

## Major climate variables and the endangered high altitude Himalayan herb *Picrorhiza kurroa* Royle ex Benth.: An insight

Manoj Kumar<sup>1,3</sup>, Aishwarya Rajlaxmi<sup>1,3</sup>, Archana Rani<sup>1,3</sup>, Anamika Roy<sup>2</sup>, Mamun Mandal<sup>2</sup> and Abhijit Sarkar<sup>2</sup>

<sup>1</sup>Environmental Sciences & Biomedical Metrology Division, CSIR-National Physical Laboratory, Dr. K. S. Krishnan Marg, New Delhi 110012, India

<sup>2</sup>Laboratory of Applied Stress Biology, Department of Botany, University of Gour Banga, Malda 732103, West Bengal, India

<sup>3</sup>Academy of Scientific and Innovative Research, Ghaziabad 201002, Uttar Pradesh, India

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The Himalayas are a global biodiversity hotspot that faces threats due to climate change, over-exploitation, and invasive agriculture. Increasing temperatures have accelerated species extinctions and shifts in their high-altitude habitats. Climate change poses a significant threat to alpine and medicinal plants, impacting their growth, composition, and survival. The endangered status of these species has socio-economic implications due to their economic importance. Therefore, it is vital to study the potential impact of climate change on medicinal plants and their ecological status. This review enlisted the research work carried out on plants with respect to altitude, temperature, elevated CO<sub>2</sub>, and drought. The present review focuses on the endangered perennial herb, i.e. *Picrorhiza kurroa* Royle ex Benth., an endangered medicinal plant that is useful in the treatment of many diseases in the Indian Himalayan region. Due to overharvesting from the wild, as well as climate change, its population is declining at an alarming rate. There are several studies that attempt to assess its availability and predict areas with high suitability for in situ conservation in the Himalayan region. The outcomes of the study will guide the herbal community, harvesters, drug manufacturers and government agencies on strategies to mitigate the rapid extinction of these plants.

**Keywords:** Climate change, Elevated CO<sub>2</sub>, Environmental stress, Secondary metabolite

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### Introduction

Evidence from climatic data of the past 100 years revealed that the global mean surface temperature has increased by approximately 2.2°C by the end of 2100 and continues to increase by 3.6°C in 2200<sup>1</sup>. Further, the earth's temperature is likely to exceed 1.5°C by the end of the 21<sup>st</sup> century, higher than at any other time during the last 1,000 years<sup>1</sup>. Carbon dioxide (CO<sub>2</sub>) concentration in the atmosphere has increased from about 284 ppm in 1832 to 407 ppm in 2016<sup>1</sup>. According to the 'National Oceanic and Atmospheric Administration' (NOAA), the global average concentration of CO<sub>2</sub> was 421 ppm in 2022<sup>2</sup>. There is an active link between the levels of such "greenhouse" gases in the atmosphere and global warming<sup>2</sup>. Thus, a continuous increase in CO<sub>2</sub> concentration is expected to bring a rise in atmospheric temperature, which affects the rainfall patterns and possibly the severity of extreme weather throughout the globe. This climatic change has a varying effect on different parts

of the world; it has been found that a mountainous region like the Himalayas is more sensitive to climate change than other land surfaces of the same latitude<sup>3</sup>. Climate models indicated that an increase in the sea surface temperature of the tropics could increase oceanic evaporation, which might be responsible for increased warming in the Himalayas<sup>4</sup>.

Climate change has emerged as a key driver of ecosystem change in recent decades due to anthropogenic activity. Global warming has significantly altered vegetation phenology, productivity, distribution, and even the vegetation-climate relationship at regional and global scales. The effect of climate change on the vegetation of the Himalayas is more pronounced than in any other part of the globe due to its high-altitude region being rich in endemic plants. The Indian Himalayan Region (IHR) has a vast area spreading across 12 states, covering approximately 2400 km in length and consisting of a wide diversity of flora with 3471 endemic species found in the region, which is 46% of the total reported in India<sup>4</sup>. In addition, IHR consists of 11 forest formations, 10 forest types, and

\*Correspondent author  
Email: bhu.manoj@gmail.com

21 vegetation types, with Berberidaceae, Saxifragaceae, Ranunculaceae, Rosaceae, and Apiaceae families comprising the highest degree of endemism<sup>5</sup>. Conservation of plant diversity in the IHR has become a significant concern due to the depletion of plants from their natural habitats, and most species are endemic with restricted distribution<sup>6</sup>. Overexploitation due to commercialisation and the impacts of climate change led to the dwindling population of the medicinal plants from the alpine and subalpine regions of IHR<sup>5,6</sup>. Therefore, most of the medicinal plants found in the Himalaya have been listed in Appendix I and II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)<sup>7</sup>.

Several studies have reported that global warming in the mountainous region leads to changes in vegetation phenology, species range, and altitudinal shifts<sup>7</sup>. These changes might affect biomass production, economic yield, and occasional extinctions of medicinal plants. Therefore, there has been increasing consciousness regarding vegetation sensitivity to global warming in mountain regions, especially for medicinal plants, and, accordingly, there is a need to quantify and monitor its potential impact. Analysing the effects of climate change on the productivity of medicinally important plants is not only of theoretical interest but might also be of practical relevance for the production of high-quality secondary metabolites in the face of climate change. Several valuable medicinal plants of the Himalayan regions, viz., *Rhododendron sp.*, *Aconitum sp.*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Picrorhiza kurroa*, and *Juglans regia*, are highly sensitive to changing climatic conditions, which can significantly influence their growth, distribution, and medicinal potency<sup>8</sup>.

The focus is on the species *P. kurroa* Royle ex Benth. This species is one of the primary non-timber forest products that generate income in the Nepalese Himalayas. It is one of the oldest medicinal plants traded from the Karnali zone<sup>9</sup>. *P. kurroa* is an endangered temperate alpine Himalayan plant, belonging to the family Plantaginaceae (formerly known as Scrophulariaceae) and is endemic to the western Himalayan region and found up to the Yunnan mountains in China<sup>9</sup>. It is a small perennial herb with underground stems and roots at nodes about 5-10 cm. Leaves are dentate and spatulate with a length of 5-10 cm. The stem is short and becomes

erect during the flowering stage. Flowers are very small, in dense spicate racemes, white or pale blue-purple in colour with exerted stamens, and the root system has a long creeping rootstock that grows in rock crevices and moist places<sup>9,10</sup>.

Most of the research on *P. kurroa* is related to proteomics, chemistry, and implications of picrosides in drug development; however, a limited study is available on its performance under climate change scenarios. Extreme climatic conditions, including elevated CO<sub>2</sub>, temperature, altitude, and drought, alter the plant's phenological, physiological, and biomolecular responses. Therefore, in the present study, our aim is to identify the effect of various climatic factors on *P. kurroa*, regarding its habitat distribution, plant performances, and secondary metabolite production, and to develop strategies for its conservation and propagation. Additionally, a particular emphasis is given to the role of altitude on picroside production in *P. kurroa*.

## General overview of *P. Kurroa*

### Morphological description

*P. kurroa* is a high-value Himalayan medicinal plant that prefers moist rock crevices and sandy soil<sup>9</sup>. In the local region of Uttarakhand Himalaya, the plant is also known as 'Kutki'. Kutki is a small, perennial creeper herb with short stems and creeping rootstocks with a characteristic odour, that attains a height of 6-10 inches. Kutki grows up to a height of 10-20 cm<sup>11</sup>. Although the stem is small, weak, leafy, and slightly hairy, it becomes erect during flowering. The rhizomes are irregularly branched, cylindrical, and greyish-brown in colour<sup>9</sup>. The plants are raised through vegetative propagation mainly from stolons, rhizomes, and offsets<sup>12</sup>. Leaves of *P. kurroa* are flat, basal, sharply dented, and blackish upon drying<sup>11</sup>; whereas, the species could be classified into two categories based on their leaf morphology; (i) narrow leaves plant, which is found near springs, boulders, rocky ravines, and steep slopes, and (ii) broad leaves plant, which prefers high humus and moisture content and found at lower altitude compared to narrow leaves variety. The inflorescence is spicate racemes, which form triangular heads. In inflorescence, zygomorphic, sessile flowers are arranged in long spikes; the flowers are small, bisexual, bluish-white in colour, occur in densely arranged terminal clusters, and they prefer cross-pollination. Fruit is an oblong capsule; seeds are many, oblong, and whitish<sup>9</sup>. During

the months of June to August, flowering and fruiting take place.

#### Anatomical description

The anatomy of the rhizome shows 20-25 layers of cork consisting of tangentially extended and suberised cells and small-sized vascular bundles surrounded by a fibrous bundle sheath<sup>11</sup>. Young roots generally have a single-layered epidermis, sometimes with unicellular hairs, and the cortex is 8-14 layered, consisting of oval to polygonal, thick-walled, parenchymatous cells<sup>12,13</sup>. The secondary phloem is poorly developed. The chromosome number of the *P. kurroa* is  $2n=34$ <sup>10</sup>.

#### Taxonomical hierarchy

Royle drew *P. kurroa* for the first time in 'Illustrations of Botany' on 24<sup>th</sup> August 1835<sup>9</sup>. Bentham described it in 'Scrophularineae Indicae' on 17<sup>th</sup> November 1835, but he mistakenly wrote the species name as *P. kurroa* Royle later corrected it to *P. kurroa*. Since Bentham was the first to describe the species, it was named "*Picrorhiza kurroa* Bentham" or "*Picrorhiza kurroa* Royle ex Bentham". The word 'picros' means bitter in Greek, and 'rhiza' means root; the species name of the plant comes from a Punjabi word 'Karu', which means bitter<sup>9</sup>.

#### Ecology

*P. kurroa* is a perennial herbaceous plant that naturally grows in cool and moist climates. Further, optimal conditions for its growth include a specific type of soil and environment that supports its unique rhizome structure and sprouting behaviour. For healthy development, it requires sandy clay soil that is porous and well-drained. This soil type allows the plant's rhizomes to spread laterally. The spread of these rhizomes is essential because it encourages the growth of aerial sprouts that rise from the soil, allowing the plant to multiply and maintain a stable population. The plant thrives in environments that are consistently moist and shaded, which helps it to avoid excessive heat and light, which can be detrimental to its health. The plant thrives in moist and shaded environments, but cannot survive under montane conditions. Additionally, while *P. kurroa* benefits from moderate rainfall; prolonged periods of intense rainfall can be harmful, causing waterlogging and suffocating the roots and rhizomes and leading to higher mortality rates. Therefore, a balanced environment with adequate but not excessive

moisture, cool temperatures, and partial shading is crucial for cultivating *P. kurroa* effectively.

#### Chemical constituents

Several active phytochemical compounds have been extracted and studied from the roots, rhizomes, leaves, stems and seeds of *P. kurroa*. The rhizome of the species contains bioactive components called iridoid glycosides, which include kutkoside, cucurbitacin, picroside I, picroside II, and picroside III<sup>14</sup>. The *P. kurroa* has a phytochemical constituent named Picroliv, which is composed of picroside I and picroside II at a 1:1.5 ratio<sup>15</sup>. Cucurbitacin has antitumorous properties<sup>15</sup>. The study also disclosed that different phytochemicals, such as pikuroside, 4-hydroxy-3-methoxy acetophenone, veronicoside, and some phenolic compounds, are also found in *P. kurroa* extracts. In addition, this plant also contains apocynin, an anti-inflammatory agent that can neutralise neutrophil's oxidative reactions, and drosin, an anti-allergic component. Together, they are responsible for the anti-cancer properties of *P. kurroa*<sup>14</sup>. Furthermore, this plant also has aromatic amino acids like cinnamic acid, ferulic acid, vanillic acid and carbohydrates like D-mannitol<sup>16</sup>. Furthermore, different populations of *P. kurroa* show different ratios of chemical components (picroside I, picroside II, cucurbitacins and iridoids), which play a vital role in determining the medicinal values of each chemotypes<sup>17</sup>. Previous studies have shown three major groups of *P. kurroa* chemotypes, *i.e.* (i) picroside I-rich chemotypes, (ii) picroside II-rich chemotypes and (iii) Intermediate chemotypes<sup>18-20</sup>. However, understanding the chemotypes of the species is essential in order to ensure the optimisation of its medicinal properties.

#### Global status

##### Geographical distribution

With a depleting population, *P. kurroa* is mostly found in the alpine Himalayan zone. It is predominant in the alpine or sub-alpine region, from Kashmir to Sikkim, at an altitude between 2800–4800 m asl<sup>8</sup>. It is abundant in the western Himalayan regions of India, Nepal, and Pakistan. The distribution of *P. kurroa* is also reported in China, Tibet, Burma, and Bhutan<sup>11</sup>. India's major *P. kurroa* growing regions are Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Arunachal Pradesh, and Sikkim<sup>21</sup>. In Nepal, it is abundant in the rocks or their crevices on the northern slopes, turfs, and cliffs<sup>21</sup>. These plants are more

common in steeper slopes, moist rock surfaces, pasturelands, and even under the canopy of shrubs. However, increasing demand for *P. kurroa* disturbs its natural habitats and decreases habitat availability in future climatic scenarios<sup>5</sup>. For a better understanding of its geographical distribution, recorded geographical distributions were collected from various published literature and the Global Biodiversity Information Facility (GBIF) web portal (Fig. 1).

#### Global production

Due to over-exploitation and climatic changes, this plant has been listed under the critically endangered plant species<sup>22</sup>. The rising demand for *P. kurroa* will result in unmanageable and over-harvesting of the plant species. However, cultivation did not meet the demands; the lack of cultivation and over-harvesting of Kutki have accelerated its entry into the Red Data Book list. Global demand for *P. kurroa* is 500 tons, whereas the production is only 375 tons per annum. India alone produces 20% of the worldwide output<sup>11</sup>. Therefore, increasing the production of *P. kurroa* has become an urgent need.

#### Therapeutic properties

*P. kurroa*, a perennial herb, is renowned for its medicinal properties, serving as an anti-malarial, anti-pyretic, hepatoprotective, and stomachic agent, and depending on the dosage, it can function as a laxative or a cathartic. Kutki is widely used in Ayurvedic medicine systems for its antimicrobial properties. The 'National Institute of Traditional Medicines' of Bhutan

uses the rhizome of the plant for the formulation of medicines<sup>23</sup>. The rhizome also treats sore throats, eye disease, high blood pressure, and gastric diseases<sup>11</sup>. It is also used in veterinary health management in Kashmir<sup>24</sup>. It is also considered to be a blood purifier and a blood pressure reducer, and is useful in asthma, jaundice, constipation, leprosy, and stomach-related ailments<sup>25,19</sup>. Compounds extracted from different parts of the species show different properties like anti-inflammatory, anti-invasion, and anti-thrombotic activity, which restrain the effects of free radicals<sup>19,26</sup>. Extract of *P. kurroa* contains neurogenic, neuroprotective, anti-diabetic, and anti-arthritic properties<sup>19,27-28</sup>. All the medicinal therapeutic uses of the species lead to the overexploitation and unsustainable harvesting, resulting in the endangered category of *P. kurroa*. *P. kurroa* belongs to the most effective hepatoprotectors in Ayurveda – a bitter tonic that reduces excess Pitta, stimulates bile formation and accelerates the regeneration of liver tissue in hepatitis, steatosis or toxic damage. Experimental and clinical studies have further validated its hepatoprotective potential, linking its specific phytochemical constituents, particularly iridoid glycosides like picrosides, to distinct pharmacological activities and therapeutic efficacy in various diseases<sup>27</sup>. Clinical and experimental studies have demonstrated a reduction in bilirubin levels, protection of hepatocytes, and immunomodulatory effects simultaneously<sup>28</sup>.

#### Responses of *P. kurroa* under extreme climatic variables

Elevated CO<sub>2</sub> concentration could drive climatic changes by increasing temperatures and reducing water availability. Earth's climatic changes have a significantly different effect on the different regions of the world; it has been reported that mountainous global hotspot regions like the Himalayas are more sensitive to global climatic changes than other land surfaces of the same latitude<sup>1,21</sup>. The greater Himalayan region is often affected by climatic changes, which will affect the population, diversity, and habitat shift of medicinal plants of this region in the long term; however, changes in phenotypic, physiological, and biomolecular characteristics of plants and yield patterns are also reported.

#### Climate change vs. habitat distribution

Various species distribution models (SDM) have been used to predict the prospective area of distribution

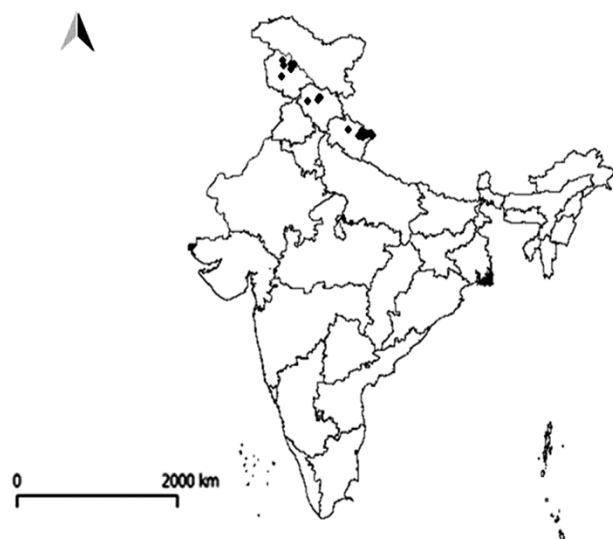


Fig. 1 — The geographical distribution of *Picrorhiza kurroa* (Data source: GBIF portal (<https://www.gbif.org/>)).

of a species in order to prioritise the areas for conservation and analyse the immediate and long-term effects of climate change on the targeted species<sup>29,30</sup>. Habitat suitability of *P. kurroa* was estimated in the Kumaon Himalaya<sup>31</sup>, Uttarakhand Himalaya<sup>21,30</sup>, Trans-Himalaya region<sup>32</sup>, indicating the change in its habitat distribution with climate change, leading to the vulnerability of the species. However, the study is limited to parts of the species entire distribution region. Furthermore, simulation with optimum resolution while using all the available temporal scales for different climate change scenarios for the entire habitat range of the species is yet to be done.

### Phenotypic responses

#### *Elevated temperature*

The consequences of the Earth's warming in the past decades have left clear imprints on vegetation. Temperature-induced species range shifts along the altitudinal gradient change the community composition and increase the regional ecosystem's complexity through biotic interaction and feedback processes, leading to the alteration of proteome and metabolic composition of *P. kurroa* for the better adaptation of the species to climate change<sup>32</sup>.

#### *Elevated CO<sub>2</sub>*

In controlled environments, pot experiments have shown the beneficial effects of elevated CO<sub>2</sub> on the productivity and quality of various products and constituents of medicinal plants. Elevated CO<sub>2</sub> enhanced the growth and biomass of *P. kurroa*<sup>32</sup>. Additionally, Chaturvedi *et al.*<sup>33</sup> reported a three times increase in aboveground biomass and an 8.25 times increase in belowground biomass of *P. kurroa* at elevated CO<sub>2</sub> (600-700  $\mu\text{mol mol}^{-1}$ ) against ambient CO<sub>2</sub> level (330-380  $\mu\text{mol mol}^{-1}$ ). They also found significant growth in various morphological parameters of *P. kurroa* under elevated CO<sub>2</sub> conditions. They reported an increase of 22.4% in plant height and an increase of 108.1 to 125.3% in leaf area at an interval of 30 days. Furthermore, elevated CO<sub>2</sub> increased dry matter production and enhanced both shoot and root growth of *P. kurroa*.

### Physiological responses

#### *Elevated temperature*

The warming treatment of 1–3°C in tundra detected a rapid response in whole plant communities after only two growing seasons<sup>32</sup>. Moreover, these results predict that warming will cause a decline in

biodiversity across a wide variety of tundras. Kumar *et al.*<sup>32</sup> found that photosynthesis-related differentially abundant proteins (DAPs) are downregulated at elevated temperatures in *P. kurroa*. These findings predict that elevated temperature significantly damages photosystems. As a result, a reduction in photosynthesis rate and downregulation of photosynthetic proteins are reported. However, the understanding of the regulation of photosynthesis and related proteins needs further investigation.

#### *Elevated CO<sub>2</sub>*

In recent years, although some CO<sub>2</sub> enrichment experiments have been performed in various ecosystems globally, very few studies have been conducted to elucidate the effect of CO<sub>2</sub> enrichment on high-altitude vegetation. However, studies on the physiological response of *P. kurroa* to elevated CO<sub>2</sub> are still limited.

#### *Drought*

Physiological traits are key influencers in determining the quality of medicinal plants. Plants with better physiological traits can tolerate environmental stress and are more efficient in photosynthesis<sup>34</sup>. Drought is an imbalance between evapotranspiration flux and water intake capacity. Drought stress has been reported to decrease photosynthetic capacity, which will reduce plant growth, yellowing of plant leaves, stomatal closure, and impact crop yield and quality in medicinal plants. Therefore, photosynthesis, the most basic yet complex physiological process, is an indicator to evaluate the impact of drought stress on the quality of medicinal plants<sup>35</sup>.

Sanjeeta *et al.*<sup>36</sup> reported a reduction in photosynthetic rate in *P. Kurroa* due to drought stress, which will further decrease photosynthetic capacity. They also reported upregulation of Cytochrome b6-f complex subunit six and downregulation of NAD(P) H-quinone oxidoreductase subunit 4L in *P. Kurroa* under drought stress. Moreover, stomatal conductance and relative water content were also reduced. However, further investigation should be done to understand plant responses under drought stress.

### Biomolecular responses

#### *Elevated temperature*

Although stress has a detrimental effect on the growth and reproduction of medicinal plants, the concentrations of economically important secondary

metabolites in plant tissues often increase in response to stress<sup>34</sup>. Moreover, some researchers found no effect of temperature on the concentration of bioactive plant contents.

Since secondary metabolites' response to elevated temperature is less studied, although an increase in the volatile organic compound has been detected<sup>32</sup>. Therefore, there is a consensus among researchers that the effect of high temperature on plant bioactive content appears to be species-specific as well as dependent on the nature of the chemical they produce. In the context of *Picrorhiza*, such studies are highly limited, but it is hypothesised that increasing temperature will shorten its growth cycles due to physiological changes and thereby reduce its active constituents<sup>32</sup>. At elevated temperatures, the leaves reported accumulated amino acids like glycine, alanine, leucine, isoleucine, phenylalanine, and tyrosine. Tyr and Phe accumulation was more significant as it was probably involved in specialised metabolites production like iridoid glycosides and phenolic acid. However, glycine, tyrosine, leucine, and phenylalanine levels significantly reduced in rhizomes. Besides amino acids, carbohydrates like mannitol, fructose, and alpha and beta glucose levels were also reduced at elevated temperatures<sup>31</sup>. They also reported that the abundance of Calcium-dependent Protein Kinase (CDPK) family proteins also increased. Hence, a detailed study of temperature-induced response to secondary metabolite production is required for *P. Kurroa* so that the long-term supply of quality raw materials to the pharma industry will not be hampered.

Recently, some researchers studied the impact of warming on picroside content; they reported that total picroside content decreased by 22% at the higher temperature as compared to the lower temperature. The reason they cited is the up-regulation of regulatory genes *pkhmgr* (3-hydroxy-3-methylglutaryl coenzyme A reductase) by 216% and *pkdxs* (1-deoxy-D-xylulose-5-phosphate synthase) by 286%, both of which are involved in the biosynthesis of secondary metabolites at low temperature<sup>32</sup>. Researchers have found that high temperature (25°C) significantly reduces the picroside content (both I & II) compared to lower temperature (15°C)<sup>32</sup>.

#### Elevated CO<sub>2</sub>

Carbon metabolism-related proteins are upregulated at elevated CO<sub>2</sub>. Since secondary metabolites are formed using primary products of

photosynthesis as a substrate, there is a possibility that when carbon accumulation increases in the plant under high CO<sub>2</sub> concentration, it would divert towards secondary metabolite production. Picroside is a monoterpene iridoid glycoside; therefore, there is a chance that the accumulation of picroside would be higher in EC conditions. Picroside content of *P. kurroa* increases at EC condition (550 ppm) in the Free Air CO<sub>2</sub> Enrichment (FACE) experiment running at Kangra Valley of the western Himalaya<sup>34</sup>. Kumar *et al.*<sup>32</sup> also found an accumulation of picroside I & II in rhizomes; however, a reduction of picroside I and an accumulation of picroside II were noticed in leaves.

#### Altitude

Altitude combines many environmental factors such as mean temperature, precipitation, wind speed, duration of snow cover, and radiation intensity. From these factors, the intensity of solar radiation and lower temperatures at high altitudes are highly responsible for the synthesis of bioactive compounds in medicinal plants. The picroside I & II content was found to be highest in the higher elevation population of *P. kurroa* in the western Himalaya<sup>18,32</sup>. On the contrary, Singh *et al.*<sup>37</sup> found that picroside content was higher at a lower altitude (3200 m) as compared to a higher altitude (4145 m). This may be due to the different ages of plants and harvesting times. Besides that, high altitudes also influence the proteomic profiles of plants. However, the role of picroside in *P. kurroa* at high altitudes is not well elucidated. Therefore, a detailed knowledge of the particular environmental factors that influence or suppress the biosynthesis of picrosides content at high altitudes is not only of theoretical interest but might also be helpful for the production of a high level of picroside in an artificial environment.

#### Drought

Plants are subjected to drought stress due to limited water uptake or excessive transpiration. Drought stress retards growth and development as well as plant metabolism. It can impact morphological, physiological, and biochemical parameters in plants. Different factors like water holding capacity, rainfall pattern, and evapotranspiration efficiency could drive drought<sup>37</sup>. Plants responses to drought vary from species to species, depending on the growing stage and other environmental factors.

Sanjeeta *et al.*<sup>36</sup> evaluated the proteomic profiles of *P. Kurroa* under drought stress. They reported that the zinc finger CCCH domain-containing protein 3, a

transcription-related protein, was upregulated in leaves under drought stress. Up-regulation of maturase K and pentatricopeptide repeat-containing protein and down-regulation of H/ACA ribonucleoprotein complex subunit 4 in the root were found. Leaves were upregulated in ATP synthesis, amino acid catabolism, and carbohydrate metabolism-related proteins like ATP synthase subunit beta, endoglucanase 9, and hexokinase-1. Caffeic acid 3-O-methyltransferase, E3 ubiquitin-protein ligase ARI5, and F-box protein were upregulated, whereas beta-D-xylosidase was found to be present only in drought stress in roots. Drought stress also negatively impacts protein synthesis; 50S ribosomal protein L2 was found to be downregulated under drought stress. Sharma *et al.*<sup>24</sup> found that up-regulation of ethylene-responsive transcription factor WIN1, a defence-related transcription factor promoting the formation of cuticles, will help plants withstand drought stress.

#### Yield responses

Plant yield is the harvested production of crops per unit area in a given time. Plant yield is subjected to different climatic variables. Different studies revealed that high-temperature stress could reduce yields and biomass significantly due to a reduction in pollen development and anther viability<sup>32,38</sup>.

Although medicinal plants have more tolerance capacity, in certain environmental conditions, their biomass and yield are also reduced<sup>32</sup>. Unfortunately, there are not enough studies on the temperature effect on the yields of medicinal plants. Very few studies have been conducted to elucidate the elevated CO<sub>2</sub> effect on high-altitude vegetation. High-altitude plants have greater efficiency for CO<sub>2</sub>, an accommodating feature to compensate for the drop in partial pressure of CO<sub>2</sub>. Therefore, the role of elevated atmospheric CO<sub>2</sub> in enhancing high-elevation plant growth is somewhat uncertain<sup>32,33</sup>. Elevated CO<sub>2</sub> enhances photosynthetic rate and capacity, which will improve plant growth and crop yields<sup>33</sup>. However, the impacts of elevated CO<sub>2</sub> on the yield of *P. kurroa* have not been reported yet and need further investigation.

Yield is basically the outcome of the different physiological processes. Drought, an inevitable factor, negatively influences most of these physiological processes. The severity of the drought and the growing stage are the main influencing factors<sup>35</sup>. Yield reduction by drought may be due to the reduction in photosynthesis rate, photosynthetic capacity, disturbed assimilate partitioning, changes in

carbon sequestration and allocation, and poor development of the flag leaf<sup>34</sup>. Drought can reduce the yield of medicinal plants in three ways: reducing photosynthetically active radiation, decreasing photosynthetic rate and capacity, and reducing harvest index (HI), which will result in yield reduction<sup>38</sup>.

#### Conservation strategies

The majority of plants demanded by pharmaceutical companies are directly harvested from the wild population, which is as threatening as global warming because it will ultimately lead to the loss of the wild gene pool. To obtain one kg of the dry weight of *P. kurroa*, around 300 to 400 individual plants are uprooted<sup>10</sup>. Indiscriminate exploitation from the wild, narrow distribution range, and endemic alpine nature lead this species to the top priority for conservation in the western Himalayas.

#### *In-situ* conservation

Conservation of plants in their natural habitats and proper management of existing endangered species are the best ways to conserve medicinal plants in their natural habitat without any loss in genetic variation. On-site conservation is the most efficient method to preserve genetic and habitat diversity. Despite protecting any single species, it can protect every existing species from extinction proficiently. However, *in-situ* conservation is not always possible due to disturbed and damaged natural habitats, area fragmentation, human interference, and anthropogenic activities<sup>22,31</sup>.

#### *Ex-situ* conservation

*Ex-situ* or out-of-natural habitat conservation of depleting plant species is very crucial for protecting them outside their normal habitats. Conservation is mainly possible through cultivating and maintaining rare or endangered plant species in parks, botanical gardens, etc. Genetic variation influences wild populations to survive, reproduce, and adapt strategies against climatic and environmental changes; so, genetic analysis is a prerequisite to conserving threatened species efficiently. Along with that, geographical distribution patterns and characteristics are also required for the conservation of the threatened species.

#### *In-vitro* conservation

Various research has been carried out in plant tissue culture, molecular biology, and agricultural

sciences for the improvement of cultivation and propagation techniques of *P. kurroa* either in *in-vitro* conditions and fields<sup>10,22,31</sup>.

#### Plant tissue culture

Different types of techniques like seed germination, vegetative propagation, and *in-vitro* propagation, i.e., root hair culture, shoot culture, axillary bud, callus culture, etc., have been used for mass multiplication of *P. kurroa*, but the success rate showed variation from around 40 to 90% in another study<sup>10</sup>. Further, instead of uprooting the whole plant, the aerial parts, like leaves, can be used for the extraction of bioactive compounds. Aromatic and medicinal plants like *P. kurroa* could be cultivated on a mass scale, and the farmer should be motivated to grow these plants. Moreover, the farmers of the Himalayan states should be incentivised and encouraged to grow these threatened plants, which ultimately benefits the local communities through self-employment.

#### Cryopreservation

Cryopreservation is also a valuable means to conserve the germplasm of endangered medicinal plants. Sharma and Sharma<sup>8</sup> successfully demonstrated the cryopreservation of shoot tips of *P. kurroa* through the vitrification technique. Besides, shoot cultures of *P. kurroa* have been stored in the repository at the National Bureau of Plant Genetic Resources, India, for over a decade.

#### Germplasm Bank

Himalayan Forest Research Institute (HFRI) established a germplasm bank of Himalayan medicinal plants, including *P. kurroa* (Indian Council of Forestry Research and Education). Mishra *et al.*<sup>31</sup> reported that 3% of Sodium alginate and 100 mM calcium chloride was most effective for encapsulation, an efficient means for conserving the germplasm of threatened plant species.

#### Biotechnological conservation

Various biotechnological methods have been applied to conserve endangered or threatened plants. Several biotechnological methods, i.e., marker technology, somatic embryogenesis, micropropagation, and organogenesis, have been used for rapid multiplication and regeneration of different species of *Picrorhiza*. Due to the increasing demand for *P. kurroa*, natural habitats are being disturbed and

destroyed; these methods could be effective measures for the conservation of *P. kurroa*.

Intercropping is another measure that can be employed and used in the case of *P. kurroa*, and its intercropping with *Foeniculum vulgare* Mill., *Solanum tuberosum* Linn., and *Digitalis purpurea* has been done successfully, as these crops provide a suitable climate for the growth and the productivity<sup>39</sup>. Most of the research on the conservation of *P. kurroa* has focused on its status assessment, *in vitro* propagation protocol and understanding its reproductive biology. Nevertheless, to assess the effect of climate change on its phenology, range shifts, and secondary metabolites production, long-term ecological research stations and phenological stations are required in IHR. The detailed study of genetic structure and variation will also be very helpful for plant preservation, management, and survival in the long term. Moreover, the use of climate modelling with the help of remote sensing and satellite imagery is another major strategy that could be used for estimating the impact of global warming on the Himalayan alpine region. Instead of predicting for a more extended period, the researchers should make a short-term prediction using different projections of climate change rate, so that the result of projections will correspond with the results of real-world events very soon, and the management for the conservation will be quicker.

#### Conclusion

Global climate warming is increasingly evident in mountain ecosystems, where rising temperatures are driving low-altitude plants upward and reducing endemic species richness. Although the warming observed so far represents only the early stage of projected future change, ecological responses are already clear. Shifts in phenology, altered distribution ranges, and increasing extinction risks threaten many wild medicinal plants. These impacts not only limit their therapeutic use but also erode irreplaceable genetic resources developed over millennia. *P. kurroa*, a high-value Himalayan medicinal species, is particularly vulnerable due to excessive and illegal wild harvesting. Its survival is further challenged by extreme weather events, rising temperatures, drought, elevated CO<sub>2</sub>, and altitudinal shifts. However, the influence of elevated CO<sub>2</sub> on its secondary metabolites remains uncertain, with studies reporting both increases and decreases. Evidence also indicates a positive

correlation between picroside content and altitude. Therefore, detailed research is needed to clarify the environmental factors regulating picroside biosynthesis at high elevations, which could support optimized production under controlled conditions. Conservation strategies should address overexploitation, enhance secondary metabolite production, and expand habitat range. Biotechnological methods such as hairy root and callus cultures offer promising alternatives for sustainable metabolite production. Overall, this review highlights the need to conserve Himalayan medicinal plants and protect their fragile mountain environments.

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### Conflict of interest

The authors declare no competing interests.

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