter Content (OM) %

Soil Organic Matter (%) was determined by Walkely and Black rapid titration method (as described in Gupta (2000)). In this method, 0.5 g air dried fine soil was taken in a clean and dry 500 ml conical flask. Then, 5 ml of 1 N $K_2Cr_2O_7$ (potassium dichromate) and 10 ml of conc. H_2SO_4 was added successively. The mixture was shaked well and then allowed to cool down for 30 minutes. 100 ml of distilled water and 5 ml of orthophosphoric acid was added on it. Then 0.5 ml of diphenylamine indicator was added in conical flask containing the mixture of soil and reagents. Lastly, the content was titrated with 0.5 N ferrous ammonium sulphate solution till the color changes from blue – violet to green. A blank solution without soil sample was also run simultaneously.

Now, the organic carbon (%) of the soil sample was determined by using following formula.

$$\text{Soil organic carbon estimated}(\%) = \frac{0.003 \times 10\,(\text{Blank reading titration reading})}{\text{Blank reading x weight of soil (g)}} \times 100$$

The organic carbon (%) obtained by above formula was multiplied by a factor 1.3 (based on the assumption that there is incomplete oxidation of the organic matter in this procedure and only 77 % recovery occurs through this method).

Hence, organic carbon (%) = Organic carbon estimated (%) x 1.3

Now, to determine organic matter content (%) of soil, this value of organic carbon was multiplied by Van Bemmelen factor of 1.724 (because organic matter is assumed to contain 58 % organic carbon).

Hence, organic matter content (%) = Organic carbon (%) x 1.724

4.1.5.3 Total Nitrogen

The total nitrogen (%) of soil was determined by microkjeldahl method (as described by Gupta 2000). This method includes the following three steps:

Digestion

1 g air dried fine soil was taken in a clean and dry Kjeldahl digestion flask. Then 3.5 g potassium sulphate and 0.4 g copper sulphate were added to the flask containing soil. Then, 6 ml of conc. Sulphuric acid was added to it and shaked gently. The flask was then placed on the preheated heating mantle for digestion. Temperature was raised to about 310^0C (after the bubbles started disappearing on the content of the flask). The end of digestion process was known as the color changed from black to brownish and ultimately greenish. Then the flask was removed from the mantle and allowed to cool for 30 minutes. 50 ml of distilled water was added to the digest and the mixture was shaked well. A blank without soil sample was also run simultaneously through this process.

Distillation

The diluted digest of Kjeldahl digestion flask was then transferred to Kjeldahl distillation flask. A 100 ml beaker with 10 ml of boric acid indicator was place below the nozzle of the condenser in such a way that the end of the nozzle dipped into the

indicator. After the digest become warm, 30 ml of 40 % NaOH solution was added in distillation flask and its mouth was closed with cork making the system air tight. The temperature of the mantle was raised to about 310^0C. The distillation was continued until the volume of distillate in beaker reached to about 50 ml.

Titration

The distillate was then titrated with 0.1 N HCl. The volume of HCl consumed in titrating distillate was recorded on the basis of which the total nitrogen content (N%) of the soil sample was calculated by using the following formula:

$$\text{Soil N}(\%) = \frac{1.4 \times \text{N} \times (\text{S} - \text{B})}{\text{M}} \times 100$$

where, N = normality of HCl

S = volume of HCl consumed with soil sample (ml)

B = volume of HCl consumed with blank (ml)

M = weight of soil taken (g)

4.1.5.4 Phosphorus

Available phosphorus was determined by Olsen's method (Gupta, 2000).

Preparation of standard curve of Phosphorus:

1, 2, 3, 4 and 5 ml of 5 ppm Phosphorus solution was taken in 50ml volumetric flasks separately. 5 ml of the extracting solution ($NaHCO_3$) was added to it. Then 10 ml of distilled water and one drop of *p*-nitrophenol indicator was added. Then 2.5 M H_2SO_4 was added to it dropwise until the solution becomes clear. At the point where indicator's yellow color disappeared, the correct pH (5.0) for the color development was attained. 8 ml of the Murphy – Riley solution was added to each flask. The volume was made to 50 ml with distilled water and was mixed thoroughly. Thus, those standards had Phosphorus concentration 0.1, 0.2, 0.3, 0.4 and 0.5 µg P / ml. A blank was also prepared with $NaHCO_3$ solution, distilled water and Murphy – Riley reagent. After 15 minutes, the intensity of the blue color was read on spectrophotometer at 730 nm. Absorbance values for the standards having 0, 0.1, 0.2, 0.3, 0.4 and 0.5 µg P / ml were used to construct a standard curve between absorbance values and the concentration of P in standards.

Method

2.5 g air dried fine soil sample was taken in a 125 ml Erlenmeyer flask. Little phosphorus free Darco-G-60 or activated charcoal was added to it. 50 ml of $NaHCO_3$ solution was added at 25^0C. Then it was shaken for 30 minutes on a reciprocating shaker at 120 strokes per minute. Simultaneously, a blank solution without soil was run. The extract was filtered using Whatman No.40 or 42 filter paper. Re-filter was done if the filtrate was cloudy. 10 ml aliquot of the extract was pipetted in a 50 ml volumetric flask and 10 ml of distilled water and one drop of *p*-nitrophenol indicator was added to it. Then the content was acidified to pH 5 by adding 2.5 M H_2SO_4 dropwise till color disappeared. After adding 8 ml of the Murphy-Riley solution, the volume was brought upto 50 ml with distilled water. After 15 minutes, the intensity of blue color was read on spectrophotometer at 730 nm (as in case of standard).

The available phosphorus was calculated by using the following formula:

$$\text{Available P}(\text{kg/ha}) = \frac{\text{volume of extractant}}{\text{volume of aliquot}} \times \frac{22.4}{\text{weight of soil}}$$

where C = µg P in the aliquot (obtained from standard curve)

4.1.5.5 Available potassium

Available potassium was determined by ammounium acetate extraction by flame photometer method (Gupta, 2000).

5 g air dried soil was placed in a 50 ml centrifuge tube. 25 ml of neutral normal ammonium acetate solution was added to it, stopper was put and the tube was shaked for 10 minutes. The tube was centrifuged at 2000 rpm for 10 minutes unitl the supernatant liquid was clear. The supernatant liquid was decanted into a 100 ml volumetric flask. Three additional extractions were made in the same manner. The combined extract was diluted to 100 ml with ammonium acetate and was mixed. The potassium in the extract was determined with the help of flame photometer using K filter after necessary setting and calibration of the instrument. The extract of sample was feed in flame photometer. The reading for sample was noted and K content in the sample was determined with the help of standard curve. A curve was drawn by plotting flame photometer readings of standard K solutions on the Y-axis against the concentration of K on X-axis.

The concentration of K in the soil sample is calculated by the following formula:

$$\text{Available K}(\text{kg/ha}) = \frac{\text{C} \times 100}{5} \times 2.24$$

where C = ppm or μg / ml of K (obtained from standard curve)

4.2 Seed biology

When the plant fruit gets matured, the fruit was taken and some were preserved for measurement of fresh weight while others were dried in shade for air dry weight. The number of seeds present in a single fruit was counted. This process was repeated for 20 – 25 times taking the samples at different distances for each locality. The color of the seed was observed in different locality through visual observation and photographs and its size was also measured.

4.2.1 Seed polymorphism

Some plants may produce different types of seeds within same fruit or individual. This type of characteristic feature of seed is known as seed polymorphism. Seed polymorphism may include difference in color and shape of the seed. It was observed by collecting seeds from different localities. The seed characters such as size, shape and color were observed.

4.2.2 Seed size and weight

Seeds from different localities were randomly collected and measurements were made for 25 seeds and the mean of which was presented as seed length and breadth. Since the seeds were neither too heavy nor too tiny, 10 lots of 10 seeds were weighed and the mean value was presented as seed weight.

4.2.3 Seed output

Seed output is the potential capacity of species to reproduce itself. Large variations are found in the seed output of different species. The seed output is measured by the following methods (Zobel *et al.*1987)

Average number of fruits or infloresences / plant = A

Average number of seeds / fruit or infloresence = B

Average number of seeds output / plant = A x B

Average density of plants (No. / m^2) = C

Average seed production per m^2 = A x B x C

But *Paris polyphylla* is a perennial herb bearing single flower or single fruit. So, the seed output per plant is measured simply by calculating the average number of seeds of a fruit / capsule of an individual plant.

i.e. average seed output / plant = average number of seeds / capsules

Counts for the seed output were made for each locality choosing the plants randomly from different places and the average value was calculated.

4.2.4 Seed germination

The seed germination test was carried out to observe the performance of the species. 10 lots of 10 seeds were kept on a moist filter paper in 10 different petridishes kept at room temperature. Observations were made daily to see the normal germination.

Gibberllic acid treatment (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm), NH_4NO_3 treatment (25, 50, 100, 200, 400 ppm) and Conc. Sulphuric acid treatment (for 5, 10, 20, 40, 60 min) was also done for germination purpose to enhance the germination of the seed. Three parallel replicates were prepared for each treatment. One controlled was also set. The germination behavior was observed everyday upto 2 months. Distilled water was used to wet the filter paper for the duration of this experiment. The range of germination period of seed and maximum germination percentage within whole period was calculated.

4.2.5 Seed viability

Seed viability of the plant was tested following Baskin and Baskin 1998. To test the seed viability samples of 30 seeds were taken. Embryos were dissected from imbibed seeds and then dipped in a 1 % solution of 2, 3, 5 triphenyl –tetrazolium chloride (TTC). After few hours, the embryo of a viable seed turned into pinkish – red. If the embryo turned into pink, the seed is considered as viable, if not as nonviable. The percentage of viability of seed is calculated as follow:

$$\text{Percentage viability} = \frac{\text{Number of viable seeds}}{\text{Total number of seeds}} \times 100$$

4.3 Antibacterial test

The rhizome and the seed of the plant were used for the antibacterial test, as literature states that it had strong antibacterial and antifungal activity. The disk diffusion assay

(Taylor *et al.* 1995) was used to screen plant's extract for antibacterial activity. This was accomplished by placing a known amount of the extract on a small paper disk. This disk was placed on an agar growth medium containing a confluent lawn of bacteria. The absence of bacterial growth around the disk shows the antibacterial activity against that particular bacterium.

a) Preparation of the Test Extracts

The rhizome and the seed dried in shade were grinded to make fine powder. Two grams of fine powder was soaked in 25 ml methanol for 24 hours and filtered using Whatman filter paper. This process was repeated for 3 times to extract the chemical substances from the plant material. The filtrate was then allowed to evaporate until completely dry. The extract was dissolved in 2 ml of methanol. The concentration of the final extract was 1 g dried root powder or seed powder per 1ml methanol.

b) Microrganisms

One species of gram positive bacteria and five different species of gram negative bacteria used for test were *Staphylococcus aureus*, *Escheria coli*, *Shigella bodyii*, *Shigella dysentrae*, *Klebsiella pneumoniae* and *Salmonella typhii* respectively. These species were obtained from National Public Laboratory, Teku and were used to test antibacterial activities of plant's extract.

c) Preparation of the Test Disks

Sterile test disks were prepared by dipping and saturating sterilized filter paper disks (6 mm in diameter) in plant extracts solution using sterile forceps. These disks were dried in the sterilized petridishes.

Positive Controls: Positive control disks were prepared by dipping sterilized filter paper disks into a solution of tetracycline (5 mg/ml in methanol) and drying on a sterilized petridish.

Negative Controls: Negative control disks were prepared by dipping the sterilized filter paper disks into methanol and drying on a sterilized petridish.

d) Culture Media and Inoculum

Agar solidified nutrient media was prepared by dissolving 2.8 g powder of agar in 100 ml water. About 25 ml of nutrient media was poured into a petridish. The

inoculum for bacteria was prepared by culturing a large number of bacteria in a tube containing 10 ml liquid media of nutrient broth and incubating over night at 37ºC. The fresh inoculums of bacteria were transferred into petridish containing solid nutrient media of agar using a sterile swab. The bacteria was spread on the media in a confluent lawn with the help of swab and by rotating the petridish at 90º. One swab was used for one species of bacteria.

e) Placing Test Disks

Dried test disks were transferred on bacterial lawn under aseptic conditions using sterilized forceps. Each disk was placed gently on the agar surface and patted with the forceps so that it sticks. The petridish was incubated upside down at 37º C for 24 hours. Resulting zones of inhibition were observed. The inhibitory zone around the test paper disks indicates absence of bacterial growth and that was recorded as positive result and absence of zone as negative. The diameter of zone of inhibition along with paper disk was also recorded. Tests were repeated three times to insure reliability of the results.

4.4 Statistical Analysis

Different statistical tools were used. Pearson's correlation coefficients were determined among different variables like density, coverage and plant height of *Paris polyphylla*, and soil characteristics. Regression analysis was carried out using 1st order Generalized Linear Model (GLM) to establish relations among significant ones which were elaborated in the results. Statistical analyses were done using statistical program for Social Science (SPSS, 2002) version 11.5 and R Development Core Team (2008).

Chapter 5
RESULTS

5.1 Altitudinal distribution

The distribution of *Paris polyphylla* was not common to all the areas and was restricted only to certain areas in all the studied populations. The plant's dominance was seen from forest at 1900m just above the huge settlement of Ghandruk, Sikyu upto 2800m at Tadapani. Though the plant showed occurrence at study site around 2900m, the number was very low. Even in Tadapani, the summit (top) of hill was devoid of this plant while its occurrence was seen 100 – 200 m below the summit. Different literature cited the plant's occurrence upto 3300m altitude but the plants occurrence was not found above 2900m in Rume, place above Chhomrong.

5.2 Plant attributes

Paris polyphylla was found in shady, well- moistened places under the dark canopy of mixed broad leaved forest (Rhododendron forest in Tadapani) in almost all the sites, except one population in Komrong danda where it was found under open canopy of mixed broad leaved forest. The ecological status of the *Paris polyphylla* is shown in Table 3.

Table 3. Ecological status of *Paris polyphylla* in different sites

S.N.	Sampling sites	Average Frequency %	Average Density (pl/m^2)	Average Coverage (%)	Average plant height (cm)
1.	Ghandruk	40	1.16	33.33	41.2
2.	Komrong	43.33	1.8	41	49.25
3.	Chhomrong	76.66	2.26	49.18	52
4.	Tadapani	83.33	1.90	42.66	48.62
Total average of all site		60.83	1.78	41.54	47.76

The density of *Paris polyphylla* was not significantly different in four sites of study area. Among the four sites, plants from Ghandruk showed least average density i.e. 1.16 individuals per sq. m. The highest density of the plant was shown by plants from

Chhomrong i.e. 2.26 individuals per sq m. The total average density of the plant in study area was obtained as 1.78 individuals per sq m.

Frequency of *Paris polyphylla* at Tadapani was found to be highest i.e.83.33 % while the lowest frequency was observed in Ghandruk i.e.40 %. The total average frequency of the plant in study area was 60.83 %.

The coverage of *Paris polyphylla* also did not differed significantly in all four sites of study area. The highest average cover value was obtained in Chhomrong (49.18) % and lowest in Ghandruk (33.33) %. The total average cover of the plant in study area was found as 41.54 %

5.3 Associated Species of *Paris polyphylla* and their frequencies

The herbs, shrubs and some trees and seedling of some flowering plants found associated growing in *Paris polyphylla*'s habitat are summarized in Table 4 along with their frequencies (%).

Table 4. Associated species of *Paris polyphylla* in Ghandruk VDC

S.N.	Scientific name of plant	Family	Frequency %
1.	*Giardiana diversifolia* (Link) Friis	Utricaceae	4.16
2.	*Viburnum erubescens* Wall ex DC.	Sambucaceae	35.41
3.	*Pilea symmeria* Wedd	Utricaceae	14.2
4.	*Senecio capa* Buch. Ham. Ex D. Don	Asteraceae	8.33
5.	*Strobilanthes atropupuraeus* Nees	Acanthaceae	33.33
6.	*Sarcococca coriaceae* (Hook)	Buxaceae	28.18
7.	*Impatiens* sp.	Balsamaceae	10.5
8.	*Polygonum* sp.	Polygonaceae	10.41
9.	*Pilea* sp.	Utricaceae	17.05
10.	*Arisaema* sp.	Araceae	31.25
11.	*Smilax* sp.	Liliaceae	4.16
12.	*Viola* sp.	Violaceae	14.58
13.	*Thalictrum* sp.	Ranunculaceae	2.08
14.	*Berberis aristata* DC.	Berberidaceae	2.08
15.	*Mauhinia nepalensis* DC.	Berberidaceae	4.16
16.	*Daphne bholua* Buch-Ham.ex D.Don	Thymelaeaceae	15.57

17.	*Trifoluim repens* L.	Leguminosae	18.75
18.	*Galluim* sp.	Rubiaceae	27.08
19.	Selaginella		18.60
20.	*Oxalis* sp.	Oxalidaceae	12.50
21.	Fern	Aspidiaceae	31.16
22.	*Bidens pilosa* L.	Asteraceae	12.5
23.	*Fragaria nubicola* Lindl ex Lacaita	Rosaceae	12.5
24.	*Centella asiatica* (L.) Urban	Umbelliferae	8.33
25.	*Geranium* sp.	Geraniaceae	6.25
26.	Moss		6.97
27.	*Rhododendern* sp.	Ericaceae	4.16
28.	*Daphniphyllum himalense* (Benth.) Muell – Arg.	Daphniphyllaceae	4.16
30.	*Lyonia ovalifolia* (Wall) Drude	Ericaceae	6.25
31.	*Garuga pinnata* Roxb.	Burseraceae	8.33
32	*Rhus* sp.	Anacardiaceae	2.08

The highest occurrence of plants found among associated species of *Paris polyphylla* were *Viburnum erubescens, Arisaema,* sp., fern, and *Sarcococca coriaceae* with their frequencies as 35.41%, 31.25%, 31.16%, and 28.18% respectively.

5.4 Estimation of Dry biomass and moisture percentage

The dry biomass and moisture percentage of rhizome of *Paris polyphylla* is presented in Table 5.

Table 5. Dry mass and moisture content of *Paris polyphylla* 's rhizome

S.N.	Name of Place	Average Dry mass (g/plant)	Average Moisture content (%)
1.	Ghandruk	8.58	65.68
2.	Komrong	7.15	61.16
3.	Chhomrong	10.77	61.94
4.	Tadapani	7.03	64.60
Total average		8.38	63.34

From this study, average dry biomass of *Paris polyphylla*'s rhizome was found to be highest for the plant from Chhomrong i.e.10.77 g/plant while the plant from Tadapani had the lowest dry biomass of rhizome i.e. 7.03 g/plant. But *Paris polyphylla* from Ghandruk had highest moisture content i.e.65.68% and the plant from Komrong had lowest moisture content 61.16% in air dry sample. The total average dry biomass of the rhizome of the plant in study area was obtained as 8.38 g/plant and the total average moisture content in air dry sample was obtained as 63.34%.

5.5 Soil Variables

Different parameters of soil tested are presented in Table 6.

Table 6. Result of Soil test of different locality

S.N.	Locality	Soil pH	Organic matter %	Total N %	Available P kg/ha	Available K kg/ha
1.	Ghandruk	3.96	12.55	0.62	33.75	611.25
2.	Komrong	3.47	12.45	0.61	46.0	449.50
3.	Chhomrong	3.87	12.02	0.60	29.25	451.25
4.	Tadapani	3.51	12.30	0.61	35.42	582.42
Total average		3.70	12.33	0.61	36.10	523.60

Soil from the habitat of *Paris polyphylla* was found to be highly acidic in all sites of the study area. Though the mean soil pH value of different locality did not differ significantly, soil of Komrong hill was found to be little more acidic, 3.47 in comparison to others. The total mean value of soil pH in *Paris polyphylla* occurring sites of the study area was obtained as 3.70.

The average organic matter content (%) in soil of Chhomrong was only 12.02 which was the lowest while that of Ghandruk was found to be 12.55 which was the highest. The total average value for total organic matter (%) of soil of *Paris polyphylla* occurring site was found to be 12.33.

Soil of Ghandruk had 0.62% of total nitrogen content which was the highest among the four localities although there was no significant difference among the different sites. Chhomrong had 0.60 % of total nitrogen content which was lowest for the study

site. The total average nitrogen content (%) of soil of *Paris polyphylla* occuring sites of study area was 0.61.

Similarly, the average phosphorus content of soil was found to be highest for Komrong i.e.46.0 kg/ha while soil of Chhomrong contained lowest phosphorus content i.e. 29.25 kg/ha. The average phosphorus content (kg/ha) of soil of *Paris polyphylla* occurring sites of study area was 36.10 kg/ha.

The mean soil potassium content (kg/ha) of Ghandruk was 611.25 and that of Komrong was 449.5, the highest and the lowest value for the study site respectively. The total average potassium content (kg/ha) of soil of *Paris polyphylla* occuring sites of study area was 523.60.

5.6 Relations of Plant (*Paris polyphylla*'s) abundance with soil attributes

The relation between different parameters (which are supposed to be linked to plant's density) determined by using Pearson's correlation is presented in Table 7. The Pearson's correlation test shows that density of *Paris polyphylla* was found positively correlated only with litter thickness (i.e. r = 0.004, P=0.01). Similarly, the plant height was found positively correlated with soil OM (i.e. r = 0.038, P = 0.05) but negatively correlated with soil K (i.e. r = 0.006, P = 0.01). The corresponding linear regression line for these relationships is shown in Figure 6.

Table 7. Pearson's Correlation Coefficients (r) between different variables.

	Density	Soil pH	Soil OM	Soil N	Soil P	Soil K	Plant height	Litter cover	Litter thickness
Density	1								
Soil pH	0.076	1							
Soil OM	-0.028	-0.328**	1						
Soil N	-0.039	-0.351**	0.846**	1					
Soil P	0.117	-0.043	0.071	0.064	1				
Soil K	-0.097	0.050	-0.419**	-0.366**	-0.222	1			
Plant height	0.202	0.017	0.241*	0.092	0.079	-0.314**	1		
Litter cover	0.043	0.032	0.202	0.106	0.250*	-0.057	0.103	1	
Litter thickness	0.333**	0.218	0.026	-0.002	0.321**	-0.201	-0.060	0.296*	1

* Pearson's Correlation Coefficients significant at the 0.05 level

** Pearson's Correlation Coefficients at the 0.01 level

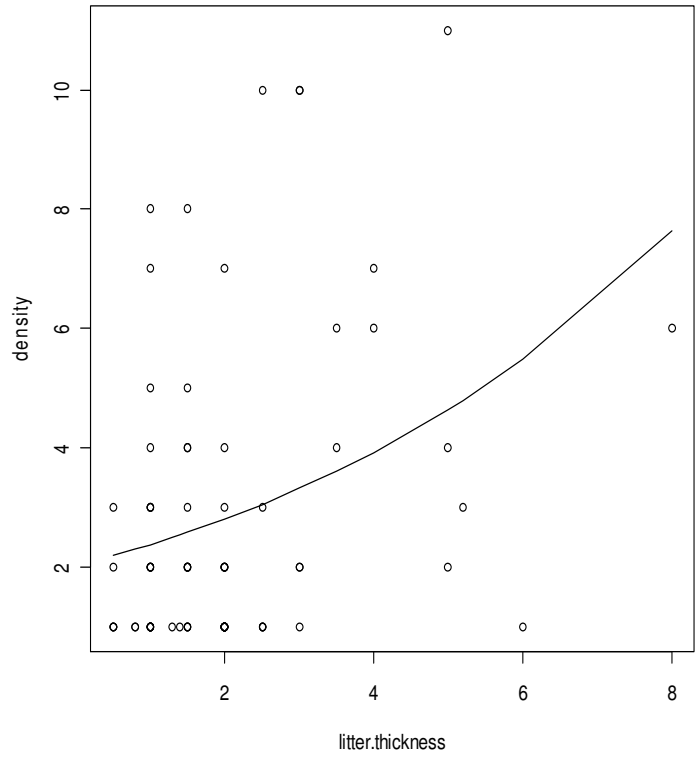


Fig. 6. Relationship between plant density and litter thickness

Table: Regression statistics using 1st order Generalized Linear Model (GLM) where response is density of *Paris polyphylla* per 1 m^2 plot

Predictor	Poly order/model	Residual degree of freedom	Residual deviance	Deviance explained (%)	Pr(>Chi)
Litter thickness	Null	73	136.74		
	1	72	121.177	11.38	< 0.0001

5.7 Seed biology

5.7.1 Seed dehiscence

Capsules (fruit) usually ruptured after maturation, however seeds did not get dispersed immediately. Sometimes it spilled 1 -2 week after the capsule ruptured and in some cases it remained on the plant until it started drying. Some seeds on the edge of capsules started drying on the plant because of late dispersal.

5.7.2 Seed shape and weight

Mature seed resembles that of Pomegranate in external morphology. They were more or less oval in structure with broad end and red in colour. Morphological variation was very less significant among different research sites. The measurements of seed size and weight are presented in Table 8.

Table 8. Measurements of seed of different locality

S.N.	Place	Average seed length (cm)	Average seed breadth (cm)	Average seed fresh weight (g)	Average seed dry weight (g)	Average seed output (per plant)
1.	Ghandruk	0.9	0.65	2.19	0.39	44.5
2.	Komrong	0.8	0.6	1.64	0.26	28
3.	Chhomrong	0.95	0.75	2.74	0.53	56.4
4.	Tadapani	0.85	0.65	2.09	0.363	38.21
Total average of all sites		0.87	0.66	2.16	0.29	41.77

The average seed length was 0.95 cm and average seed breadth was 0.75 cm for Chhomrong which was highest among the four sites. The size of seed was smaller for Komrong which has seed length as 0.8 cm and seed breadth as 0.6 cm. Similarly, dry seed weight per 10 seeds was found highest for Chhomrong 0.53 g while lowest dry seed weight was found for Komrong 0.28 g. The total average seed length for the study area was 0.87 cm, seed breadth 0.66 cm, seed fresh weight 2.16 g and seed dry weight 0.29 g.

5.7.3 Seed output

The average seed output was 56 seeds per plant for Chhomrong which was maximum among the four sites. Minimum seed output was for Komrong i.e 28 seeds per plant. In total average seed output for the study area was 41.77 seeds per plant.

Average density of plants for the study area (No. / m^2) = 1.78 pl / m^2

So, average seed production per m^2 = 1.78 x 41.77 = 74.35 seeds per sq. m.

5.7.4 Seed germination

At first, normal germination (without treatment) was carried out in laboratory conditions. After the seeds failed to germination in normal condition, different pretreatments like gibberllic acid treatment, ammonium nitrate treatment, concentrated sulphuric acid treatment were given to break the dormancy and enhance the germination but the seeds did not germinate.

5.7.5 Seed viability

Seed viability test measured with 2, 3, 5 triphenyl-tetrazolium chloride (TTC) showed that very few seeds were viable. Only 29 % of total seeds treated with TTC were found to be viable (Fig.7)

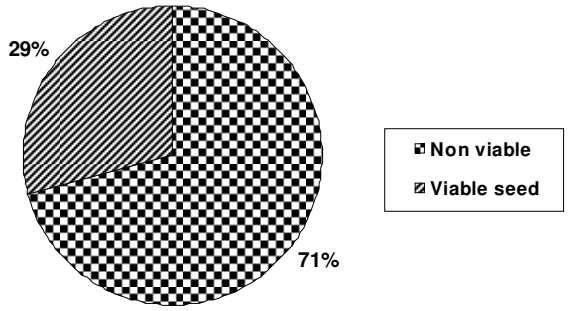


Fig. 7 Seed viability test

5.8 Phenology

Paris polyphylla plant starts germinating in April and flowers in April / May. Seeds mature in October. After the seeds mature, the capsule bursts but the seeds do not disperse immediately but instead remain on plant for long time. Seeds can be harvested from mid October to last of October. By the beginning of November to mid November, most of the plant part dies out and all seeds get dispersed on ground. But before the plant dies, and after the seed gets matured, bud sprouts on rhizome which remains underground for the whole winter until next germination period (sprouting season) arrives. So, the rhizome is dormant for almost five months. Germination of seeds seems negligible on field. People reported that very few seeds germinate. Even it germinates, it starts flowering late and takes many years to produce good yield.

5.9 Local Uses

Paris polyphylla has been used by local inhabitants traditionally since ancient times. They use it as traditional medicine especially for fever and headache. They also use it for burns, wounds and many livestock diseases mainly to neutralise poison when the livestock feed on poisonous herbs (bikh). Some people also used it as a medicine for Jaundice.

5.10 Harvesting Pattern

Paris polyphylla was found to be harvested for its underground rhizomes. The rhizomes were harvested mainly for medicinal purposes. In the past, the rhizomes were said to be traded. The harvested rhizomes were taken to Pokhara city or Kathmandu and traded from there to other parts of the country and sold at Rs 200 per kg. Some local people made a professional job of collecting these rhizomes for some traders living in the city. It was told that they left the job later because of low wages and some left after the area was designated as a conservation area. People are now afraid of wild collection of the plant rhizome as they fear being caught by the members of different conservation committees.

No trade of *Paris polyphylla* occurs at present but people still harvested the rhizomes of the plant for domestic use as medicines. From the survey, mainly two seasons were found for the harvest of the plant. Usually people harvest the rhizome at the fruiting

season (October), just before the plant dies. They prefer this season because the yield would be high at this time and also because the plant is easily recognizable at its fruiting time (*Paris polyphylla* and one species of *Arisaema* are found to be very similar and is difficult to distinguish except at the time of fruiting). But the local people especially the Gurungs believe that the plant harvested on last Tuesday of Chaitra month is more effective as a medicine than those harvested at any other season. So, some people harvested the rhizome of *Paris polyphylla* at this time, when the plant has just germinated. Besides, these two main seasons, people harvest the rhizome in between these period too whenever they visit the forest for fodder collection or for different purposes.

Seeds of the plant were found to be edible and local people ate the seeds of the plant just as other wild fruits but not for medicinal purposes. Medicinal value of seeds or other aerial parts have not been reported yet from the study area. After collecting *Paris polyphylla*'s rhizome, soil is removed from it and is dried in either sun or shade before it is stored for future use for medicine.

5.11 Antibacterial test

Since *Paris polyphylla* is used to treat wounds, burn (infected by *Pseudomonas aeruginosa*), fever (caused by *Salomonella typhii*), dysentery (caused by *Shigella dysenteriae*), diarrhoea (caused by *Eischeria coli*, *Shigella boydii*), the methanolic plant's extract of it was screened for their antibacterial property against different strains of bacteria using disc diffusion assay (Taylor et. al. 1995). The result of antibacterial test is summarized in Table 9:

Table 9. Zone of inhibition shown by plant's extract against bacterial strain

S.N.	Name of bacteria	Control (mm)	Rhizome extract (mm)	Seed extract (mm)
1.	*Escherichia coli*	14	10	10
2.	*Klebsiella pneumoniae*	19	8	16
3.	*Salomonella typhii*	8	9	9
4.	*Shigella boydii*	24	24	25
5.	*Shigella dysenteriae*	8	-	8
6.	*Staphylococcus aureus*	30	9	-

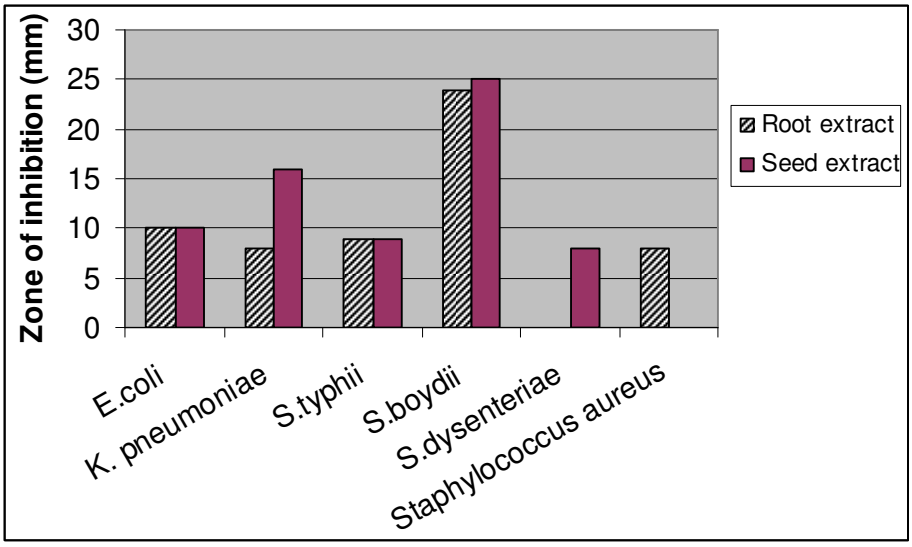


Fig.8. Antibacterial activity of the plant's extract

Both the rhizome and the seed extract produce the zone of inhibition against *Shigella boydii, Escherichia coli, Klebsiella pneumoniae, Salmonella typhii*. But no zone of inhibition was produced by rhizome extract against *Shigella dysenteriae* but seed extract showed some zone of inhibition against it. Similarly, no zone of inhibition was produced by seed extract against *Staphylococcus aureus* but rhizome extract showed some zone of inhibition against it.

Chapter 6

DISCUSSION

The present study aims to assess the ecology, distribution pattern, soil analysis of *Paris polyphylla* growing areas and its local use in Ghnadruk VDC. The emphasis has been given to *Paris polyphylla* since it is one of the most valuable medicinal herbs of temperate belt which has been categorized as vulnerable species.

6.1 Plant distribution

Though, *Paris polyphylla* is reported to be found up to 3300m in the literature, from our study site its distribution was found to be only up to 2800 m starting from 2000m – 2100m. Highest population of the plant was observed between 2300 m to 2700m which may be due to suitable environmental condition for the plant. Even though different literatures cite the plant's occurrence up to 3300 m, the plant was not found above 2900m in Rume, place above Chhomrong residents. Population was not very high in higher altitude or in lower altitude. It can be concluded that density of *Paris polyphylla* increases with increase in altitude up to certain altitudinal limit (2700 m) and then decreases gradually with further increase in altitude. Similar pattern of density distribution was also found in other medicinal plant species like *Kutki* (Gahire, 2003), *Swerita angustifolia* (Bhatt *et al.* 2007). Also, the distribution of herb was not common to all the areas but restricted to certain areas / pockets. This pattern of distribution correspond with some reports on other medicinal plant species like *Houttuynia cordata* (Bhattacharya and Sharma, 2008), *Nardostachys grandiflora, Arnebia benthami, Pleurospermum angelicoides* (Uniyal *et al.* 2002), *Swertia angustifolia* (Bhatt *et al.* 2007), *Nardostachys jatamansi* (Airi *et al.* 2000).

6.2 Plant attributes

Paris polyphylla plant is a perennial herb with rhizome of medicinal value. It reproduces through seeds as well as through vegetative propagation by rhizome. The plant is listed as vulnerable under IUCN threat category and CAMP.

Paris polyphylla grows under canopy of forest in full shade to partial shade. It was mostly found on the moist north facing slope although it shows some presence in a few sites in south facing slope too. The plant thrives well with moist and humus rich soil. Temperature decreases with increasing altitude. The study sites remain moist at

higher altitude under thick canopy of forest which receives low solar radiation which may be the reason for higher frequency and density of the plant in higher altitude in comparison to lower altitude (near 2000 – 2100 m) like Ghandruk. Even in Ghandruk, this plant is found in moist sites such as near streams. The presence of thick litter 1 – 3 cm deep with almost 40 – 65 % ground coverage on *Paris polyphylla*'s habitat also indicate that the plant is adapted to moist and humus rich soil. The plant found under open canopy coverage of forest on Komrong was also under dark shade formed by *Viburnum erubescens* and *Sarcococca coriacea*. Thus, this study shows that *Paris polyphylla* is a shade tolerant plant and area receiving direct solar radiation like pastureland is not suitable for the growth of this plant.

This species was mostly found in patches with numbers varying from 2 , 3 to 10 individuals in a patch of size ranging from (0.15m x 0.07m) to (6.4m x 6.5m) area. Various factors like altitude, aspect, slope, soil, vegetation type and anthropogenic activities influences the plant density. The higher density of *Paris polyphylla* was at the middle belt of the temperate zone i.e. 2300m to 2700m which may be due to favourable moist climate for the plant. The lowest density of this plant was found in Ghandruk. This might be due to lower altitude and excessive human encroachment of the huge settlement of Ghandruk village lying below. Average density of *Paris polyphylla* of Chhomrong was highest among the four sites. The plant was found from 2100m to 2600m i.e. the plant's abundance was higher in Chhomrong though the altitudinal difference was near to that of Ghandruk. This might be due to higher precipitation than other sites as it lies more near to Himalayan range. Moreover, fewer plants were found to be harvested by local people in Chhomrong in comparison to Ghandruk although the settlement of Chhomrong was as high as that of Ghandruk. Average plant density in Tadapani was better than in Ghandruk and Komrong. This might be due to less human encroachment as there are only four households in the area all running their own hotels, who have mostly migrated from nearby places. Only few people residing there were aware of the uses of this plant. So, very few plants were harvested from the area annually.

Overall, plant density (1.16 – 2.26 ind / m^2) of *Paris polyphylla* in Ghandruk VDC is very low i.e. average plant density = 1.78 ind / m^2 only. The density of another endangered plant, *Dactylorhiza hatagirea* was also found to be low (0.7 – 1.8 ind /

m^2) in Tungnath, alpine meadows of Garhwal Himalaya, India (Giri *et al.* 2008). *Angelica glauca*, medicinal plant of western Himalaya facing severe threat also showed comparatively low plant density 0.7 – 1.7 individual /m^2 in Garhwal Himalaya, Uttaranchal, India (Vashistha *et al.* 2006). But several earlier researchers reported the species density in high altitude region to be very high (Nautiyal *et al.* 1997; Nautiyal *et al.* 2000). This indicates that plant number is decreasing with time. There is no previous data on *Paris polyphylla* plant so the comparison could not be done but according to local people of Ghandruk, there was greater population in the past and the plant could be found easily nearer to the villages. But currently, people have to explore wider area to find a small population. This also indicates that the population of *Paris polyphylla* is declining with time. Frequency of occurrence was relatively better (40-83.33%) although population density was low. Results show similarity with data of another high value medicinal plant *Swertia angustifolia* where density ranges between 0.8 - 1.95 individual/m^2 while frequency ranges between 55 to 90%. Low population density across the surveyed population indicates poor availability of the species but higher frequency of occurrence indicates that species have better potential for better performance in the region (Bhatt *et al.*, 2007).

6.3 Species association

The associated species of *Paris polyphylla* in all sites of study area were almost similar. *Viburnum erubescens*, *Arisaema* sp., *Pilea* sp., *Sarcococca coriaceae* and fern were the most frequent associates of *Paris polyphylla* in all sites. The strong association of *Paris polyphylla* was seen with the *Arisaema* species as it was found that the *Paris polyphylla* plant's absence or presence in an area was easily indicated by the presence and absence of *Arisaema* in all the study sites. But the other associated species mentioned above were found to be distributed all over the forest while the *Paris polyphylla* and *Arisaema* species were found to be distributed in specific areas.

6.4 Biomass Production

Due to the low population density of the *Paris polyphylla* plant in the study site, only five fully mature individuals were harvested from each sampling site for studying the dry biomass production of rhizome to cause less damage to its wild population. The average dry biomass of *Paris polyphylla*'s rhizome calculated from the study area was

found to be 8.38 g / plant. The maximum dry biomass of *Paris polyphylla* rhizome was found in plant from Chhomrong which might be due to more favourable climate for the rhizome growth of the plant. Not only the rhizome growth but the shoot growth was also highest in Chhomrong site in comparison to the other site. The maximum plant height observed throughout the study area was 85 cm tall found in Chhomrong site.

In the present study, the moisture percentage on the basis of fresh weight could not be calculated because of lack of weighing machine in the field. But the moisture percentage in air dry sample was calculated. The highest value of moisture of *Paris polyphylla* rhizome was recorded in the sample of Ghandruk. This might be due to the plant's distribution which is mostly restricted to the streamside.

Paris polyphylla is a perennial herb and its rhizome attain larger dimension with age. So, its biomass increases with the age of the plant. Local people count its age by counting the scars / ridges found on the rhizome and the plant is said to live for 12 – 13 years.

6.5 Soil Analysis

Through soil analysis it was found that *Paris polyphylla* prefers highly acidic soil with average pH value around 3.7. Soil analysis of *Paris polyphylla*'s habitat reveals that plant occurring area is slightly acidic than the soil where this plant is absent. Similarly, soil nutrients like organic matter, nitrogen and phosphorus of soil of *Paris polyphylla*'s habitat were higher than the nutrients of soil where the plant is absent. But the potassium content of soil was found to be just the opposite. Though the nutrient contents of soil of different localities are more or less similar, the total nitrogen content (%), organic matter (%) and potassium contents of the soil of *Paris polyphylla* occurring areas of Ghandruk were higher in comparison with other sites (Table 7). This might be due to high litter accumulation in Ghandruk compared to other sites as litter is the nutrient reserve that becomes available for the plant to use as a result of weathering and mineralization of humus. But the phosphorus content of soil of that area was slightly lower than other two localities. The low amount of phosphorus may be due to interaction between different soil parameters.

6.6 Seed biology

All seeds matured by the end of September. Seed capsules usually rupture after maturation but seeds do not get dispersed immediately. Dispersal period of seeds varied within locality. Early seed dispersal takes place in Komrong followed by Ghandruk. Late seed dispersal takes place in Chhomrong. This might be due to the microclimatic factors and the encroachment in the forest.

Seed germination is extremely rare in nature. Local people reported that seeds germinate occasionally in nature but this is very rare. Seed germination did not take place in green house or in laboratory even by maintaining favorable temperature and treating it with different chemicals like Gibberlic acid, ammonium nitrate and concentrated sulphuric acid. It is mentioned that seeds produce primary root about seven months after sowing and then leaves about four months later / second year. Some seeds can remain dormant for a number of years (Vassilopoulos, 2009). The failure to break the dormancy of seed may be due to inappropriate measures for the type of seed dormancy of *Paris polyphylla*. It is reported that seed dormancy in *Paris polyphylla* is caused by dynamic changes of several endohormones (Absicic acid decreased, Gibberllic acid, Indole Acetic acid content increased), development of inhibiting substances, and the increase in material accumulation during embryo physiological ripening period (Yun *et al.* 2006). Moreover, the result of seed viability shows that only 29 % of total seeds tested were viable which is comparatively very low. This may be the one cause for negligible seed germination.

Because of few viable seeds and difficulty in germination, propagation by seeds is probably not the chief means of proliferation of this species even though the seed output is quite good. If we can enhance the seed germination, grow the plants in nursery beds and successfully transplant it in the wild, its conservation would be secure for the future as seed might be the principal method of increasing distribution / population of plant. The plant reproduces easily by vegetative propagation through rhizome which seems to be the common mode of regeneration. But a single offspring is produced from a single mother plant which in turn is harvested unscientifically leading to rapid decline in the number of this important plant species. Another

threatened medicinal plant like *Dactylorhiza hatagirea* also shows similar results (Ghimire *et al.* 1999).

6.7 Local knowledge on collection and use of plant

Local knowledge of the people about the resource availability, distribution, extraction process, amount use and management along with bio-physical, socio- economic and cultural historical elements of their immediate environment plays a significant role in determining the long term sustainability and management of resources (Cunningham, 1997).

Paris polyphylla has been used by local inhabitants in traditional ways since long period of time. They use it as traditional medicine especially in fever and headache. Some young people of the village are unaware of its uses and hence rely more on modern medicine like cetamol for the treatment of fever instead of the plant's rhizome. There is one health post running successfully in the VDC. There are also local people especially professional traders who visit the Pokhara city (nearby city) daily or frequently to buy goods needed to run the hotels in the study sites and buy allopathic medicine along with them which are transported upto the village by mule. Despite these, large number of local people still rely on *Paris polyphylla*'s rhizome for the treatment of various diseases.

Local people just uproot the plant for collection of the rhizome. They were unaware about harvesting the plant in a sustainable way. Mainly two seasons were preferred by the local people to harvest the plant. Usually local people harvest this plant from September to October before the plant dies as they believe that the yield will be high at this time. This is the appropriate time because by this time, the bud emerges from the rhizome which remains dormant underground till next germination period. If some portion of the rhizome with bud is left underground while harvesting the rhizome of the plant, it will be more sustainable and would help in conserving the plant population in near future. Moreover, this is also the appropriate harvest time for the plant according to different literature (Bhattarai and Ghimire, 2006; Shrestha and Shrestha, 2064). But according to Gurung culture, the plant harvested on Tuesday of mid April (i.e. last Tuesday of Chaitra month) will be more effective medicinally than the plant harvested in other seasons. So, the Gurungs, major inhabitants of the study

area, harvest the plant during this time period which is the rapid growth period of the plant. This mode of harvest is unsustainable and directly affects the plant population.
No commercial collection or trade was found from Ghandruk VDC at present. It is banned for commercial collection in this region as it falls under Annapurna Conservation Area. Strict regulation is followed by the ACAP's staff and the local committee's members. But some local people reported that there used to be a large scale collection of *Paris polyphylla*'s rhizome from the study site 10 - 15 years ago. The rhizomes were traded to Pokhara and Kathmandu city at Rs. 100 – 200 per kg. It is said that even the people from Gorkha and near by VDC like Dansing used to come to Ghandruk VDC to collect the *Paris polyphylla*'s rhizome and sell it at a reasonable price.

The trade of *Paris polyphylla* is reported from other parts of the country. According to records of Department of Forest, Ministry of Forest and Soil Conservation (MoFSC) from fiscal year 2059 / 2060 to 2064 / 2065, the main commercial centers for the export of *Paris polyphylla* in Nepal was Bajhang, Darchula, Jajarkot, Bajura, Mugu, Jumla, Kalikot, Achham. An average of 2,091 kg of *Paris polyphylla* is reported to be exported from the Nepal legally in the fiscal year 2064 / 2065 collecting total revenue Rs 18,790. The royalty rate is Rs. 5 / kg of dry rhizome and it ranges up to Rs 10 / kg at major trading centers in Nepal (GoN, 2008).

6.8 Antibacterial test

Rhizome and seed extracts of *Paris polyphylla* screened for antibacterial activity shows that extract prohibit the growth of *Shigella boydii* and *Escherichia coli* both of which are the causal organism for the diarrhoea. Seed extract was found to inhibit the growth of *Shigella dysentery* that causes dysentery while the rhizome extract produce no zone of inhibition. Both kinds of extracts were found to inhibit the growth of *Salmonella typhii* that cause different kinds of fever. So, the plant extract is effective for the treatment of diarrhoea, dysentery and fever which matches with the ethnobotanical practice of the local people. Both kinds of extract also inhibit the growth of *Klebsiella pneumoniae* which can cause severe bronchopneumonia, urinary tract and other human infections. Plant extracts show less response to gram positive bacteria than gram negative bacteria. Regarding the zone of inhibition produced, seed extract was found to be more effective than rhizome extract. Panthi (2006) found

similar kinds of result, root extract of the plant was effective in inhibiting the growth of *Escherichia coli* and *Shigella boydii* but ineffective with gram positive bacteria *Staphylococcus aureus.* Contrasting result was obtained by Parajuli (2001) who stated that *Paris polyphylla*'s rhizome extract did not show the zone of inhibition with any of the test bacteria *(Bacillus subtilus, Staphylococcus aureus, Pseudomonas aeruginosa*) including *E. coli*. Pokhrel (2000) also found similar negative results. Such negative results may be due to chemical degradation during drying the plant part, degradation of chemicals during extraction process or due to test concentration.

Present study shows that zone of inhibition produced was more in seed extract than rhizome extract. This shows seed extract has higher antibacterial property than rhizome extract which is in contradiction with the local use of the plant. People use the rhizome of the plant for treatment of different diseases while the seed is used just as it is edible. The seed showed medicinal property like that of rhizome which may be due to the presence of steroid saponins like that present in rhizome (Chen *et al.* 1990) though it is not in use in traditional health practice. All the literature mentioned the high use of the rhizome of the plant and many researches have been done regarding the chemical constituents present in the rhizome of the plant (*Deng et al.* 2008; *Yin et al.* 2008; Wang *et al.* 2007; Devkota, 2005). But very few research have been done regarding the seed constituents which is felt necessary as it is also found to effectively control the growth of certain bacteria. Further research has to be done regarding its antibacterial / antifungal / antiviral activity which can be a good source to pharmaceutical industry. Vassilopoulos (2009) have stated that the whole plant can be used as febrifuge.

6.9 Threat, Management and Conservation

Human encroachment was more in the study site especially in Ghandruk and Chhomrong where the human settlement was large. Except Tadapani, all the other sites were found to be grazed by the livestock. People take their herds of sheep and goats to the pasture land in June / July and from September / October as the temperature goes on decreasing in high altitude, they slowly move down to the forest with their livestock for grazing. They stayed at forest for 1 – 2 month slowly moving down residing at different places and grazing their livestock in the forest. People reported that among the livestocks, sheep and goat eat the plant's (*Paris polyphylla*'s)

shoot and fruit but cow and buffaloes do not. Goats prefer it more than sheep does. Similar case was found for *Aconitum naviculare* (Chandrashekar, *et al.* 2007 as cited in Shrestha *et al.* 2007 b). Besides that, trampling of livestock destroys the plant habitat. These might be the one reason for very low population of the plant.

Overharvesting in the past, unscientific collection of rhizome (in which all the underground parts are removed without leaving any fragment), harvesting of plant before seed maturity, lower number of viable seed production and long dormancy of seeds or very poor seed germination seems to be the major threats to the plant regeneration in the study area. As a consequence, population is declining day by day. People are aware of the declining population of *Paris polyphylla* but they are unaware about its sustainable harvest. Although different conservation committee and ACAP's staff look after the natural resources in this area including this medicinal plant, the management is poor.

Paris polyphylla can be cultivated in the study area by creating suitable environment but there has been no such efforts done yet. In Rasuwa district, people of Thulo Syaphru and Brabal VDC have started the small scale cultivation of *Paris polyphylla* (Prasai, 2007). In China, the Yi healers in Chuxiong of Central Yunnan have traditionally grown one variety of *Paris polyphylla* viz. *Paris polyphylla* var. *yunnanensis* in their own agroforestry systems. Fruit trees and crops were the major component in the system with *Paris polyphylla* being the byproduct from the system (Long, *et al.* 2003). Large scale cultivation of *Paris polyphylla* var *yunnanensis* are being tested in Lameirong village and Dian Nan Village of North West Yunnan, China (Shengji *et al.* 2006).

Chapter 7
CONCLUSION AND RECOMMENDATION

7.1 Conclusion

Paris polyphylla is a threatened medicinal plant found in temperate belt. In the study area, the plant is found to be distributed from 2000m- 2800m under forest canopy in dark shade to partial shade but is completely absent from open canopy forest or pastureland. The plant grows in moist, acidic soil with high nitrogen, organic matter, phosphorus and potassium content. The average plant density recorded in the study area is low which might be due to overharvesting of the rhizome in the past and unsustainable harvesting from past to the present, grazing impact, negligible seed germination and poor management. The plant's rhizome is widely used in Ghandruk VDC especially for the treatment of fever and headache. They also use it for burns and wounds and feed it to livestock to counteract poison when they graze on poisonous herbs. Among the four localities studied Chhomrong shows higher density, seed output (per plant) and better growth of the plant in comparison to other sites. This reveals that the environmental conditions of Chhomrong are best suitable for the plant. The red colored fleshy seeds show late dispersal after the rupture of capsule or seed maturation. The seed shows negligible germination in the nature which might be due to low viability of the seeds and long dormancy period of seeds. The plant regenerates mostly by vegetative propagation through rhizome which is harvested annually for local uses without leaving any sign of it. Even though no trade of rhizome is found at present or recent past as it falls under the Annapurna Conservation Area, the plant's population seems to be declining due to lack of conservation awareness among local people, disturbances on plant's habitat like grazing, overharvesting in the past etc.

The ecological condition of the study area is best suitable for the plant's (*Paris polyphylla*) growth but its population is at risk which needs to be conserved in time. Cultivation practice inside the forest and sustainable use of the rhizome should be promoted in order to increase the declining population.

7.2 Recommendations

- The conservation / awareness program on the conservation and importance of natural resource, sustainable utilization, management and proper harvesting of the medicinal and aromatic plants (MAPs) at local level need to be conducted.
- Harvesting of MAP should be done in sustainable way such as to leave behind about 20 – 25 % of the total portion of rhizome so that further regeneration is assured as its parts used as resource (rhizome) is the most important part required for its regeneration. Seeds shows very poor germination and the rhizome is the only source of effective regeneration.
- Because of rapid declination of population of *Paris polyphylla*, it is urgent to develop rapid propagation method (eg. Tissue culture, mycorrhizal techniques)
- Cultivation technique of the plant and its extension in the mountain region of Nepal should be promoted.
- Further explorations should be carried out to assess their status in other different parts of the country.

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Annex I

General Description of Bacteria

Escherichia coli

Escherichia coli is the type species of the genus *Escherichia.* They are gram negative, straight rods, motile by petritrichous flagella and non spore forming. They are facultatively anaerobic and are chemoorganotrophic, having both a respiratory and a fermentative type of metabolism. *E. coli* occur as normal flora in the lower part of the intestine of warm blooded animals and are thus generally taken as indicator of faecal pollution. *E. coli* are found to be associated with opportunistic infections. Certain strains of *E. coli* are recognized as gastrointestinal pathogen which contains enterotoxins or the virulence factors including invasiveness and colonization factors which cause diarrhoeal disease. *E. coli* is a major cause of urinary tract infections and nosocomial infections including septicaemia and meningitis. The strains producing enterotoxins cause food poisoning (Holt *et al.* 1994; Collee *et al.* 1996).

Klebsiella pneumoniae

Klebsiella pneumoniae is the type species of genus *Klebsiella.* They are gram negative, capsulated straight rods, non – motile and facultatively anaerobic. They are chemoorganotrophic, having both a respiratory and fermentive type of metabolism. *K. pneumoniae* contains a large polysachharide capsule so it forms large, grayish – white, mucoid colonies or the laboratory medium. *Klebsiella* sp. occur in human faeces and clinical specimens, soil, water, grain, fruits and vegetables. *K. pneumoniae* can cause severe bronchopneumonis, bacteremia and urinary tract and other human infections. They frequently cause noscomial infections in urological, neonatal, intensive care and geriatric patients. (Holt *et al.* 1994).

Salmonella typhii

Salmonella typhii is a pathogenic enterobacteria. They are gram negative bacilli, non capsulated and non sporing usually motile by peritrichous flagella. They are facultatively anaerobic and chemoorganotrophic. They occur in human, warm and cold blooded animals, foods and the environment. They are pathogenic for human and many species and they cause typhoid fever, enteric fever, gastroenteritis and septicaemia (Holt *et al.* 1994; Collee *et al.* 1996).

Shigella dysenteriae

Shigella dysenteriae is the type species of the genus *Shigella.* They are gram negative, straight rods, facultatively anaerobic and non – motile. They are chemoorganotrophic. *Shigella* are divided into four group species: *S. dysenteriae*, *S. flexneri*, *S.boydii* and *S. sonneii. S. dysenteriae* are intestinal pathogens of humans and other primates causing bacillary dysentery (Holt, *et al.* 1994; Collee *et al.* 1996).

Shigella boydii

Shigella boydii is the type species of the genus *Shigella.* They are enterobacteria that are non-motile, non – sporing, non – capsulate gram negative rods. They infect the intestinal mucosa to cause dysentery in man. A positive finding in case of diarrhoeal illness is practically diagnostic of *Shigella boydii*. *Shigella* may however be present in the faeces of chronic carriers and so may occasionally be found in the faeces of a patient with diarrhoea due to a cause other than Shigellosis (Holt. *et al.* 1994).

Staphylococcus aureus

It is a spherical facultatively anaerobic, gram-positive bacteria, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo (may also be caused by Streptococcus pyogenes), boils, cellulitis folliculitis, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, Toxic shock syndrome (TSS), and septicemia (Holt. *et al.* 1994).

Annex II

Questionnaire

1. What about the distribution and availability / abundance of the plant at present and in the past?
2. Which part of plant is used and for what purposes?
3. When do you harvest the plant and what tools and techniques are applied?
4. How much amount is harvested annually?
5. Who are involved in the plant collection?
    - All the local villagers
    - Only the primary users
    - Distant people also
    - Trekkers
6. Is there any commercial collection of the plant and trade found illegally at present and in the past?
7. What about the trend of amount harvested from past to the present? Decreasing or increasing? So, why?
8. What about the regeneration of the plant? Condition of regeneration from seed and rhizome?
9. Who do you think are more responsible for the depletion of the plant?
10. Do you use the modern medicine? Is it accessible easily?
11. Which one do you prefer best and which one first?
12. What kinds of other medicinal plants are present in the area and what's their use?
13. Which other kinds of highly valuable medicinal plants do you use in traditional health care that may not be available in the village? From where do you get them? What difficulties / constraints in getting them?
14. What type of NTFPs / MAPs based industries are present in the area and what about their status?
15. In your opinion, how the plant can be conserved? What should be done from local level / ACAP / Governmnet?
16. Do you follow the conservation practice, if yes how? If no, what can be done?
17. Do you want to cultivate the plant on your private land?

Annex III

Climatic data

Average maximum and minimum temperature of Lumle station from 2003 / 2007

S.N.	Month	T max (^{o}C)	T min (^{o}C)
1	January	14.66	5.32
2	February	16.06	7.04
3	March	20.48	9.72
4	April	23.24	12.12
5	May	23.92	13.84
6	June	24.42	16.32
7	July	42.14	17.7
8	August	24.64	17.54
9	September	23.58	16.58
10	October	21.8	12.98
11	November	18.42	8.92
12	December	15.58	5.98

Average rainfall of Ghandruk station from 2003 – 2007

S.N.	Month	Rainfall (mm)
1	January	41.06
2	February	69.8
3	March	68.84
4	April	176.32
5	May	169.14
6	June	389.5
7	July	762.36
8	August	755.72
9	September	442.1
10	October	68.4
11	November	13.84
12	December	7.4

PHOTO PLATE

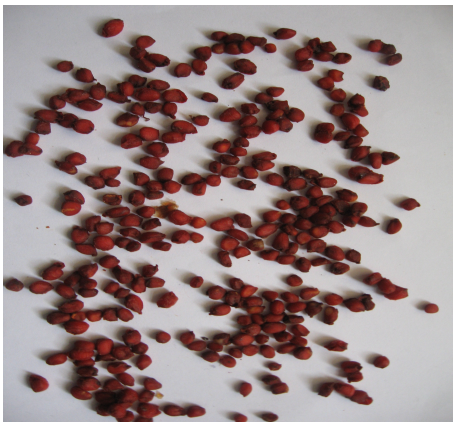
a. Flowering Stage

b. Post Flowering Stage

c. Plant without flower

d. Fruiting Stage

e. After seed dispersal



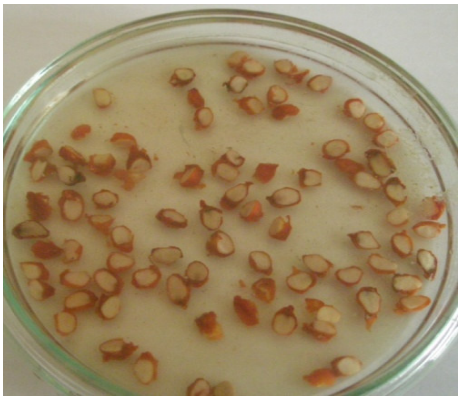
f. Dry seeds

Plate 1. *Paris polyphylla* (Satuwa) Plant

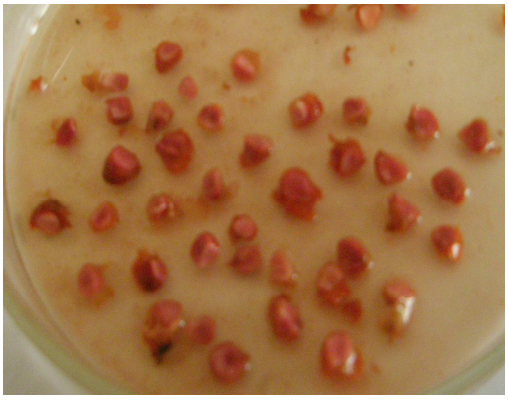
PHOTO PLATE

g. Rhizome

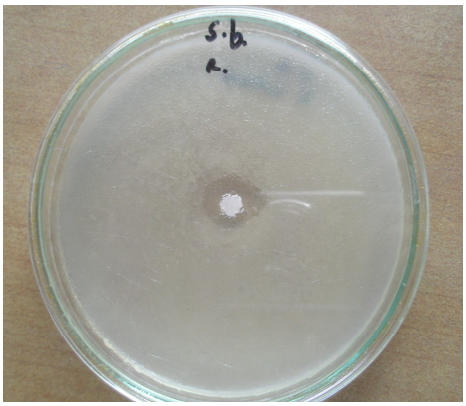
h. Rhizome powder



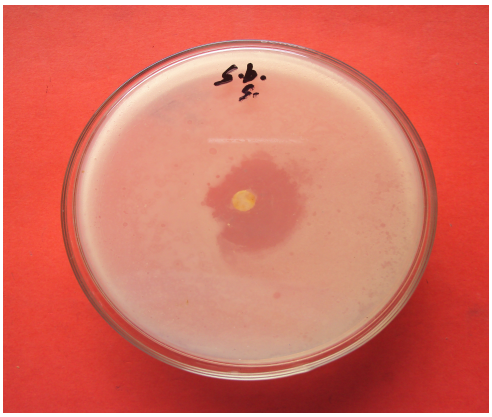
i. Test for seed viability



j. Viable seed



k. Antibacterial test of rhizome extract with *Shigella boydii*



l. Antibacterial test of seed extract with *Shigella boydii*

Plate 2. Rhizome and seeds of *Paris polyphylla*