Population Status, Distribution, Antioxidant Properties and Antibacterial Activity of Threatened Herb *Gentiana kurroo* Royle

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ABSTRACT: Sustainable utilization and conservation of the threatened species is a major challenge for conservationists. For the conservation and reintroduction of species, comprehensive information on the ecological elements, potential habitats and pharmaceutical importance of the species is essentially required. This has necessitated initiating studies on habitat and population ecology of the threatened species. In view of the above, the present study investigated populations of *Gentian kurroo* representing different habitats and aspects in Himachal Pradesh. Among the populations, mean density of *G. kurroo* 80.75 Ind per 100m^2 and range varied from 63.0-110.0 Ind 100m^{-2}. The density showed direct relation with soil, slope, aspect and habitat. The particular geographical distribution of the species was sub tropical indicated species habitat specificity. Biological features, aspects, slope and pressure contributed to the critical population status of the *G. kurroo*. The present investigation clearly showed that *G. kurroo* populations occurred in fragmented pattern in the sub-tropical ecosystem. Species re-introduction should therefore carefully select suitable habitat with suggested setting in this study. The present study confirmed that population ecology, habitat distribution modelling provide assistance in the species recovery plan. The study would not only help in eco-restoration of the species and habitats but also in recovering the species population and improving its conservation.

Keywords: *Gentiana kurroo;* Niche, Ecology; Phytochemical; Antibacterial Activity; Conservation; Indian Himalaya.

INTRODUCTION: One fifth of the plant species of globe to the brink of extinction because of changing climate, habitat fragmentation, overexploitation, and escalating human population (Brummitt and Bachman, 2010; Barnosky *et al.*, 2011). The loss of biological diversity in the world's forests has experienced a colossal depletion in the recent past. The International Union for Conservation of Nature and Natural Resources (IUCN) has estimated about 10% of the vascular plants are varying degree of threats and nearly 25% of the floral species in the world may become extinct within the next 50 years (Schemske *et al.*, 1994). In the Indian Himalayan Region (IHR), over exploitation and habitat degradation are among the major factors leading to species vulnerability (Samant et al., 2007; Rana and Samant, 2010). *Gentiana kurroo* is one of the species which face the high degree of threat due to unsustainable collection. *Gentiana kurroo* Royle belongs to the family Gentianaceae and genus *Gentiana* which represented 360 species in the northern temperate areas of the world (Judd, 1999; Struwe, 2002; Behera, 2012). It is en-

demic and critically endangered to the north-western Himalayas and found on dry and rocky grasslands and scrubs habitat in the south facing slopes between 1500–3000 m asl (Raina, 2003; Khuroo, 2005). During the period of last ten years > 80% of the populations were declined (Goraya, 2013).

The word *Gentiana* has been derived from "Gentius", a king of Europe, who discovered the medicinal value of the *Gentiana* roots and specific name from the indigenous word "Karu" means bitter. The shoot system of plant is represented by flowering branches with culine leaves. The stem is a modified rhizome and root system is rhizome and adventitious root. Flowering starts from the last week of August to first week of November and ideal time for seed harvest is the first fortnight of November (Raina, 2003).

The root and rhizome are rich source of Iridoid glycosides-gentiopicrine, gentiamarin, amaroswerin, and the alkaloid gentianine (Niiho 2006). The roots contain 20% of a yellow, transparent, and brittle resin (Coventry, 1927; Anonymous, 1956), aucubin, cata-

lpol, 6-O-vanilloyl catalpol, 6-O-cinnamoyl catalpol, (Sarg, 1991; Raina 2011). However, the leaves also contain some of the important bitter compounds; noticeably moreso than the roots (Wani, 2011). Some other components isolated are morroniside and gentiopicroside (Lin, 2008).

The medicinal values of *G. kurroo* well known from when human beings starts got to know herbal treatment for different diseases from natural products. In folkloric system species is used to cures many diseases such as ulcer, skin fungal infection, stomach-ache, urinary infections, liver complaints, headache, bronchial asthma, cough, leucoderma, leprosy, dyspepsia, colic flatulence, blood purifier, indigestion, gastric infections, anorexia and high fevers (Gilani, 2006; Sharma 2000; Kirtikar, 1935). Gentianine possesses anti-infammatory, analgesic, anticonvulsant, hypotensive, antipsychotic, sedative, diuretic, antimalarial, anti-amoebic and antibacterial properties and Amaroswerin acts as gastro-protective (Sharma, 2006; Sarg, 1990). The drug obtained from plant is helpful in removing all kinds of weakness and overtiredness of body from prolonged illness and in the preparation of tonics for stomachic (Pullaiah, 2002).

MATERIALS AND METHODS:

Study area: The present study on *Gentiana kurroo* has been carried out in Himachal Pradesh (30º 22' 40" to 33º 12'40" N latitudes and 75º 47'55" to 79º 04'20" E longitudes) of Trans and North-Western Himalaya (Map 1). It is bounded by Tibet in the East, Jammu and Kashmir in the North, Uttarakhand in the South-East, Haryana in the South and the Punjab in the West. Physiographically, it is divided in three conspicuous zones, namely Outer Himalaya or the Shiwaliks, Inner Himalaya or mid mountain and the Greater Himalaya or alpine zones. Five rivers namely, Sutlej, Beas, Ravi, Yamuna, and Chenab with a large number of their tributaries flow through the State. It is known for its salubrious climate and experiences considerable variations in the distribution of rainfall and temperature due to varying aspects and altitude. Precipitation declines from West to East and South to North (Singh, 2007).

Assessment of populations: The field surveys were conducted in Himachal Pradesh to assess the populations of species. For the quantitative assessment of *Gentiana kurroo* populations, representative sites were selected and sampled in different habitats and aspects. For each site, altitude, latitude, longitude, aspect, habitat and dominant species were noted. In each site a plot of 20x20m was laid and species were

sampled by randomly placed quardrats. For the analysis of density Singh and Singh (1992), Dhar et al. (1997) and Samant *et al.* (2002) were followed. From each site samples of each species were collected and identified with the help of local and regional flora. Five soil samples, one from center and four from four corners were randomly collected from each site. Soil was cored up to 20cm depth. These samples were mixed together and a composite sample weighing 200g of the homogenized soil was collected in airtight polythene bags and brought to the laboratory for physical and chemical analysis. Moisture (%) and pH of the soil were measured. Soil was air dried and sieved with 2mm mess and, used for analysis of total nitrogen, organic carbon and organic matter by following Allen (1974).

Map1: Study area and population of *G. kurroo*.

Ecological Niche: Point distribution, bioclimatic, elevation, slope, aspect, environmental and physiographic variables were utilized for the prediction of potential areas of species with the help of MaxEnt version 3.3.3k Ecological Niche Modelling package (Phillips *et al.*, 2011). Point distribution records were collected through primary field surveys from the Himachal Pradesh. Predictive 22 variables selected for the habitat suitability of species retrieved from different databases. Nineteen climatic raster data were obtained from WorldClim at 30 arc-second resolution (Hijmans *et al.*, 2005). Topographic variables were achieved from ASTER Global Digital Elevation Model (1 arc-second resolution; http://gdem.ersdac.jspace systems.or.jp). All the predictive variables rescaled to~250m pixel dimension in ArcGis 9.2. For the geographical distribution of species, MaxEnt version

3.3.3k was run using auto features, with output set to logistic, easier to interpret than raw or cumulative formats (Phillips and Dudik 2008). To validate the robustness, 20 replicated models were run with a threshold rule of 10 percentile training presence and in the replicate cross validation technique were run. Other parameters were set to default as the program is already calibrated on a wide range of species datasets (Phillips *et al*., 2006; Phillips and Dudik 2008). A jackknife procedure run to get an alternate measure of the importance of environmental variables by calculating several models with each variable omitted in turn, and models with variables used individually. (Phillips et al., 2006; Phillips and Dudik 2008). Model quality was evaluated based on Area Under Curve (AUC) value (Thuiller *et al*., 2005). The MaxEnt output in ASCII format was then exported to DIVA-GIS var.7.3 software for further analysis and map composition. Logistic threshold of 10 percentile training presence value was categorized into five classes for map composition of potential habitats distribution i.e., very high (0.762–1), high (0.572–0.761), medium (0.381–0.571), low (0.325–0.380) and not suiTable (0–0.324) (Adhikari *et al*., 2012).

Biochemical activities: The total phenols were determined by Folin - Ciocalteau reagent method described by Wolfe *et al*., (2003) and total flavonoids were estimated using Ordonez *et al*., (2006) method. The antioxidant activities in methanol extracts of leaf, stem and root were measured in terms of DPPH, ABTS radical scavenging following the methodology of Mensor *et al*., (2001), Re *et al.* (1999) and Benzie and Strain (1996).

For the estimation of biochemical activities, plant samples were collected from the study site. Samples were washed 2-3 times using tap water to remove the dust particle and contamination and chopped in small pieces using chopper or cutter. One g of leaf, stem, fruit and root will be weighed accurately, and then were crushed in 10 ml of 80% (v/v) methanol using motar and pestle. The extract were kept in refrigerator for 72 h and then centrifuge at 5000 rpm for 5 min and the supernatant were used for the total phenolic and total flavonoid contents and in vitro assays for antioxidant activities. For the concentration versus DPPH and ABTS inhibition assays of plant species, different concentrations of the extracts of leaf, stem and root were prepared and analyzed.

Antibacterial Activity: The fresh and healthy root, stem, and leaves samples of the of species were collected from study site during the months October, 2018, brought to the laboratory, air dried, converted into fine powder, and stored at 4-8 °C. Root and leaf were extracted (separately) in two solvents i.e. methanol and water taking in a ratio of 1:5 (dry powder: solvent). The mouth of conical flask was sealed with para-film. Samples were macerated in a rotary shaker (Remi) at 160 rpm for 48 h.

One (1) Gram +ve bacteria (*Bacillus subtilis* (NRRLB-30408); two (2) Gram –ve bacteria (*Escherichia coli,* and *Serratia marcescens* (MTCC4822) and one actinobacteria (*Nocardia tenirefensis* (MCC2012) were used for the antibacterial activity. These microorganisms were taken from the Microbiology Lab of the Institute (GBPNIHESD). Accession numbers shown in the parentheses have been allocated by the National/International depositories.

For qualitative estimation of antimicrobial potential of *G.kurroo* extracts, agar plate based bioassays were performed using disc diffusion method. Bacterial and actinobacterial culture suspensions were prepared in tryptone yeast extract (TYE) agar. 100 µl of all the test microorganisms (separately) were spread uniformly on the respective agar surface agar plates for bacteria and actinobacteria with the help of a glass spreader. Sterilized 5 mm filter paper (Whatman No. 1) discs were placed over the agar surface with the help of sterile forceps. 15 µl of extract was loaded over the agar disc. The plates were then incubated at 25 ºC. The results were recorded measuring the zone of inhibition (mm) after 24 h for bacteria and 120 h for actinobacteria and fungi. All the experiments were performed in triplicates.

Minimum inhibitory concentration **(**MIC was) determined following Clinical and Laboratory Standard Institute Methodology (Wayne, 2008). Bacterial and actinobacterial culture suspensions were prepared in TYE broth. For determination of MIC, 1 ml extract was diluted using different concentration ranging from 100 to 1000 µg/ml, 1 ml test microorganism and 8 ml broth was taken in sterile test tube, and then incubated at 25 °C for 24 h for bacteria and 120 h for actinobacteria. Control was prepared in two sets; one containing broth medium and test microorganism while the other containing broth medium and extract. After 24 h, the MIC values were recorded on the basis of the lowest concentration showing absence of growth in the tubes. The test was further confirmed by plating on TYE agar medium.

RESULTS AND DISCUSSION:

Physical Attributes: Total 4 populations of *Gentiana kurroo* were studied between 1423-1821m amsl in the Himachal Pradesh. These populations were fallen in

different aspects viz., West, Northeast and North; and the sites/populations were represented by dry and rocky habitats. The sampling of the vegetation was done between 30.771° N to 30.816° N latitudes and 77.196 to 77.279° E longitudes (Table 1).

Table 1: Populations physical characteristics.

Species name	Locality	Habitat	Altitude(m)	Latitude (°N)	Longitude(°E)	Aspect	Slope (°)
Gentiana kurroo	Pab	Rocky	1659	30.816	77.196	N	20
Gentiana kurroo	Baru	Rocky	1821	30.771	77.262	NE	48
Gentiana kurroo	Mangarh	Dry	1460	30.796	77.279	w	10
Gentiana kurroo	Madesh	Rocky	1423	30.811	77.211	NW	35

Chemical properties of the soil: Population wise pH, moisture content (%), total nitrogen (%), total organic carbon (%), and total organic matter (%) of the studied threatened plants have been presented (Table 2). In the studied populations, moisture content ranged from 5.05-14.70%, pH 7.99-8.31, total nitrogen 0.65-0.81%, organic carbon 2.56-5.34% and organic matter 4.41-9.21%. In the studied populations, maximum total nitrogen (%) was recorded in Madesh (0.81), followed by Pab (0.70), Mangarh (0.69) and Baru (0.65); maximum total carbon (%) in Pab (5.34), followed by Baru (3.24), Madesh (3.21) and Mangarh (2.56). Soil of all populations was alkaline in nature i.e., Mangarh (8.31), Baru (8.16), Pab A (8.03) and Madesh (7.99).

Table 2: Moisture content (%), pH, total nitrogen (%), total organic carbon (%) and total organic matter (%) of the soil.

Species	Population	Moisture	pH	Total Nitrogen%	Organic Carbon (%)	Organic Matter (%)
Gentiana kurroo	Dab	5.05	8.03	0.70	5.34	9.21
Gentiana kurroo	Baru	14.7	8.16	0.65	3.24	5.58
Gentiana kurroo	Mangarh	10.32	8.31	0.69	2.56	4.41
Gentiana kurroo	Madesh	9.2	7.99	0.81	3.21	5.53

Population Status: Population wise richness; total density, Concentration of dominance and Species diversity are presented in Table 3. Among the populations, richness of species ranged from 12-25; total trees density 17-162 Ind ha^{-1} ; total shrubs density 530-710 Ind ha^{-1}; total herbs density 45.35-73.10 Ind m^{-2}; Concentration of dominance for trees 0.03-1.00, shrubs 0.25-0.34 and herbs 0.05-0.44 and Species diversity (H') for trees 0.0-0.52, shrubs 1.23-1.53 and herbs 1.02-2.36 (Table 4). Mean density of the populations was 80.75 Ind per 100m^2 and range varied from 63.0-110.0 Ind 100m^{-2}. In the studied populations, maximum density was recorded in Baru (110.00 Ind 100m^{-2}), followed by Pab (80.00 Ind 100m^{-2}), Mangarh (70.00 Ind 100m^{-2}) and Madesh (63.00 Ind 100m^{-2}).

Table 3: Total density, diversity and Concentration of dominance of herb, shrub and tree layers and relative density of studied threatened plants populations in Himachal Pradesh.

| Species name	Locality	SR	Herbs			Shrubs			Trees		
			Den*	H'	Cd	Den	H'	Cd	Den	H'	Cd
Gentiana kurroo	Dab	12	45.35	1.22	0.37	7.10	1.39	0.31	0.17	0.00	1.00
Gentiana kurroo	Baru	16	73.10	1.02	0.44	7.00	1.32	0.34	1.62	0.34	0.03
Gentiana kurroo	Mangarh	17	63.00	2.36	0.05	5.30	1.23	0.25	1.36	0.52	0.25
Gentiana kurroo	Madesh	25	58.50	1.51	0.31	6.40	1.53	0.32	1.30	0.00	1.00

Ecological Niche: The model test for *Gentiana kurroo* yielded satisfactory results (AUC$_{test}$= 0.826 ± 0.110). Amongst the predictor bioclimatic variables, Precipitation of Driest Period (BIO 14); Temperature Annual Range (BIO 7 (Maximum Temperature of Warmest Period - Minimum Temperature of Coldest Period)) and Temperature Seasonality (BIO 4, Coefficient of Variation) were the most influential and con-

tributed 38.1%, 35.4% and 24.4%, respectively to the Maxent Model (Table 4). Considering the permutation importance, Ttemperature Annual Range (BIO 7 (Max Temperature of Warmest Period - Min Temperature of Coldest Period)) had the maximum influence on the habitat suitability model and contributed to 51.4%, while Precipitation of Driest Period (BIO 14) contributed to 41.7% (Table 4).

Potential habitats with high suitability thresholds were distributed in the lower elevations of the Shimla, Mandi, Solan and Sirmaur districts of Himachal Pradesh in Trans and Northwestern biogeographic provinces of the Indian Himalaya (Figure. 1). Primary field surveys revealed that the predicted potential habitats were mostly located in the dry grassland, pine and oak forests of Himachal Pradesh.
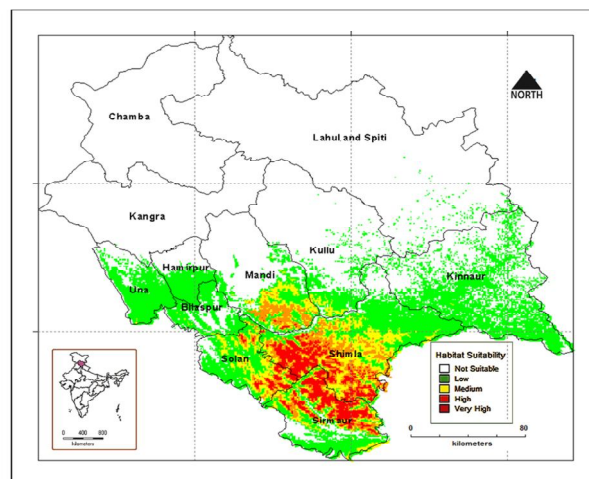
Figure 1: Habitat suitability and distribution of *Gentiana kurroo* in Himachal Pradesh.

Table 4: Estimates of relative contributions and Permutation importance of the predictor variables to the Maxent Model.

Variable	Name of predictor variable	Percent Contribution	Permutation importance
bio_1	Annual mean temperature	0.0	0.0
bio_10	Mean Temperature of Warmest Quarter	0.0	0.0
bio_11	Mean Temperature of Coldest Quarter	1.4	6.6
bio_12	Annual Precipitation (mm)	0.0	0.0
bio_13	Precipitation of Wettest Period (mm)	0.0	0.0
bio_14	Precipitation of Driest Period (mm)	38.1	41.7
bio_15	Precipitation Seasonality (Coefficient of Variation)	0.0	0.0
bio_16	Precipitation of Wettest Quarter (mm)	0.0	0.0
bio_17	Precipitation of Driest Quarter (mm)	0.0	0.0
bio_18	Precipitation of Warmest Quarter (mm)	0.0	0.0
bio_19	Precipitation of Coldest Quarter (m)	0.0	0.3
bio_2	Mean diurnal range (max temp – min temp) (monthly average)	0.0	0.0
bio_3	Isothermality (BIO1/BIO7) * 100	0.7	0.0
bio_4	Temperature Seasonality (Coefficient of Variation)	24.4	0.0
bio_5	Max Temperature of Warmest Period	0.0	0.0
bio_6	Min Temperature of Coldest Period	0.0	0.0
bio_7	Temperature Annual Range (BIO5-BIO6)	35.4	51.4
bio_8	Mean Temperature of Wettest Quarter	0.0	0.0
bio_9	Mean Temperature of Driest Quarter	0.0	0.0
aspect	Aspect(degrees)	0.5	0.0
dem	Elevation(m)	0.0	0.0
slope	Slope (degrees)	0.0	0.0

MaxEnt is capable of giving maximum accuracy rate with 5 to 25 sample sample points as comparing the other modeling methods (Hernandez *et al*., 2006). Thus, present study stated that niche of G. kurroo an endangered, high value medicinal plants can be modeled using small number of occurrence record and environmental variables in MaxEnt. AUC value was more than 0.8 for the studied species suggesting that the model fit was good, far closer to a perfect fit than

a random one, indicated an excellent and accurate prediction. Ecological niche modelling is a useful technique to determine suiTable habitats, especially high potential areas, model output predicts the low altitude region of Shimla, Solan, and Sirmour highly capable of supporting the growth of species with favorable climatic and topographic conditions.

These most suitable habitats make the actual platform for designing effective conservation strategies for this

threatened species, including establishment of plant conservation priority zones, reintroduction in the highly suitable areas and their short and long term monitoring.

Contribution of environmental variables indicated that precipitation of driest period was the most influential variable out of 22 variables in determining habitat suitability of species and contributed 38.1%. These results clearly indicated that only few environmental variables were affecting the growth and distribution of the highly valuable species. Result also indicated strong correlation between bioclimatic variables and spatial distribution of species.

The results showed that the predicted suitable habitats through MaxEnt almost always appeared as over predicted in some areas compared to the realized niche of the species. Because, MaxEnt considers only niche-based presence data, it estimates the species fundamental niche (different from occupied niche) rather than realized niche (Kumar and Stohlgren, 2009; Yang *et al.*, 2013; Ardestani *et al.*, 2015). The overall study provides perfect baseline information for planning conservation and management strategies to protect such unique medicinally important plant species of Himalaya. It also helps us to prepare database for the target species, provides new localities where natural habitats can be protected and restored in order to promote natural regeneration, which further can be used to monitor population status, thereby, useful in minimizing threats as well as creating awareness. Hence, ecological niche modelling prediction makes species survival approach more effective than other practices.

Biochemical Activities:

Total Phenolic and Flavonoid Contents: The total phenolic content of the leaf and root methanolic extracts of G. kurroo was determined by the method described above. The total phenolic content for the root extract was found to be 86±2.4 (GAE)/g DW and for the leaf extract 43±1.5 (GAE)/g DW (Table 5). The total flavonoid content of *G. kurroo* root and leaf extracts is given in Table 5. The total flavonoid content for the root extract was found to be higher (51±2.2 rutin equivalent/g DW) than the leaf extract (30±1.3 rutin equivalent/g DW).

Table 5: Total phenolic and flavonoid contents of root and leaf methanolic extracts of *Gentiana* kurroo.

Extract	Total phenolic content[a]	Total flavonoid content[b]
Leaf extract	43±1.5	30±1.3
Root extract	86±2.4	51±2.2

Each value is a mean of three biological replicats.
[a] =mg gallic acid equivalent (GAE)/g DW.
[b] =mg rutin equivalent/g DW.

Antioxidant Activity: The DPPH is one of the best scavenging method, because it is easy, fast and reliable. It is a sTable diamagnetic molecule and the reduction capability was determined by the decrease in absorbance induced by plant antioxidants. For each sample, four concentrations (50–400 μg mL^{-1}) of the extracts were tested. IC$_{50}$ value is the concentration of the extract required to scavenge the free radicals to 50% of the control. IC$_{50}$ values were negatively correlated with antioxidant activity, the lower the IC$_{50}$ value, the higher and the antioxidant activity of tested sample. In the *G. kurroo* DPPH radical scavenging activity in rhizome extracts was higher in leaf with IC$_{50}$ value 119.82 μg mL^{-1} (Table 6). The antioxidant activity of leaf and root extracts was increased with increasing concentration as shown in Table 6.

Table 6: DPPH radical scavenging of leaf and root parts of *Gentiana kurroo* in methanolic and water extracts.

Concentration (µg /ml)	DPPH radical scavenging ability of leaf and root extracts (% inhibition)			
	Methanolic extracts		Water extracts	
	Leaf	Root	Leaf	Root
25	49.85	33.43	1.58	14.83
50	70.09	32.84	0.95	39.75
75	76.83	43.99	7.57	41.32
100	79.47	46.04	20.50	44.16
125	81.52	46.63	16.09	48.90
150	81.52	52.49	29.34	52.68
IC 50	**26.06**	**70.70**	**119.82**	70.42

ABTS radical scavenging activity: The ABTS assay is one of the best scavenging methods as it is easy to carry out. Similar to the DPPH assay, ABTS assay is also chemically sTable diamagnetic molecule and used to measure the decrease in absorbance induced by plant antioxidants. The ABTS radical scavenging

activity of leaf and root extracts of *G. kurroo* was increased with increasing concentration. The highest ABTS radical scavenging effect was obtained in leaves with the IC_{50} value 66.03 μg mL^{-1} of water extracts. While in case of root extract, the highest ABTS radical scavenging effect was obtained methenolic with the IC_{50} value 56.56 μg mL^{-1} (Table 7).

**Table 7: ABTS radical scavenging of leaf and root parts of *Gentiana kurroo* in   methanolic and water extracts.**

Concentration (µg /ml)	ABTS radical scavenging ability of leaf and root extracts of all populations (% inhibition)			
	Methanolic extracts		Water extracts	
	Leaf	Root	Leaf	Root
50	72.91	58.60	58787	51.63
100	67.38	59.45	61.38	62.70
200	82.35	75.24	78.12	78.96
400	75.20	89.25	82.06	86.66
IC_{50}	175.66	**56.56**	66.03	32.67

The extracts of leave and root of G. kurroo showed high phenolic and flavonoid content. Phenolic compounds are important plant constituents for their free radical scavenging ability, enabled by their hydroxyl groups, and the total phenolic concentration might be used as a source for rapid screening of antioxidant activity and are also involved in the oxidative stress tolerance of plants. Flavonoids are highly effective scavengers of most oxidizing molecules concerned with several diseases. On the other hand, flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species, up-regulate and protect antioxidant defences. The methanolic extracts of root as compared to the methanolic extract of leaves showed comparatively high antioxidant activity, which could be related to the total flavonoid and phenolic content of the two extracts.

Antibacterial Activity: Figure 2 shows the yield of root and leaf plant parts of *G. kurroo* extracted in methanol and water solvents according to their polarity (water> methanol) following maceration. The extraction yield was recorded highest in root, followed by leaf. Water was found to be the best solvent for root obtaining highest yield and methanol for leaf parts of *G. kurroo*. Extract yields were found to be higher in

root as comparison of leaf in both extracts. These results coincide with several previous reports [Felhi et al. 2017, Tatiya et al. 2011]. Higher yield in water can be attributed to their high di electric constant which responsible for solubility of bioactive compounds [Adhikari, 2018]. This result indicates toward the role of solvent system in obtaining the extract yield of *G. kurroo*.

Qualitative test (Plate based bioassays): Extracts of root and leaf showed antimicrobial activity against bacteria and actinobacteria it's due production of antimicrobial metabolites of *G. kurroo* are shown in figure 2. Methanolic and aqueous extracts inhibited the growth of bacteria, Gram +ve and Gram -ve. Antimicrobial activity, recorded in different plant parts, was in the order: leaf> root. Maximum inhibition was recorded in case of *S. marcescens* (9±0.07mm) in leaf methanolic extract, followed by leaf methanolic extract (8±0.51 mm) against *B. subtilis*. In comparative assessment with respect to the bacteria, *S. marcescens, E. coli,* and *B. subtilis* leaf methanolic extract were the most effective (figure 2). Aqueous and methanolic extracts of plant showed inhibition of the actinobacterial species *N. tenirefensis*. This indicates that both the solvent have capability for detection of actinobacterial compound(s) (Table 8 and Figure 2).
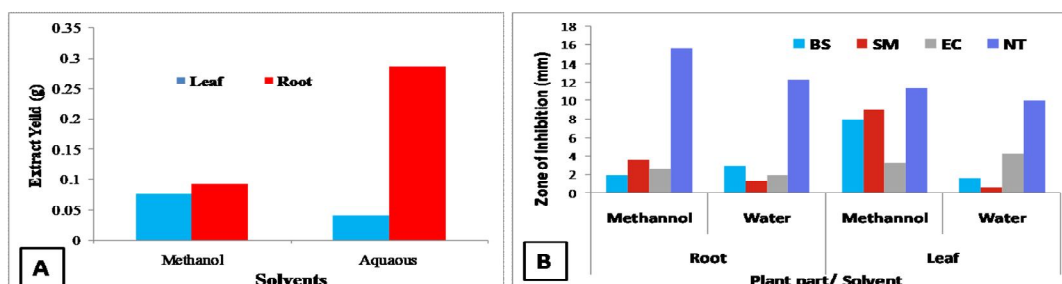
Figure 2: G. *kurroo* root and leaf (A). Extract yield and (B) Antibacterial activity of aqueous and methanolic exatrcts (BS=*B. subtilis*, EC=*E.coli*, SM=*S. marcescens* and NT= *N. tenirefensis*).

In the MIC experiments, out of two solvents, methanol root extract and aqueous leaf extract showed strong inhibition of bacterial species. The most affected group was actinobacteria which was supported by the low MIC values of methanol and aqueous extracts of root and leaf (Table 8). Significant variations in MIC recorded in all the extracts demonstrated the role of selection of solvent as well as the type of test microorganisms.

Table 8: Minimum inhibitory concentration ug/ml of *G. kurroo* plant extracts.

Microorganism		Root extracts (ug/ml)		Leaf extracts (ug/ml)	
		Methanol	Aqueous	Methanol	Aqueous
Gram(+)ve	*B. subtilis*	600	100	900	400
Gram(-)ve	*S. marcescens*	200	700	700	800
	E. coli	300	600	300	800
Actinobacteria	*N. tenirefensis*	200	400	900	500

CONCLUSION: Based on the present study it is confirmed that population ecology and habitat distribution modelling are the pre-requisite for the species recovery plan and also species is reach source of antioxidant & antibacterial chemical. The study would not only help in eco-restoration of the habitats, but also in recovering the species population and improving its conservation. Therefore, the outcome of the study will help the state Government, particularly Forest Department for developing strategies for the conservation of *G. kurroo*.

REFERENCES:

1. Adhikari, D.; Barik, S. K.; Upadhaya, K. Habitat distribution modelling for reintroduction of Ilex khasiana Purk., a critically endangered tree species of northeastern India. *Ecol. Eng.* **2012**, 40, 37-43.

2. Adhikari, P.; Pandey, A.; Agnihotri, V.; Pande, V. Selection of solvent and extraction method for determination of antimicrobial potential of *Taxus wallichiana Zucc. Res. Pharm.* **2018**, 8, 01-09.

3. llen, S.E. *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, Oxford, 1974, pp. 368.

4. Anonymous. The Wealth of India: A Dictionary of Indian Raw Material and Industrial Products; Publication and Information Directorate CSIR: New Delhi, India, 1956, pp. 123–125.

5. Ardestani, E. G.; Tarkesh, M.; Bassiri, M.; Vahabi, M. R. Potential habitat modeling for reintroduction of three native plant species in central Iran. *J Arid Land.* **2015**, 7(3), 381-390.

6. Barnosky, A. D.; Matzke, N.; Tomiya, S.; Wogan, G. O. U.; Swartz, B.; Quental, T. B.; Mar-shall, C., McGuire; J. L., Lindsey; E. L., Maguire, K. C.; Mersey, B.; Ferrer, E. A. Has the Earth's sixth mass extinction already arrived? *Nature.* **2011**, 471, 51–57.

7. Behera, M. C.; Raina, R. *Gentian kurroo* Royle-A critically endangered bitter herb. *Int. J. Med. Arom. Plants.* **2012**, 2, 22–29.

8. Benzie Iris, F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power". The FRAP assay. *Analyt. Biochemi.* **1996**, 239, 70-76.

9. Brummitt, N., Bachman, S. Plants under pressure a global assessment. The first report of the IUCN Sampled Red List Index for Plants. Royal Botanic Gardens, Kew, UK, 2010.

10. Coventry, B. O. Wild Flowers of Kashmir (Series-III); Raithby, Lawerence and Co., Ltd.: London, UK, 1927.

11. har, U.; Rawal, R. S.; Samant, S. S. Structural diversity and representativeness of forest vegetation in a protected area of Kumaun Himalaya, India: implications for conservation. *Biodiver. Conser.* **1997**, 6, 1045 -1062.

12. Felhi, S.; Daoud, A.; Hajlaoui, H.; Mnafgui, K.; Gharsallah, N.; Kadri, A. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *Food Sci. Technol.* **2017**, 37(3), 483-492.

13. Gilani, S. A.; Qureshi, R. A.; Gilani, S. J. Indegenious uses of some important ethnomedicinal herbs of Ayubia national park, Abbottabad, Pakistan. *Ethnobot. Leafl.* **2006**, 10, 385–393.

14. Goraya, G. S.; Jishtu, V.; Rawat, G. S.; Ved, D. K. Wild Medicinal Plants of Himachal Pradesh: An

Assessment of their Conservation Status and Management Prioritization. Himachal Pradesh Forest Department, Shimla, India 2013.

15. Hernandez, P. A.; Graham, C. H.; Master, L. L.; Albert, D. L. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*. **2006**, 29(5), 773-785.

16. Hijmans, R.J.; Cameron, S.E.; Parra, J. L.; Jones, P.G.; Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*. **2005**, 25, 1965-1978.

17. Judd, W. S.; Campbell, C. S.; Kellogg, E. A.; Stevens, P. E. Plant Systematics: A Phylogenetic Approach. Sinauer Associates, Inc.: Sunderl and, MT, USA, 1999.

18. Khuroo, A. A.; Dar, G. H.; Khan, Z. S.; Reshi, Z. A. Observations on Gentiana kurroo Royle, a critically endangered medicinal plant from Kashmir Himalaya, India. *Endanger. Sp. Update*. **2005**, 22, 139–143.

19. Kirtikar, K. R.; Basu, B. D. Indian Medicinal Plants; Bishen Singh Mahendra Pal Sigh: Dehradun, India., 1935, 3, 1661–1662.

20. Kumar, S.; Stohlgren, T. J. Maxent modeling for predicting suiTable habitat for threatened and endangered tree *Canacomyrica monticola* in New Caledonia. *J. Ecol. Nat. Environ.* **2009**, 1(4), 94-98.

21. Lin, C. T.; Chen, C. J.; Lin, T. Y.; Tung, J. C.; Wang, S. Y. Anti-inflammation activity of fruit essential oil from Cinnamomum insularimontanum Hayata. *Bioresour. Technol.* **2008**, 99, 8783–8787.

22. Mensor, L. I.; Menezes, F. S.; Leitao, G. G.; Reis, A. S.; Dos Santos, T.; Coube, C. S.; Leitao, S. G. Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. *Phototherapy Res.* **2001**, 15, 127-130.

23. Niiho, Y.; Yamazaki, T.; Nakajima, Y.; Yamamoto, T.; Ando, H.; Hirai, Y.; Toriizuka, K.; Ida, Y. Gastro protective effects of bitter principles isolated from Gentian root and Swertia herb on experimentally induced gastric lesions in rats. *J. Nat. Med.* **2006**, 60, 82–88.

24. Ordonez, A. A.; Gomez, J. G.; Vattuone, M. A.; Isla, M. I. Antioxidant activities of Sechium edule swart extracts. *Food Chemi*. **2006**, 97, 452-458.

25. Phillips, S.; Dudik, M.; Schapire, R. Maxent Software, ver. 3.3.3k, 2011.

26. Phillips, S. J.; Dudík, M. Modeling of species distributions with MaxEnt: new extensions and a comprehensive evaluation. *Ecography*. **2008**, 31, 161–175.

27. Phillips, S. J.; Anderson, R. P.; Schapire, R. E. Maximum entropy modeling of species geographic distributions. *Ecol. Modell*. **2006**, 190, 231–259.

28. Pullaiah, T. Medicinal Plants in India; Regency Publication: New Delhi, India, 2002, 1, 267.

29. Raina, R.; Behera, M.C.; Chand, R.; Sharma, Y. Reproductive biology of Gentiana kurroo Royle. *Curr. Sci.* **2003**, 85, 667–670.

30. Raina, R.; Chand, R.; Sharma, Y. Conservations strategies of some important medicinal plants. *Int. J. Med. Aromat. Plants*. **2011**, 1, 342–347.

31. Rana, M. S.; Samant, S.S. Threat Categorization and Conservation of Floristic Diversity in the Indian Himalayan Region: A State of Art Approach from Manali Wildlife Sanctuary. *J. Nat. Cons.* **2010**, 18(1), 159-168.

32. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radi. Biol. Medi.* **1999**, 26, 1231-1237.

33. Samant, S. S.; Joshi, H. C.; Arya, S. C.; Pant, S. Studies on the structure, composition and changes of vegetation in Nanda Devi Biosphere Reserve of west Himalaya. Final Technical Report submitted to the Ministry of Environment and Forests, New Delhi, 2002.

34. Samant, S. S.; Pant, S.; Singh, M.; Lal, M.; Singh, A.;Sharma, A.; Bhandari, S. Diversity, distribution pattern, indigenous uses and conservation prioritization of medicinal plants of Himachal Pradesh, India. *Int. J. Biodiver. Sci. Manag.* **2007**, 3, 234-251.

35. Sarg, M.; El-Domiaty, M.; Salama, O. M.; Bishr, M. M.; El-Gindy, A. R. Pharmacognostical study of the rhizomes and roots of Gentiana kurroo Royle. *Mansoura J. Pharm. Sci.* **1900**, 6, 49–72.

36. Sarg, T.; Salama, O.; Domiaty, M. E.; Bisher, M.; Mansour, E. S.; Weight, E. Iridoid Glucosides from Gentiana kurroo Royle. *Alex. J. Pharm. Sci.* **1991**, 5, 84–86.

37. Schemske, D. W.; Husband, B. C.; Ruckelshaus, M. H.; Goodwillie, C.; Parker, I. M. Bishop, J. G.. Evaluating approaches to the conservation of rare and endangered plants. *Ecol.* 1994, 75, 584-606.

38. Sharma, G. K. Medicinal plants folklore and Ayurvedic system of medicine in the Indo-Tibetan outer Himalayas. *J. Tenn. Acad. Sci.* **2000**, 75, 38–41.

39. Sharma, P. K.; Sethi, G. S.; Sharma, S. K.; Sharma, T. K. Ethnomedicinal observations among the inhabitants of cold desert area of Himachal Pradesh. *Ind. J. Tradit. Knowl.* **2006**, 5, 358–361.

40. Singh, A. Assessment of plant diversity and conservation status of forest vegetation in a proposed cold desert biosphere reserve of the Western Himalaya. Ph. D. Thesis. Kumaun University, Nainital, 2007.

41. Singh, J. S.; Singh, S. P. *Forests of Himalaya: Structure, Functioning and Impact of Man.* Gyanodya Prakashan, 1992, Nainital.

42. Struwe, L.; Albert, A. Gentianaceae Systematics and Natural History; Cambridge University Press: Cambridge, UK, 2002.

43. Tatiya, U.A.; Tapadiya, G. G.; Kotecha, S.; Surana, J. S. Effect of solvents on total phenolic, antioxidant and antimicrobial properties of *Brideliaretusa*Spreng. Stem bark. *Indian J. Nat. Prod. Res.* **2011**, 442-447.

44. Thuiller, W.; Richardson, D. M.; Pyˇsek, P.; Midgley, G. F.; Hughes, G. O.; Rouget, M. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Global Change Biol.* **2005**, 11, 2234–2250.

45. Wani, B. Ramamoorthy D.; Rather, M.A.; Ganai, B.A.; Masood, A.; Zargar, M.A.; Wani, I.H. Headspace solid-phase microextraction (HS-SPME) Gas Chromatography Mass Spectrometric (GC-MS) analysis of the volatile aroma components of Gentiana kurroo Royle. *J. Pharm. Res.* **2011**, 4, 2943–2945.

46. Wayne. 2008. Clinical and Laboratory Standard Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeast, 3rd edition. Approved Standard M27-A3, CLSI.

47. Wolfe, K.; Wu, X.; Liu, R. H. Antioxidant activity of apple peels. *J Agri. Food Chem.* **2003**, 51: 609- 614.

48. Yang, X. Q.; Kushwaha, S.; Saran, S.; Xu, J.; Roy, P. Maxent modeling for predicting the potential distribution of medicinal plant, *Justicia adhatoda* L. in Lesser Himalayan foothills. *Ecol. Eng.* **2013**, 51, 83-87.