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\textsuperscript{2} and range varied from 63.0-110.0 Ind 100m\textsuperscript{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 endemic 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 “Genius”, 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 withculine 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, gentianarim, amaroswerin, and the alkaloid gentianine (Nihio 2006). The roots contain 20\% of a yellow, transparent, and brittle resin (Coventry, 1927; Anonymous, 1956), aucubin, cata-
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-inflamatory, analgesic, anticonvulsant, hypotensive, antipsychotic, sedative, diuretic, antimalarial, anti-amoebic and antibacterial properties and Amaroserin 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 (30° 22’ 40” to 33° 12’ 40” N latitudes and 75° 47’ 55” to 79° 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 quadrats. 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).
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 Gkuroo 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 °C. 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 1423-1821 m 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 name | 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 100m² 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 (AUCₜₑₜₜ = 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-
distributed 38.1%, 35.4% and 24.4%, respectively to the Maxent Model (Table 4). Considering the permutation importance, Temperature 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 Sirmour 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.

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<sup>a</sup> | Total flavonoid content<sup>b</sup> |
|------------|------------------------------------|-----------------------------------|
| Leaf extract| 43±1.5                             | 30±1.3                             |
| Root extract| 86±2.4                             | 51±2.2                             |

Each value is a mean of three biological replicates. 
<sup>a</sup> =mg gallic acid equivalent (GAE)/g DW. 
<sup>b</sup> =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 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<sup>−1</sup>) of the extracts were tested. IC<sub>50</sub> value is the concentration of the extract required to scavenge the free radicals to 50% of the control. IC<sub>50</sub> values were negatively correlated with antioxidant activity, the lower the IC<sub>50</sub> 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<sub>50</sub> value 119.82 µg mL<sup>−1</sup> (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                  | 29.34 | 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 a 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 methanolic 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 leaf 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 dielectric 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 extract](image)

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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*.

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