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Ecological Parameters and Diversity Indices of Marsh Orchid (*Dactylorhiza hatagirea D.Don*) and its Associates in Lete Village of Mustang District, Nepal

C.B. Khadka¹, A.L., Hammet², A. Singh³, Y.P. Timilsina⁴ and M.K. Balla⁵

Abstract

This paper focuses on the ecological parameters and diversity indices: Simson's Index (C), Simson's Index of Dominance (D), Shannon-Weaver Index (H), Shannon Evenness (E_{shannon}) and Simson Evenness (E_{Simson}) of *D. hatagirea* and its associates-*Rheum australe, Rumex nepalensis* and *Allium wallichii kunth* in Lete village of Mustang District within the Annapurna Conservation Area. The inventory was conducted during the monsoon season (June/July) of 2013 within an area of 4.5 ha. Altogether, 100 circular plots, each with 25 m² area, were laid out purposively with the sampling intensity of 5.55%. The relative frequency, relative density, abundance, relative coverage and the Important Value Index of *D.hatagirea* were found to be 70.27, 49.64, 0.44, 70.27 and 190.18, respectively. Similarly, the value of C, D, H, E_{Shannon} and E_{Simson} of *D. hatagirea* were 0.35, 0.65, 1.18, 0.85 and 0.71, respectively, indicating relatively even and relatively diverse community. The study showed relatively higher values of all the parameters of *D. hatagirea* as compared to its associates indicating good ecological value. The Rank abundance curve showed uneven plant community in the study site. However, threats remain due to the unsustainable harvesting, illegal trading and overgrazing in the study site.

Keywords: Biodiversity, Conservation, Medicinal Plants, Abundance.

Introduction

Orchidaceae is regarded as the largest family of plant kingdom comprising 25,000-35,000 species (Dressler, 1993; Hossain, 2011). Scientists have traced orchids as far back as 120 million years ago. The history of orchids might have started with their uses in the medicinal purpose. Chinese were the first to cultivate and describe orchids (Jalal et al., 2008). Nepal constitutes a unique and enormous diversity of flora and fauna within a relatively small geographical area due to variations in topography, altitude and climate. In spite of being a small country, it possesses around 7000 species of vascular plants having 2000 species of medicinal plants (Shrestha and Shrestha, 1999). Baral and Kurmi (2006) have compiled and described 1792 medicinal plants. According to Bhattarai and Ghimire (2006), 49% of the traded medicinal plants are herbs, 29% tress, 14% shrubs and 8% climbers. So, Nepal is veritable treasure trove of medicinal plants (Phoboo et al., 2008).

Medicinal and aromatic plants of high altitude region are an invaluable resource not only to local communities and the nation, but also to the global Orchids are nature's most extravagant group of flowering plants distributed throughout the world from tropics to high alpine (White and Sharma, 2000). They exhibit incredible range of diversity in shape, size and color of their flowers. They are important aesthetically, medicinally and also regarded as ecological indicators (Joshi *et al.*, 2009).Out of many MAPs, *Dactylorhiza hatagirea* (*D.Don*) Soo commonly known as marsh orchid in English (Warghat, 2015) and *panch aunle* in Nepali has been identified as the endangered species listed by Convention on International Trade in

¹Ranger Chhatra Bahadur Khadka

Ministry of Forest and Soil Conservation, Department of National Parks and Wildlife Conservation, Chitwan National Park, Nepal. ²Professor A.L. (Tom), Hammet, Virginia Polytechnic Institute, Blacksburg, USA Department of Sustainable Biomaterials, 230 E Cheatham, Blacksburg, VA 24061.

³Professor Abadhesh Singh, Institute of Forestry, Tribhuvan University, Pokhara Campus, Nepal.

⁴ Yajna Prasad Timilsina, Institute of Forestry, Tribhuvan University, Pokhara Campus, Nepal.

⁵Mr. Mohan Balla Institute of Forestry, Pokhara Sankhamul, Kathmandu, Nepal.

community at large. They have high ecological values as well as poor rural communities are highly dependent on them for their health and economic benefit derived from harvesting for trade. The Master Plan for Forestry Sector (1988) and the tenth five year plan (2003- 2008) has emphasis the development of Medicinal and Aromatic Plants (MAPs) as a priority programme for poverty alleviation. These show the concernment of the government for conservation and management of medicinal plants. Rare and high priced medicinal herbs are on the top priority for domestication, research and cultivation, processing and marketing.

^{*} Corresponding author: Ranger Chhatra Bahadur Khadka

^{*} e-mail: chhatra10@gmail.com

Endangered Species (CITES), vulnerable species listed by Conservation Assessment and Management Plan (CAMP) and critically rare by IUCN (Samant, 2001). Dactylorhiza hatagirea is a Himalayan endemic medicinal orchid which is found in Hindu Kush Himalayan range. Its occurrence is sub-alpine and alpine zones from 2800-4200 m above from sea level (IUCN, 2004). Other than Nepal Himalayas, it occurs in the same altitudinal ranges of India, Pakistan, Bhutan and China also. The government of Nepal has prioritized 30 important medicinal plants for research and management. Among these, 12 plants have been selected for agro-technology. Dactylorhiza hatagirea is one of them (DPR, 2006). According to Forest Act 1993 and Forest Regulation 1995, Nepal Government has banned the rhizome of *Dactylorhiza* hatagirea to collect, trade and process.

As with many other terrestrial orchids, populations of *Dactylorhiza* have decreased due to habitat loss. Another threat to *Dactylorhiza* is the collection of their tubers to make salep (starchy sweet substance that can be added into bread or make a drink), used as food and medicine. This is a particularly important threat in the Himalayas (Srivastava and Mainera, 1994), where *D. hatagirea* or "panchaunle" is judged critically endangered (Biodiversity Conservation Prioritisation Project, 1998) due to over-collection. Thus, several species of *Dactylorhiza* are declining, and some are already protected at a national scale, e.g., in Belgium, Luxembourg, Nepal, and the United Kingdom (UK) (Pillon *et al.*, 2005).

The basic idea of a diversity index is to obtain a quantitative estimate of biological variability that can be used to compare biological entities, composed of discrete components, in space or in time. Species richness is a measure of the total number of species in the community (but note already that the actual number of species in the community is usually immeasurable). Evenness expresses how evenly the individuals in the community are distributed over the different species (Peet, 1974). The concept of diversity, including biodiversity itself as well as the narrower concept of species diversity, is a human construct without any unique mathematical meaning. The simplest measure of species diversity is species richness, but a good case can be made for giving some weight to evenness as well. Diversity indices are mathematical functions that combine richness and evenness in a single measure, although usually not explicitly. Although there are many others, the most commonly used diversity indices in ecology are Shannon diversity, Simpson diversity, and Fisher's a. Both Shannon and Simpson diversities increase as richness increases, for a given pattern of evenness, and increase as evenness increases, for a

given richness, but they do not always rank communities in the same order. Simpson diversity is less sensitive to richness and more sensitive to evenness than Shannon diversity, which, in turn, is more sensitive to evenness than is a simple count of species (richness, S) (Colwell, 2009).

Very few studies have been conducted especially regarding ecological status and diversity of *Dactylorhiza hatagirea* in Nepal. There is lack of management and conservation plan from the government side. Similarly, lack of awareness of importance regarding *D. hatagirea* among rural people is leading towards the extinction of this valuable species. Although this is a banned species, its unwise harvesting, unscientific use and illegal trading are being practiced. This is resulting in improper use of *D. hatagirea* and also reducing the net income of the primary collectors and reducing national income. Therefore, the study focused on finding out the values of the ecological parameters of *D. hatagirea* and its associates and estimating diversity indices of *D. hatagirea* and its associates in the study site.

Materials and methods

Study site

The study was conducted in Paplekharka, an alpine grass land situated in Ghasa of Lete Village Development Committee (VDC) of Mustang District (Plate 1) which is one of the working areas of the

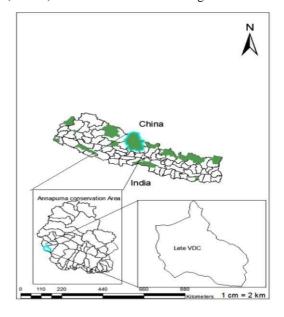


Plate 1. Geographic Information system (GIS) map of the study area.

Annapurna Conservation Area (ACA). Mustang District lies between 28°24' N to 29°20' N latitude and 83°30' E to 84°15' E longitude. The altitudinal range varies from 1,372 m to 8,167 m above the mean sea level representing subtropical, temperate and alpine types of climate (Ranpal, 2009). Lete VDC lies within the Jomsom Unit Conservation Office (UCO) of the Lower Mustang which is a transition between the Trans-Himalaya and the Inner Himalaya. The VDC receives 1,545 mm rainfall per annum (ACAP, 2009). It consists of deep gorges made by the Kaligandaki River.

Sampling Design

The inventory was carried out in 4.5 ha during the monsoon season (June/July) in 2013. A 21 m x 21 m grid (plot to plot distance) was laid on the map and, altogether, 100 circular sample plots were established using the Arc Map Geographical Information System

(GIS) Software of version 10; the sampling intensity being 5.55% (the greater intensity was due to the small area of the study site). Among the 100 plots, *D. hatagirea* plants were found in 26 plots only. The circular sample plots were chosen purposively so that the plots where *D. hatagirea* plants and its associates were found would not be left during the field inventory as the topography in some plots was either rocky or stiff or with dense forest cover or barren (Plate 2).

All the plants of *D. hatagirea* found in 26 plots were measured. The number of plots in which *D. hatagirea* was absent was 15 (in which other herbs were present) excluding the plots with barren, forest, rocky and stiff topography. Similarly, out of the total 100 plots, 20 plots included barren area, 16 plots forest area and 23 plots rocky and stiff area in which inventory was not possible.



Plate 2. Google image (26-10-2016) of Lete VDC and sample plots (placemarks) of marsh orchid.

Species accumulation curve and sampling

Species accumulation curve is a curve of rising biodiversity in which the x-axis is the number of sampling units (individuals or samples) from an assemblage and the y-axis is the observed species richness. The species accumulation curve rises monotonically to an asymptotic maximum number of species (Figure 1). The curve is steepest in the early part of the collecting, as the common species in the assemblage are detected relatively quickly. The curve continues to rise as more individuals are sampled, but the slope becomes shallower because progressively

more sampling is required to detect the rare species. As long as the sampling area is sufficiently homogeneous, all of the species will eventually be sampled and the curve will flatten out at an asymptote that represents the true species richness for the assemblage (Gotelli and Chao, 2013). Similar trend was found while plotting the curve of number of species (x-axis) with number of collected samples (y-axis) in study.

Primary data collection

The primary data was collected through reconnaissance survey and herb inventory. The reconnaissance survey was carried out for general field observation,

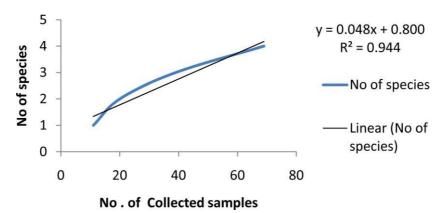


Figure 1. Curve showing number of species *vs* number of collected samples of species.

rapport building with the local people and the ACAP personnel about the location where D. hatagirea was found. A participatory sketch map was prepared for carrying out the field inventory smoothly. Generally, 1 m × 1 m sample plots are used for inventory of herbs. However, as D. hatagirea is a low abundant herb, circular sample plots of 25 m² were established as recommended by Ravindranath and Premnath (1997). All the plants of D. hatagirea and its associate species were counted and their mean height, mean collar diameter (5 cm above the ground) and mean age were measured within the sample plots. The mean age of D. hatagirea was calculated by summing up all the ages of D. hatagirea plants and dividing by the number of plots where D. hatagirea occurred. Similar process was repeated to calculate the mean height and the mean collar diameter of the species. A Global Positioning System (GPS) was used to locate the sample plots and a 20-meter Reel Tape was used to measure the radius of the circular sample plot. Similarly, Vernier Calliper was used to find out the collar diameter of the herb whereas a 5- foot Steel Tape was used to detect the height of the herb. An Inventory Sheet was developed to record the details for calculating the ecological parameters and the diversity indices of the species. The frequency, relative frequency, density, relative density and abundance were calculated as recommended by Ravindranath and Premnath (1997) whereas coverage, relative coverage and Important Value Index (IVI) were calculated as recommended by Department of Forest (DoF) (2013). Species Rank versus Abundance, Species Rank versus Log Abundance and Species Rank versus proportion curves was plotted to identify the nature of plant community (evenness or unevenness) in the study area.

Secondary data collection

The secondary data were obtained from the annual reports, newsletters, bulletins, journals, dissertations, publications, maps and so on available in the ACAP Libraries as well as at the Department of Forest Research and Survey (DFRS), the Institute of Forestry (IoF) and the International Centre for Integrated Mountain Development (ICIMOD). Additional information was also acquired through internet.

Data analysis and interpretation

The information obtained from the herb inventory was analyzed using Microsoft Excel and the Statistical Package for Social Science (SPSS). The quantitative data was analyzed by using these formulas:

i) Frequency =
$$\frac{\text{No.of plots where species occcurs x 100}}{\text{Total no. of plots}}$$

ii) Relative Frequency = $\frac{\text{Frequency of species x 100}}{\text{Sum of all frequency}}$

iii) Density = $\frac{\text{No.of species in all plots x 10,000 m}^2}{\text{Total no. of plots x area of plot}}$

iv) Relative Density = $\frac{\text{No.of species in all plots x 100}}{\text{Total no. of individuals of all species}}$

v) Abundance = $\frac{\text{No. of species in all plots x 10,0000m}^2}{\text{No. of plots in which species was found x area of plot}}$

vi) Coverage (%) = $\frac{\text{Area occupied by species A x 100}}{\text{Area of sample plot}}$

vii) Relative Coverage = $\frac{\text{Coverage of species A x 100}}{\text{Total coverage of all species}}$

viii) Importance Value Index (IVI) = Relative Frequency + Relative Density + Relative Coverage

ix) Simpson's (1949) Index (C) = $\sum (P_i)^2$ and

(Department of Forest, 2012)

Simpson's index of Dominance (D) = $1 - \sum_{i} (\tilde{P}_{i})$ Where D = Simson index of Dominance,

P_i= the proportion of individuals found in the i species

 $(P_i = n_i/N, n_i)$ is the number of individuals of i species and N is the total number individuals of all species)

The Simpson's index values range between 0 and 1. The closer to 0 the value is, the more diverse the plot is. A plot with only one species would have a Simpson's index value of 1.0. Trends are opposite to those found for Shannon-Weaver values since Simpson's Index

values decrease with increased diversity (Reich et al., 2001). In practice values below 0.5 indicate a relatively even community, while high values are indicative of communities dominated by one or a few species.

x) Shannon-weaver (1949) index (H) =
$$-\sum_{i=1}^{s} (pi) * \ln(pi)$$

Where H= index of species diversity

 P_i = the proportion of the number of individuals of i species

 $(P_i = n_i/N, n_i)$ is the number of individuals of i species and N is the total number of individuals of all species) ln = Natural logarithm

Due to its logarithmic nature, the Shannon-Weaver Index is sensitive to uncommon plant species and less sensitive to very common species (Krebs, 1989). The Shannon-Weaver index can theoretically range from zero (a community with only one species, which is technically just a "population") to infinity. In practice

though, a value of 7 indicates an extremely rich community while values under 1 suggest a community with low diversity. Often values above 1.7 are taken to indicate a relatively diverse community. The qualitative data was analysed descriptively.

xi) Shannon Evenness (
$$E_{Shannon}$$
) = $\frac{H}{Ln(S)}$
n-Weaver index, S= Number of species, Lr

Where H = Shannon-Weaver index, S= Number of species, Ln=Natural logarithm

If diversity is a mixture of richness and evenness, then removing richness should produce evenness. This is the logic behind Shannon's evenness measure; the highest value of H when all species are equally

abundant can readily be seen to be ln(S) so dividing by ln(S) will give an index from 0 to 1 (Maurer and McGill, 2011).

xii) Simson Evenness (
$$E_{Simson}$$
) = $\frac{1}{D/S}$
Where D= Simsons' Index of Dominance, S= Number of species

Table 1. List of scientifically identified herbs found in the project site.

S.no.	Local	English Name	Scientific Name	Family	Nature
1	Name Panch aunle	Himalayan Orchid	Dactylorhiza hatagirea (D.Don) Soo	Orchidaceae	of Plant Herb
2	Padamchal	Himalayan Rhubarb	Rheum australe D.Don	Polygonaceae	Herb
3	Halhale	Nepal Dock	Rumex napalensis Spreng.	Polygonaceae	Herb
4	Dungdunge	Himalaya Onion	Allium wallichii Kunth	Amaryllidacea e	Herb

The same logic applies to Simson Evenness. Evenness is a measure of how different the abundances of the species in a community are from each other (Smith & Wilson, 1996). A community where every species had the same abundance would be perfectly even. All natural communities are highly uneven, so evenness is a relative statement. Most evenness indices are scaled to approximately run from 0=maximally uneven to 1 = perfectly even (Maurer and McGill, 2011).

Results

Distribution of D. hatagirea

A total of three scientifically identified herbs viz. Himalayan rhubarb (*Rheum australe*), Nepal dock (*Rumex nepalensis*) and Himalyan Onion (*Allium wallichii kunth*)were found in the project site with

dominance of D. hatagirea (Table 1).

Mean height, collar diameter, number of leaves and age of *D. hatagirea*

The height, collar diameter and age of each *D. hatagirea* plant were measured in each plot in which a total of 69 *D. hatagirea* plants were found in 26 measured plots. From the inventory, it was found out that the mean height of *D. hatagirea* was 91.08 cm. The mean collar diameter at 5 cm above the ground was found to be 1.63 while the mean age was found to be of 2 years and the mean number of leaves was found to be 5.

Frequency of D. hatagirea and its associates

The study shows 26 % occurrence of *D. hatagirea*, 7 % of *Rheum australe*, 3 % of *Rumex nepalensis* and 1 % of *Allium wallichii* in the sample plots.

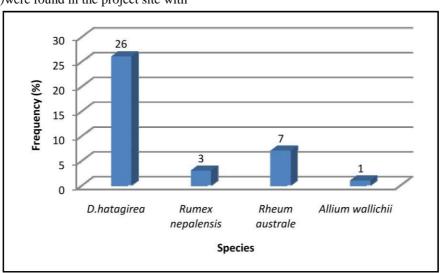


Figure 4. Frequency percentage of species.

Relative frequency of D. hatagirea and its associates

Relative frequency is the frequency of a species in relation to other species. The relative frequency of *D. hatagirea* was high (70%) followed by *Rheum australe* (19%), *Rumex nepalensis* (8%) and *Allium wallichii* (3%) compared to its three associates.

Density of D. hatagirea and its associates

This study showed the highest density of 276 per ha $(0.03 \text{ individuals (ind)}/\text{m}^2)$ of the marsh orchid (D. hatagirea) as compared to its associates (Table 2).

Relative density of D. hatagirea and its associates

Relative density is the density of a species with respect to the total density of all species (Ranpal, 2009). In the study site, *D. hatagirea* was found to have the

highest relative density (49.64 %) as compared to those of its associates (Table 3).

Abundance of D. hatagirea and its associates

Allium wallichii Kunth was found to have the highest abundance of 0.44 followed by Rumexnepalensis with 0.27. Rheum australe had abundance with 0.22 while D.hatagirea had the least abundance of 0.11 (1061.54 per ha).

Rank Abundance and Rank Proportion Curves of *D.hatagirea* and its associates

Figures 8, 9 and 10 explain the geometric nature of curve. Rank 1 (Table 4) is given for the species which has higher abundance i.e. *Allium wallichii Kunth* followed by *Rumex nepalensis* (2), *Rheum australe* (3) with *D.hatagirea* having least rank 4, respectively.

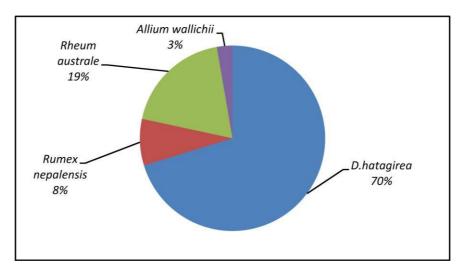


Figure 5. Relative frequency of species

Table 2. Density/ha of *D. hatagirea* along with its associates.

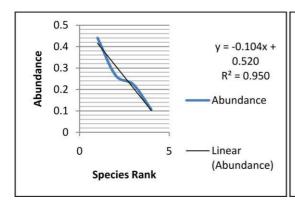
S.NO.	Species	Density/ha
1.	D.hatagirea D.Don Soo	276
2.	Rheum australe	80
3.	Rumex nepalensis	156
4.	Allium wallichii kunth	44

Table 3. Relative density of *D. hatagirea* along with its associates.

S.No.	Species	Relative Density (%)
1.	D.hatagirea	49.64
2.	Rheum australe	14.39
3.	Rumex nepalensis	28.06
4.	Allium wallichii kunth	7.91
	Total	100

Table 4. Rank Abundance and Rank Proportion values of D.hatagirea along with its associates

Species	Rank	Abundance/ha	Abundance	Log(Abundance)	Proportion
Allium wallichii	1	4400.00	0.44	-0.36	0.42
kunth					
Rumex	2	2666.67	0.27	-0.57	0.26
nepalensis					
Rheum australe	3	2228.57	0.22	-0.65	0.22
Dactylorhiza	4	1061.54	0.11	-0.97	0.1
hatagirea					
D.Don					
Total		10356.78	1.04	-2.56	1.00



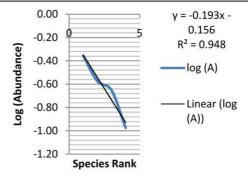


Figure 6. Species Rank Abundance curve for *D.hatagirea* and its associates.

Figure 7. Species Log Abundance curve for *D.hatagirea* and its associates.

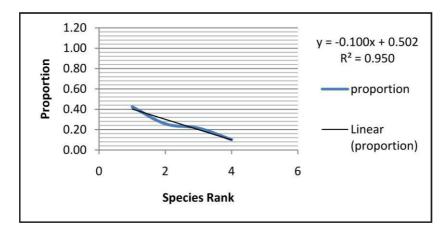


Figure 8. Rank Proportion Curve for *D.hatagirea* and its associates.

Table 5. Coverage and Relative Coverage of marsh orchid along with its associates.

S .No.	Species	Coverage (%)	Relative coverage (%)
1.	D.hatagirea	26	70.27
2.	Rumex nepalensis	3	8.11
3.	Rheum australe	7	18.92
4.	Allium wallichii kunth	1	2.7
5.	Total	37	100

Table 6. Important Value Index of D. hatagirea along with its associates

S.No.	Species	IVI
1.	D.hatagirea	190.98
2.	Rumex nepalensis	30.60
3.	Rheum australe	65.90
4.	Allium wallichii kunth	13.32
5.	Total	300

Diversity Indices Value Remarks Simpsons' Index (C) 0.35 Relatively even community Simpsons' Dominance of Index (D) 0.65 Higher dominance of one species i.e. D.hatagirea Shannon-Weaver Index (H) 1.18 Relatively diverse community Shannon Evenness (E_{shannon}) 0.85 Uneven community Simpson Evenness (E_{simson}) 0.71 Uneven community

Table 7. Simpson's index (C), Simpsons' Index of dominance (D), Shannon-Weaver Index (H), Shannon Evenness ($E_{Shannon}$) and Simpson Evenness (E_{Simson}) for *D. hatagirea* and its associates.

Coverage and relative coverage of *D. hatagirea* and its associates

D. hatagirea was found to have the highest coverage (26%) and highest relative coverage (70.27%) as compared to those of Rumex nepalensis, Rheum australe and Allium wallichii (Table 5).

Important Value Index (IVI) of *D. hatagirea* and its associates

D. hatagirea was found to have the highest IVI (190.98) as compared to its two associates (Table 6).

Diversity indices for *D. hatagirea* and its associates

The Simson's Index (C) and the Simson's Index of Dominance (D) were found to be 0.35 and 0.65,

respectively (Table 6). Similarly, the Shannon-Weaver Index (H) was found to be 1.18. The Shannon Evenness ($E_{Shannon}$) value was 0.85 whereas Simpson Evenness (E_{Simson}) value was 0.71.

Discussion

The altitudinal range of habitat distribution of D.

hatagirea in the study site was from 3,200 m to 3,600 m above sea level, which was similar to the study done by

Ranpal (2009). The aspect of habitat distribution of *D. hatagirea* in the study site was South-West. The mean

height was greater than 41.97 cm found by Ranpal (2009) and 60 cm by Dutta (2007). Ranpal (2009) used three individual plants (tall, medium and short) to calculate the mean height of *D. hatagirea*. Similarly, the diameters (at 5 cm above the ground) of *D. hatagirea* plants were measured, and the mean diameter was calculated. The difference in height might be due to the methodology used as well as the age, topographic, soil and climate factors. The greater number of *D. hatagirea* was found in the Southwest aspect. Ranpal (2009) has indicated its occurrence in Paplekharka as 71%.

47%) and Warghat, 2015 (49.23) for *Allium species* (Figure 6). The difference might be due to the area of the study. According to Ranpal (2009), relative frequency of *D.hatagirea* was 17%. The species having the lowest frequency were *Rumex nepalensis* (8%), *Rheum australe* (19%) and *Allium wallichii* (3%). Similarly Ranpal (2009) and Warghat (2015) found it for *Allium species* to be 12% and 5.44%, respectively (Figure 7). Density of *D. hatagirea* was comparatively much lower than 1,671 per ha or 0.17 ind/m² indicated by Ranpal (2009). The difference might be due to the smaller area

(4.5 ha) of the study site taken for the research. The least number was that of *Allium wallichii kunth* with 44 per ha which might be due to the least distribution of this species in the plot and heavy grazing pressure. The

density of *D. hatagirea* was reported to be 0.2 individuals per m² in Samar Lek of the Upper Mustang (Chhetri and Gupta, 2006). The next reported density of *D. hatagirea* was 2.66 per m² in the grazed sites and 3.2 per m² in the ungrazed sites at Tungnath, India (Nautiyal*et al.*, 2004). Bhatt*et.al.* (2005) found density of *D. hatagirea* i.e. 1.13 ind/m² (Lata) and 2.19 ind/m²

(Nagtal) of India whereas Giriet.al.(2008) found 0.7 ind/m² in the Garhwal himalayas of India. Rinchenet.al.

(2012) found least density of *D. hatagirea* (8.00 and 6.1 ind/m²) among 11 associates in Suru Valley of Jammu

and Kashmir, India while Warghat (2015) found 6.9 ind/m² in trans-himalayan region of Ladakh region,

India. The low density in the unprotected areas might be due to heavy grazing pressure. Ranpal (2009) indicated the relative density of *D. hatagirea* to be quite low, only 9% or 0.09. The bigger difference in the relative density of this species might be because of the smaller study area (4.5 ha). Ranpal (2009) reported higher abundance of 0.24 (2,367 per ha) than in this study; the difference might be due to the least number of marsh orchid plants found in the study area. The geometric nature of Species Pank Abundance curve is found in higher altitudes.

Similarly, *Rheum australe* was found to have an occurrence of 7% as compared to 65% found by Ranpal (2009). On the other hand, *Rumex nepalensis* had an occurrence of 3% while that of *Allium wallichii* 1 % which was lower than study done by Ranpal 2009 (

where alpine grassland type of habitat exists. Since, the curve is not horizontal the species found in the area have uneven distribution and there is high dominance of one species i.e. *Allium wallichii Kunth*. The geometric series yields a straight line on a plot of log abundance

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against rank. The communities described by it are very uneven, with high dominance of the most abundant species. It is not very often found in nature. Whittaker (1972) found it in plant communities in harsh environments or early succession stages. The highest coverage of *D. hatagirea* was due to the highest number of plots in which it occurred as compared to its associates. Warghat (2015) found the cover percentage of Allium species to be6.8 % in Ladakh region of India which was greater (1%) than Allium wallichii Kunth found in this study. The higher value of IVI of D.hatagirea indicates its dominance in the study site. Similarly, Warghat (2015) found the value of IVI of Allium species to be10.26 in Ladakh region of India which was lower (13.32) than the study found. The basic difference might be due to the area of the study taken including environmental factors. The values of Simpson's Index (C) and the Simpson's Index of Dominance (D) indicate relatively even community and higher dominance of D. hatagirea in the study site. Similarly, the value of Shannon-Weaver Index (H) indicates relatively diverse community in the study site. According to study done by Warghat, 2015 the value of C, H and evenness in herbaceous meadows type of habitat of D.hatagirea in Ladakh region of India were 0.8928, 2.396 and 0.646, respectively which were comparatively higher than the values in this study. The values of $E_{Shannon}$ and E_{Simson} indicates uneven community of plants in the study area. The difference was due to the presence of the greater number of D. hatagirea individuals as compared to its three associates. The diversity of the species in the study site was, therefore, not satisfactory.

Conclusions

The study revealed that frequency, relative frequency, relative density, relative coverage and Important Value Index of D. hatagirea were higher as compared to its three associates- Rhuem australe ,Rumex nepalensis and Allium wallichii. This indicated the good ecological status of D. hatagirea in the study area. Rank Abundance Curve indicated uneven distribution of the species in the study area. However, the value of Simson's Index (C) indicated the relatively even community and the Simson's Index of Dominance (D) indicated the dominance of one species i.e. D. hatagirea while the value of the Shannon-Weaver Index (H) indicated relatively diverse community i.e. the plant diversity was found to be not satisfactory, suggesting for necessary actions for the conservation of the diversity of D. hatagirea in the study area. The values of

Simson Evenness (E_{Simson}) and Shannon Evenness $(E_{Shannon})$ indicated uneven community of the species in the study area. Further research is recommended on an

annual basis so as to maintain database on the population dynamics, distribution pattern in different possible habitats and the harvesting level of this valuable medicinal plant. Research on genetic diversity using molecular marker technique is also recommended to compare the genetic diversities of the populations of this orchid at different locations of Nepal. To identify the chemical hotspots of threatened orchid, phytochemistry research is also recommended since it would help in isolation and molecular characterization in different locations of Nepal. In addition to that study on physical and chemical properties of soil, leaf dynamics is also highly recommended. Although the occurrence of D. hatagirea was found to be higher than its three associates, the unsustainable harvesting and illegal trading of this valuable orchid including overgrazing in the study site are likely to bring it to extinction. Therefore, awareness programmes about the in-situ and ex-situ conservation of the endangered medicinal orchid D. hatagirea should be conducted in the study area.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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