Chapter 1

Introduction
INTRODUCTION

The Himalayan region is a major hotspot of biodiversity as it is rich in floral diversity and is infinitely used by various section of the community. In the North India, the Himalayas cover 18% of Indian subcontinent, accounts for more than 50% of India’s forest and contain 30% of India’s endemic species. The area harbours about 8000 species of higher plants, of which 1748 are used for medicinal purposes (Samant et al., 1998 and Gaur et al., 1983). There are many remote areas in the Indian Himalaya that contain a rich traditional knowledge on the use of medicinal plants which is still not documented (Semwal et al., 2010), Uttarakhand is one among them. Uttarakhand is largely a hilly state located at foothills of the Himalayan mountain ranges, having international boundaries with China (Tibet) in the North and Nepal in the east. On its north-west lies Himachal Pradesh, while on the south is Uttar Pradesh. The state is divided into two divisions, Kumaon and Garhwal, located above 1850 m from sea level, with the total of 13 districts, lies between 28°53’24” – 31°27’50” N and 77°34’27” – 81°02’22” E and occupies an area of 53,483 km² (Tiwari et al., 2012). Uttarakhand is well known for its biodiversity richness and diverse cultural mosaic. The climate of Uttarakhand varies from subtropical to alpine. It is relatively cool and humid compared to rest of the Western Himalaya. Except inner dry ranges, much of the state receives high precipitation during monsoon and heavy snow during winter at higher altitudes (above 2,000 m). The area is endowed with high floristic diversity, most of the plants have high medicinal properties. The inhabitants of this region are familiar with the economic properties of the existing plant species. The knowledge of utilization of medicinal plants is very high among the people of Uttarakhand (Kala et al. 2004).

The instant rising demand of plant-based drugs and indiscriminate use of many indigenous and exotic endemic plants of greater economic importance are unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges (Nautiyal et al., 2002), therefore they are more prone to extinction (Jablonski, 2004). In this context, one of the highly valuable medicinal plant species which also requires its conservation and dissemination in Uttarakhand is Berberis species.
Berberis is a genus of shrubs and small trees. The name Berberis derived from “Berberys” the Arabic name for the fruit. It is the largest genus in Berberidaceae (Ahrendt, 1961) and is commonly known as “barberries or Pepperidge bush”. Berberis has two centres of diversity, Eurasia with ca. 300 species mainly in Himalayas and in China; and South Africa with ca. 200 species (Ahrendt, 1961). In India, the family is represented by 3 genera and 68 species. Largest among them is genus Berberis L. that has 55 species, majority (above 95%) of them are distributed in the Himalayan region, Nilgiri hills, hilly tracts of Jharkhand and Madhya Pradesh (Rao et al. 1998a). Berberis has about 650 species worldwide, of which nearly seventy seven species have been reported in India (Wealth of India, 1988) and out of which 54 have been reported from Indian Himalaya (Sharma et al., 1993). In Uttarakhand, 22 species of Berberis and about 29 distinct taxa have been reported (Rao et al. 1998b). But this number is likely to be more, given the topographic and altitudinal variation in the state. Out of these, seven species are endemic to state (Tiwari and Adhikari, 2011). According to Red Data Book of Indian plants (Nayar and Sastery 1987-88), Berberis lambertii is characterized as vulnerable and Berberis affinis, Berberis osmastonii as rare. All these three species are endemic to a few pockets of Uttarakhand. The family also includes genera, which are quite distinct from one another.

Several species of Berberis are grown in gardens for their ornamental leaves and edible berries. Berberis species are characterized by long or short dimorphic shoots, which form the structure of the plant. The flowers are produced singly or in racemes and are yellow or orange in colour. The sepals are usually coloured like the petals. The fruit is a small berry. The root and inner bark have been used for yellow dyeing (Strausbaugh and Core, 1978). The wood is bright yellow, hard to very hard, moderately heavy to very heavy, usually straight grain with very fine textures. Almost all parts of plant have been investigated chemically and biologically (Dobhal et al., 2007, Saied et al., 2007, Tomosaka et al., 2008, Ivanovska et al., 1996).

In India, mainly Berberis angulosa, Berberis aristata DC, Berberis asiatica Roxb., Berberis coriaria Royle ex. Lindl., Berberis chitria Lindl, Berberis tinctoria Lesch., Berberis umbellata, Berberis virescens, Berberis coriaria Royle, Berberis lycium Royle, Berberis floribunda are found and are being planted as hedges due to
their straggling habit. Barberry bushes generally bloom from February to June and attract bees for the pollen and nectar. The honey obtained is dark and has a strong flavour similar to molasses (Singh, 1954; Sabnam, 1964). *Berberis asiatica*, one of the most common species in the middle hills of Western Himalaya, was known to be the alternate host of dreaded wheat rust *i.e.* *Puccinia graminis tritici*. Hence, during the early phase of green revolution there was a movement to eradicate *Berberis* spp. from the Himalayan region. Similarly, several species of *Berberis* have been eradicated from the Himalayan region in order to reclaim the hill slopes for agriculture or to extract valuable drug ‘Berberidine’ from the roots and stem of *B. asiatica, B. aristata* and *B. lycium*.

**Therapeutic and commercial value**

Barberry has played a prominent role in herbal healing for more than 2,500 years (Kala *et al.*, 2007). As an herbal remedy, it has no match in serving human race since ancient times. Most of the *Berberis* species have medical uses because of the presence of alkaloid, ‘Berberine’ an isoquinoline alkaloid (Morales, 1992; Khosla, 1992; Rastogi *et al.*, 1993) known for its activity against cholera (Rabbani, 1996), diarrhoea (Yamamoto *et al.*, 1991), amoebiasis and latent malaria (Ghosh *et al.*, 1985). British pharmacopoeia patented a drug made from Berberine is ‘Orisol’ (Tripathi *et al.*, 2013). It is the most widely used drug in Homeopathic system of medicine for kidney pain and for removal of kidney stones.

In traditional folk medicine, barberry has been used to treat diarrhea, reduce fever, improve appetite, relieve upset stomach, and promote vigor as well as a sense of well-being (Bergner, 1996). It is also said to be used as an excellent preservative. *B. aristata* or Indian barberry is used in the treatment of urinary troubles caused as a side effect of the anti-cancer chemotherapy drug called as ‘Cisplatin’. ‘Rasaut’ (also known as Rasanjana or Rasavanti), a thick extract is being made from the root-barks, roots and lower stem-wood of *B. aristata* by boiling them with water. The drug is regarded as bitter tonic and is reported to be used as cholagogue, stomachic, laxative, diaphoretic, antipyretic and antiseptic. It is useful in eye diseases particularly in conjunctivitis, indolent ulcers and in haemorrhoids (Rehman *et al.*, 1983). The crude drug alone or in combination with other ingredients shows efficacy against allergic
conjunctivitis, acne vulgaris, and rheumatic pain, premature ejaculation, fungal infection and viral hepatitis.

*B. aristata* possesses stomachic, astringent, antiperiodic and diaphoretic properties. Crude extract of the leaves exhibits hepatoprotective activity against paracetamol–induced liver damage in rats. The fruits either fresh or in dried form exhibited hypocholesterolemic activity (Gilani and Janbaz, 1995). The whole plant extract forms one of the constituents of herbal cream, ‘**Dermocept**’ used against sarcoptic mange lesions. It also forms one of the constituents of a crude Ayurvedic drug formulation exhibiting inhibitory effect against *Salmonella typhi* in vitro. The plant extract with another crude drug exhibited anti-amoebic activity against *Entamoeba histolytica*.

*B. asiatica* constitutes a composite drug which minimizes the lesions of hepatotoxicity of paracetamol poisoning. *B. lycium* Royle (also known as Daruharidra) roots are used for the treatment of intestinal colic and pharyngitis. The root-bark has astringent property and used for healing internal wounds, cracked bones and urine burning and also as a tonic in pregnancy (Wealth of India, 2006). Various parts of *B. vulgaris* have been used for the treatment of gall bladder and liver disinfections, leishmaniasis, malaria, stomach problems and urinary tract diseases (Arayne *et al.*, 2007). A berry tea is used for poor appetite, also as a diuretic, expectorant and laxative, used for jaundice, hepatitis, haemorrhages and diarrhoea. It is also considered as an astringent, diaphoretic and antiseptic. A tincture of root bark was used for arthritis, rheumatism and sciatica. In Chinese medicine, barberry is used to increase white blood cell and platelets after chemo or radiation cancer therapy. The ripe fruit is also considered a wild food source and can be used as a cooked fruit (Foster and Duke, 2008) as well as for the preparation of Jellies, marmalades, and wines (Crovetto, 1980).

*Berberis* leaves, stems and root aqueous extracts, have purported antimicrobial activity *in vitro* on Gram-positive and Gram-negative bacteria and fungi (Freile *et al.*, 2003). Berberine has antioxidant effects similar to that of vitamin E in the riboflavin system (Haisong *et al.*, 1990). Barberries also are of great value for wildlife food, cover, and erosion-control planting (Decker *et al.* 1991). However, the common
barberry, as an invasive exotic, is considered by many to be a noxious weed (Mack, 1991). Besides high medicinal value, the plant can also be used as fuel wood, fodder, edible fruits and as live-fence (Bottini et al, 2002). Several barberry species are grown as ornamentals because of their handsome foliage and often attractive flowers or fruits (Schlosser et al., 1992).

The species of Berberis are distributed between altitudinal ranges of 1850-3300 m above mean sea level spreading over the Himalaya and show considerable genetic diversity both with regard to qualitative and quantitative traits (Tiwari et al., 2012). The levels of genetic diversity within the populations of Berberis reflect the genetic resources necessary for short-term ecological adaptation and for long-term evolutionary change. Species must have available pool of genetic diversity if they are to survive environmental pressures exceeding the limits of developmental plasticity. Because environmental changes are unpredictable, it is critical that natural populations of Berberis should secure sufficient genetic diversity to permit the species to continuously evolve in response to environmental changes (Prajapati et al., 2003).

Numerous techniques have been performed which contribute to a better knowledge of Berberis species. Morphological, ecological, cytogenetic, and biochemical studies have allowed the delimitation of these taxa and the postulation of homoploid and polyploid speciation (Bottini et al., 2000b). Massive loss of valuable plant species in the past centuries and its adverse impact on environmental and socio-economic values has triggered the conservation of plant resources. Appropriate identification and characterization of plant materials is essential for the successful conservation of plant resources and to ensure their sustainable use. Molecular tools developed in the past few years provide easy, less laborious means for assigning known and unknown plant taxa. These techniques answer many new evolutionary and taxonomic questions, which were not previously possible with only phenotypic methods.

The taxonomy of the genus Berberis is still somewhat uncertain, despite the large number of studies performed due to extremely high morphological variations, probably affected by environment and natural hybridization (Bottini et al., 2007). The occurrence of hybridization and some degree of introgression in transitional zones has
produced intermediate forms that cause difficulties in *Berberis* taxonomy (Bottini *et al*., 1999b). Overlapping of characters, especially in leaves, stem, flower and berry size make field identification often difficult. Several classification systems have been proposed for the extensive character variation in habit, floral morphology and fruit type, due to this reason many species of *Berberis* are morphologically so similar as well as have great variation among them that’s why their taxonomic identification becomes very difficult (Tripathi *et al*., 2013). To overcome this problem studies at molecular level becomes mandatory. Phylogenetic analysis seeks to identify monophyletic groups, the members of which share a common ancestor. With recent advances in both sequencing techniques to produce taxonomic characters and cladistic methods to infer natural relationships between organisms, molecular systematics has become a paradigm in biology. While going through the literature, no phylogenetic work has been conducted for the genus in Uttarakhand. Population studies and genetic diversity studies in the family is almost non-existent. Taxonomically the genus is complicated due to difficulty in their correct identification of species (Rao *et al*., 1998, Tripathi *et al*., 2013). Ribosomal gene (rDNA) is the most widely used nuclear locus studied for determining angiosperms molecular systematics (Randall *et al*., 2004). Appropriate nucleotide sequence variation for systematics of *Berberis* was found in the internal transcribed spacer (ITS) of the nuclear rDNA gene (Kim *et al*., 2007). The phylogenetic analysis using nuclear ribosomal ITS sequences in this thesis provides an important framework to understand the role of Himalayan orogeny in diversification of *Berberis* in the Uttarakhand, the number of independent lineages within the genus which are found in Uttarakhand, and the influence of ecology on *Berberis* diversification. Phylogenetic studies with internal transcribed spacer region gave insights in relationship among species of *Berberis* (Roy *et al*., 2010). Hence, this research work was undertaken to study the phylogenetic relationships of the various taxa of the genus *Berberis* occurring in Uttarakhand by comparing the sequence of the ITS regions or by DNA based markers.

Habitat loss, fragmentation and degradation are pervasive processes threatening several species of *Berberis* all over the world. Many species are endangered due to direct or indirect human-related factors such as loss and alteration of habitat and introduction of species (Kreivi, 2009). The genetics of threatened species have been of great interest to both evolutionary and conservation biologists.
Phylogeny of genus *Berberis* and genetic diversity of its two threatened species across the altitudinal zone in Uttarakhand.

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for long time (Avise, 1994; Young and Clarke, 2000; Hedrick, 1999). Collecting and working with endemic species itself is a challenging task as these species are endemic to few pockets of Uttarakhand. As predicted by population genetic theory, loss of genetic variation is a major threat to endangered species with small populations located in narrow geographic areas. A low level of genetic variability often results in minor fitness of individuals (Oostermeijer et al., 1994; Fischer and Matthies, 1998; Luijten et al., 2000; Hansson and Westerberg, 2002), reduces the viability or adaptability of populations in changing environments (Young et al., 1996), and in extreme cases causes the extinction of species. These effects may be most pronounced in species that are self-compatible and have limited seed dispersal ability (Ellis et al., 2007). Therefore, the two threatened species *B. osmastonii* and *B. lambertii* were taken up for genetic diversity study which specifically focused on determining the degree of genetic variation and differentiation within and among populations of each species. Genetic variability studies may contribute insight into the underlying causes of reduced population numbers and reduced fitness. This information may provide evidence of inbreeding, genetic drift and conditions that may contribute to the endangered status of these species (Kim et al., 2005).

Keeping in view the importance of the *Berberis* species and the vulnerability of the plant genetic resources of the species in Uttarakhand the present investigation was carried out with following objectives:

- Study the phylogenetic relationship of the genus *Berberis* involving the species found in Uttarakhand.

- Estimate genetic diversity in *Berberis lambertii* Par. and *Berberis osmastonii* Dun. across the altitudinal zone in Uttarakhand.