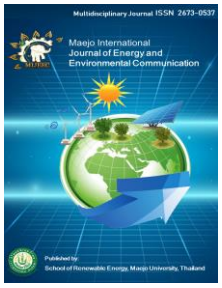
Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Study on phytochemical, antibacterial and antioxidant properties of *Genitiana kurroo* Royle

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ARTICLEINFO

Article history:
Received 27 February 2022
Received in revised form
19 March 2022
Accepted 27 March 2022

Keywords:
Genitiana kurro Royle
Endangered species
Phytochemical screening
Antimicrobial activity
Antioxidant activity

ABSTRACT

Gentiana kurroo Royle is an endangered bitter medicinal plant of Indian subcontinent region. This medicinal plant mostly grows in Kashmir, Himachal Pradesh and North-west Himalayas. The medicinal plant's root and rhizome are frequently used by native people for various local remedies. The leaf and root samples of the plant were studied for its phytochemicals screening, antibacterial and antioxidant properties. The methanolic root extract as comparison to methanolic leaf extract was detected with high concentration of phytochemicals like alkaloid, flavonoids, saponins, phenol, glycosides whereas, other phytochemicals like carbohydrates, tannins, terpenoids showed equal concentration in methanolic root and leaf extracts. In antibacterial study, the methanolic root extract was found to exhibit maximum zone of inhibition (33± 1) against *E. fecalis* and minimum zone (17 ± 2) against *E. coli*. The methanolic leaf extract showed maximum zone of inhibition (31 ± 1) against *E. fecalis* and minimum zone (18 ± 2) against *K. pneumoniae*. The antioxidant activity of *G. kurroo* revealed that the methanolic extracts of root as compared to the methanolic extract of leaves showed comparatively high antioxidant activity and this is due to the presence of high phenol and flavonoid content.

1. Introduction

A bitter plant native to the Indian subcontinent named *Gentiana kurroo* Royle is an endangered species among florae. In the North-West Himalayas, in Kashmir and Himachal Pradesh, it grows between 1500 and 3000 meters above sea level (Behera and Raina, 2011). Despite being called "Neilkanth" in Kashmir Himalayan, it is most known as "Karu" in Hindi, which means bitter, and in Sanskrit it is called by the name "Traayamaana". It is a member of the Gentianaceae family, which includes about 300 species of flowering plants worldwide. This plant is named "*Genitiana kurroo"*, which comes from "Genitus," a monarch of Illyria (Europe), as it is believed that the therapeutic properties of *Genitiana kurroo* were discovered by him (Jain, 1968). The perennial herbs category includes *Genitiana kurroo*. Many

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pharmaceutical companies are heavily dependent on natural herbs that have medicinal properties, which are causing their extinction. As a result, this medicinal herb is classified as endangered by the Red Data Book of Indian Plants, and because of its potent therapeutic qualities, this herb is often referred to as "Ram Vaan" (Sharma et al., 2000). It is found in dry, rocky grasslands (shown in Figure 1) and on south-facing slopes of scrub habitats between 1500 and 3000 meters, as well as in the north-western Himalayas, where it is endemic and seriously threatened. More than 80% of the population has decreased over the past ten years.

Figure 1 *G. Kurroo* Royle collected from Village Shaglahan, District: Sirmour, Himachal Pradesh

G. kurroo Royle has been found to have excellent medicinal properties by several researchers. It is highly considered that the rootstock of *G. kurroo* contains the most well-known bitter compounds, such as amaroswerin and gentian (Sharma et al., 2000). This plant species has analgesic, anti-malarial, diuretic, antibacterial, anti-inflammatory, anticonvulsant, and gastro-protective amaroswerin properties (Palanisamy et al., 2022). Rhizomes are classified in the Indian medical system as blood purifiers, anti-inflammatory medications, bitter tonics, anti-periodic, sputum and antipsychotics (Chang et al., 2002). Additionally, it is used as a medication to treat bronchial asthma, indigestion, skin conditions, vitiligo, leprosy, urinary tract infections, anorexia, helminthosis, constipation, inflammations, and hemorrhoids (Gilani et al., 2006). It plays a significant role in the formulation of numerous stomach tonics (Sharma, 2008). Oil is combined with Neilkanthi leaf powder in folk medicine, and it is applied topically to treat fungal infections and ulcers. The medication helps to treat anorexia, improves the digestive system, and helps get rid of all forms of physical weakness and fatigue brought on by long-term disease (Sharma, 2008). The plant is used in the Amchi medical system to treat fever, hepatic conditions, and cough (Sharma et al., 2006; Palanisamy et al., 2021).

The medicinal plant's roots and rhizomes are extensively extracted and are listed in the Indian pharmacy codex. Due to its small geographic range, extensive exploitation of its natural habitats (Palanisamy et al., 2022), lack of cultivation, and other factors, this species is at a serious risk of going extinct. According to the Indian Red Data Book, this species is critically endangered; hence it must be protected (Red Data Book,2015). Due to extensive extraction from its natural habitat, this herb is in danger of going extinct. Therefore, as indicated in Notice No. 2 (RE-98), dated April 13, 1998, covering the years 1997 to 2002, it has been added to the exports' negative list by the Ministry of Commerce, Government of India (Data,1998). The method of cultivating this species is unknown, and it is now designated as endangered by the IUCN on a national level (IUCN, 2015). The lower elevations of the Mandi, Sirmour, Solan, and Shimla districts of Himachal Pradesh in the North-Western biogeography provinces of the Indian Himalaya were discovered to contain potential habitats with high suitability thresholds. In primary field investigations, potential habitats were typically discovered in Himachal Pradesh oak and pine woods as well as dry grasslands (Lal et al., 2019). This species of plant is found in a few isolated pockets in District Sirmour, Himachal Pradesh at an altitudinal range of 1552 m above sea level, and the sample for the present study is taken from the village situated in District Sirmour. In Figure 2, the natural habitat or natural niche has been shown that is desirable for the natural distribution of this plant all over the state. It is so surprising to know that this species of plant is only present in a narrow range of distribution in the world, which is in Himachal Pradesh, India, and this narrow range of habitat makes this plant more vulnerable to extinction.

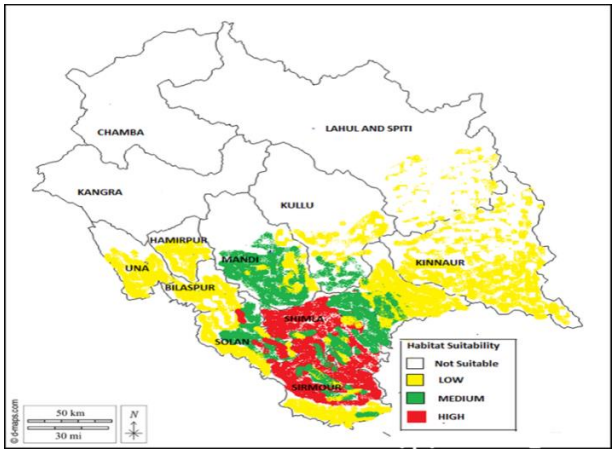

Figure 2 Habitat, suitability, and distribution of *Genitiana kurroo Royle* in Himachal Pradesh

2.0 Materials and methods

2.1 Study Area

The study has been carried out in Village Shaglahan, Mangarh Hills, Dist. Sirmour, Himachal Pradesh, India.

2.2 Plant and microbe's collection

The fresh and healthy root and leaf samples of the species were collected from a plant in the starting month of spring season (April 2022). The bacterial cultures used to check antimicrobial, and antioxidant activities were procured from the Shoolini Institute of Life Science and Business Management, Solan, Himachal Pradesh. All the cultures were sub-cultured and stored on agar plates for further experimentation (Palanisamy et al., 2022b). Table 1 represents the employed microbes with corresponding positive control (antibiotics) in the present study

Table 1 Used microbes with corresponding positive control (antibiotics) in the present study

Microorganism	Antibiotics used
Escherichia coli	Ciprofloxacin
Klebsiella pneumoniae	Ciprofloxacin
Salmonella typhi	Ciprofloxacin
Staphylococcus aureus	Ciprofloxacin
Pseudomonas aeruginosa	Ciprofloxacin
Enterococcus faecalis	Ciprofloxacin

2.3 Preparation of Plant Extract

Collected roots and leaves of the plant were washed thoroughly with distilled water. The roots and leaves were dried under shade at room temperature. Dried roots and leaves of *G. kurroo* were finely grounded into a powder using an electrical grinder and stored in airtight containers at 4–8°C for further use (Kumar et al., 2017).

2.3.1 Preparation of root extract

In this study, two type of plant extract were prepared: aqueous and methanolic (Mudzengi et al., 2017). Figure 3 represents the process of making plant extracts by making powder of plant parts (a) preparation from dried dried roots (b) preparation from dried leaf extract.

2.3.2 Preparation of aqueous and methanolic extract

The extract was prepared by mixing 10g of powdered leaves and roots with 100ml of distilled water and methanol respectively in a round-bottom conical flask for 24 hours on a rotator shaker at a temperature of 25°C and 124rpm. Then extracts were filtered through Whatmann filter paper No.1. The homogenate was then centrifuged at 5000rpm for 10 minutes and the supernatant was collected in airtight containers, kept at 4°C for further use.

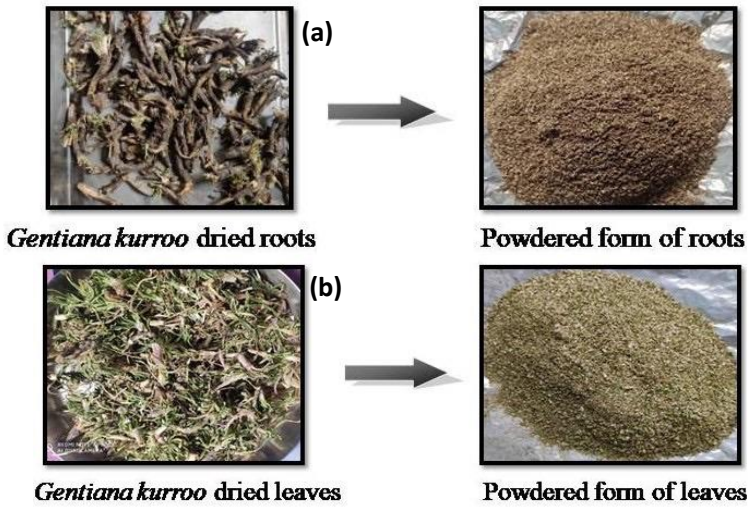

Figure 3 Process of making plant extracts by making powder of plant parts (a) preparation from dried dried roots (b) preparation from dried leaf extract

2.4 Phytochemical Screening

The methanolic extracts of leaves and root were tested for the presence of alkaloids, carbohydrates, reducing sugar, flavonoids, phenolic compounds, cardiac glycosides, saponins, terpenoids and tannins (Shaikh et al., 2020; Bhuyar et al., 2020a, b).

2.4.1 Assays for Antimicrobial Activity

Antimicrobial activity was evaluated by agar well diffusion method (Chaman et al., 2013; Ramli et al., 2020, 2021). The strains that had been incubated for 24hr were used for the assay. A sterile cotton swab was dipped into the bacterial suspension and then evenly spread over the entire surface of a sterile Mueller Hinton agar plate to obtain a uniform inoculum. Wells were punched on the seeded plates using a sterile borer (8 mm), and the plates were allowed to dry for 15 min. About 50 µl methanolic and aqueous extracts were dispensed into each well using a sterile micropipette. Methanol and distilled water were used as negative controls and ciprofloxacin was used as a positive control. The plates were incubated overnight at 37°C and the antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm).

2.4.2 Assay for Antioxidant Activity

The antioxidant activity of the plant extracts and standard was assessed based on the radical scavenging effect of the stable 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 20µl to 100µl. A 0.004% DPPH solution in methanol was prepared. Then 1ml of this solution was mixed with 3ml of sample solutions (ranging from 20µg/ml to 100µg/ml) and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min, and the optical density was measured at 517 nm using a spectrophotometer against methanol. The control used was 3ml of methanol with 1ml of DPPH solution (0.004%) (Kumar et al., 2017; Bhuyar et al., 2021a, b). The DPPH radical scavenging ability of the sample was determined according to the following equation:

$$\textbf{\textit{DPPH scavenging effect}}\ (\%) = \frac{\textbf{\textit{Control OD}} - \textbf{\textit{Sample OD}}}{\textbf{\textit{Control OD}}} \times \mathbf{100}$$

Equ. (1): DPPH Scavenging effect percentage formula

2.4.3. IC50 Value (Half-maximal inhibitory concentration)

The IC50 value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, the lower the IC50 value, the higher the antioxidant activity (Nahak and Sahu, 2010; Wongsa et al., 2022). The IC50 value was calculated by using the equation 1:

$$Y=MX+C \quad (1)$$

Where y = 50% of the maximum inhibition,
X = the concentration at which 50% oxidation inhibition can be achieved.

The "M and C" values are generated by the graph plotted between concentration and % scavenging activity.

Then X was calculated by using the equation.

$$X = Y-C/M. \quad (2)$$

3.0 Result and discussion

3.1 Phytochemical screening of methanolic leaf and root extract

Among the phytochemicals of *G. kurroo* reveals higher concentration of some phytochemicals in methanolic root extracts as comparison to methanolic leaf extracts like alkaloid, flavonoids, saponins, phenol, glycosides and other phytochemicals like carbohydrates, tannins, terpenoids showed equal concentration in methanolic root and leaf extracts shown in Table 2. The phytochemical screening was also reported previously, and it showed strong presence of flavonoids, tannins, phenolics, cardiac glycosides and terpenes and moderate presence of carbohydrates, alkaloid and saponins (Skinder et al., 2017).

Table 2 Phytochemical screening of methanolic leaf and root extract

Phytoconstituents	Methanolic Root	Methanolic Leaf
Alkaloids	++	+
Carbohydrates	+	+
Flavonoids	++	+
Saponins	++	+
Tannins	+	+
Phenol	++	-
Glycosides	++	+
Terpenoids	+	-

3.2 Antimicrobial Activity

The antibacterial activities of the extracts of the leaves of *G. kurroo* plant obtained with 4 different solvents (methanolic leaf, methanolic root, aqueous leaf, and aqueous root) were evaluated by the agar well diffusion method.

3.2.1 Antimicrobial activity of methanolic leaf and root extract.

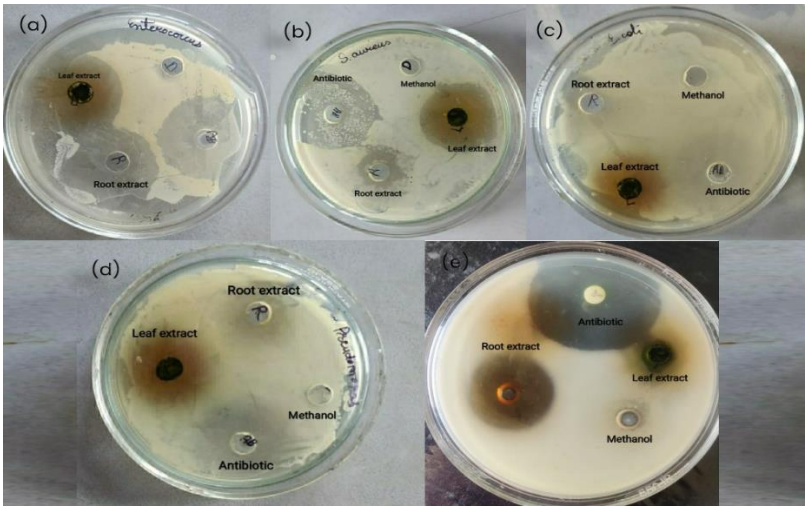

Figure 4 Antimicrobial activity of methanolic leaf and root extract against Gram positive bacteria(a) *Enteroccocus faecalis*(b) *Staphyloccocus aureus* and Gram-negative bacteria(c) *Klebsilla pneumoniae (d)Escherichia coli (e) Pseudomonas aeruginosa.*

Table 3 Mean zone of inhibition of methanolic root and leaf extract

Microorganism	Leaf Extract(mm)	Root Extract(mm)	Antibiotic (Ciprofloxacin)	Methanol (Negative Control)

Enteroccocus	31 ± 1	33 ± 1	27.66 ± 1.52	-
Staphyloccocus	26 ± 1	19 ± 1	25 ± 1	-
Klebsilla	18 ± 2	21.33 ± 2.081	33 ± 1	-
Escherichia coli	21 ± 2	17 ± 2	20.33 ± 1.52	-
Pseudomonas	22 ± 1	18.33 ± 1.52	20.33 ± 2.51	-

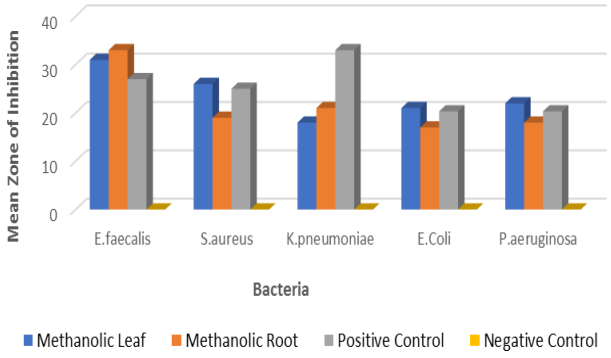

Figure 5 Mean zone of inhibition of methanolic leaf and root extract

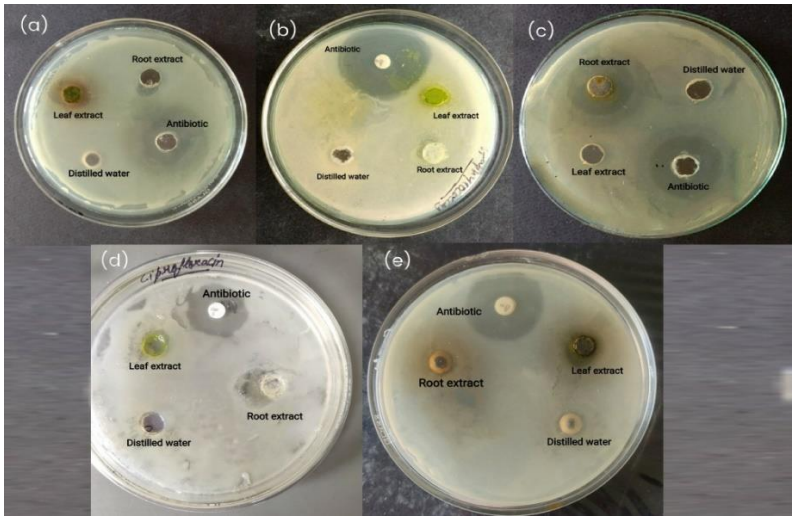

Figure 6 Antimicrobial activity of aqueous leaf and root extract against gram positive bacteria (a) *Enteroccocus faecalis (b) Staphyloccocus aureus*) and gram-negative bacteria (c) *Escherichia coli (d) Pseudomonas aeruginosa (e) Klebsilla pneumoniae*

Table 4 Mean Zone of Inhibition of Aqueous Extracts

Microorganism	Leaf Extract(mm)	Root Extract(mm)	Antibiotic (Ciprofloxacin)	Methanol (Negative Control)
E. faecalis	15 ± 1	15 ± 1	20 ± 1	-
S. aureus	16 ± 1	14.5 ± 0.70	25.33 ± 0.57	-
K. pneumoniae	15.5 ± 0.70	14.5 ± 0.70	21.33 ± 2.081	-
E. coli	16.66 ± 0.57	14.33 ± 0.57	24.33 ± 1.52	-
P. aeruginosa	15.66 ± 1.52	15.66 ± 1.52	20 ± 1	-

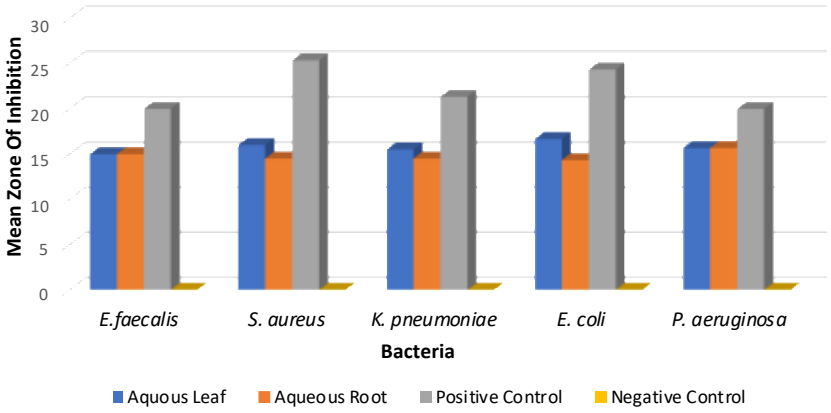

Figure 7 Mean zone of inhibition of Aqueous extracts

3.2 Antimicrobial activity of aqueous leaf and root extract

In the present study the antibacterial activity of methanolic and aqueous extract of leaves and roots of *G. kurroo* was investigated against gram positive (*E. fecalis* , *S. aureus*) and gram negative (*E. coli, K. pneumoniae, S. typhi*) bacterial species. Methanolic root and leaf extract showed more zones of inhibition in comparison to aqueous leaf and root extract against gram positive bacteria than gram negative bacteria given in Figure 4, Table 3 and Figure 5. The methanolic root extract exhibit maximum zone of inhibition (33 ± 1) against *E. fecalis* and minimum zone (17 ± 2) against *E. coli*. The methanolic leaf extract showed maximum zone of inhibition (31 ± 1) against *E. fecalis* and minimum zone (18 ± 2) against *K. pneumoniae.* Aqueous Extract showed much less antibacterial activity as shown in Table 4, Figure 6 and 7. The roots and leaves extracts of *G. kurroo* possessed relatively higher antibacterial activity against Gram positive bacteria than the Gram-negative bacteria (Skinder et al., 2017). The antibacterial activity of root extracts was found to be comparatively higher than that of leaf extracts. The possible reason for antibacterial activity is due to high content of flavonoids, involved in the inhibition of nucleic acid biosynthesis and metabolic processes.

3.3 DPPH scavenging activity of Ascorbic acid, Methanolic root and leaf extract

From the present study, antioxidant activity of *G. kurroo* reveals that the methanolic extract of root as compared to the methanolic extract of leaves showed comparatively high antioxidant activity. The different concentration of methanolic leaves and root extract were taken in the same amount (ul) as shown in Table 5 & Figure 8. O.D. was taken at 517nm and IC50 value of methanolic root and leaf was calculated as shown in Figure 9. The possible reason in the difference of the antioxidant activity is due to the total flavonoid and phenolic content of the two extracts. According to numerous research, *G. kurroo Royle* has extraordinary therapeutic qualities. In folkloric medicine, *G. kurroo* (Neilkanth) leaf powder is combined with oil and applied on ulcers and fungus infections (Gilani et al., 2006). However, the root of *G. kurroo* is used to treat stomachaches and urinary infections (Sharma, 2000) and the root mixed with ginger root powder is also used to treat high fevers (Kirtikar and Basu, 1935). Some traditional doctors use the whole plant against cough, fever, headache, liver ailments and as a blood purifier. Fresh leaves and root of *Genitiana kurroo* were collected in the month of April from Village Shaglahan Dist. Sirmour, Himachal Pradesh, India. *G. kurroo's* methanolic extracts of root as compared to the methanolic extract of leaves showed comparatively high antioxidant activities shown in Figure 11.

The different concentration of methanolic leaves and root extract were taken in the same amount (ul) as shown in Table 4 and Table 5 and O.D. was taken at 517nm.The possible reason in the difference of the antioxidant activity is due to the total flavonoid and phenolic content of the two extracts. The same study has been conducted by Skinder et al.,2017 and the result showed that methanolic extract of root had higher DPPH scavenging activity than methanolic extract of leaf. 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. 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 defenses (Lal et al., 2017).

Table 5 DPPH scavenging activity of Ascorbic acid, Methanolic root and leaf extract (517nm)

S.no.	Concentration of Ascorbic acid, Methanolic Leaf and Root(µl)	DPPH scavenging activity of Ascorbic acid %	DPPH scavenging activity of Methanolic Root %	DPPH scavenging activity % of Methanolic Leaf %
1.	20	86.6%	30.9%	15.2%

2.	40	88.0%	34%	26.2%
3.	60	89.9%	42.5%	31.9%
4.	80	90.7%	54.1%	41.4%
5.	100	91.9%	69.6%	61%

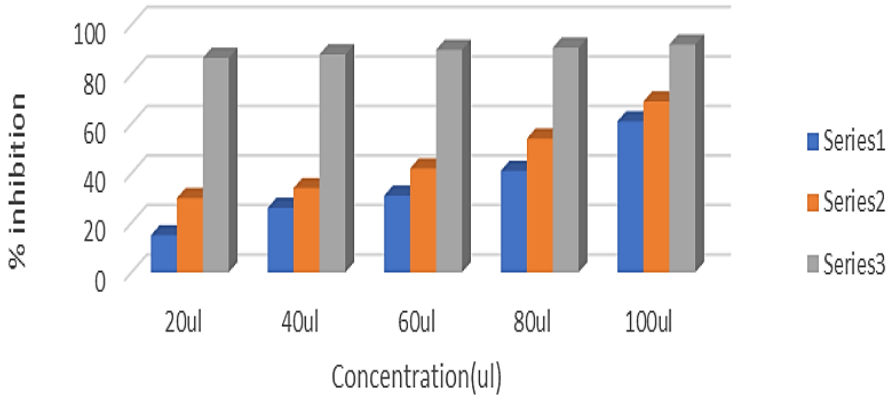

Figure 8 Free radical scavenging activity of methanolic leaf and root extract

3.2.2 IC_{50} Value (Half-maximal inhibitory concentration)

The IC50 Value of methanolic root observed is 68.86ug/ml and IC50 Value of methanolic leaf calculated is 87.8277ug/ml, which indicates the better scavenging effect of roots of the plant. With an IC50 value of 66.03 g mL^{-1} of water extracts, leaves had the greatest radical scavenging efficacy. While among the root extract, methanolic had the greatest level with an IC50 value of 56.56 g mL^{-1} for its radical scavenging effect (Lal et al., 2019).

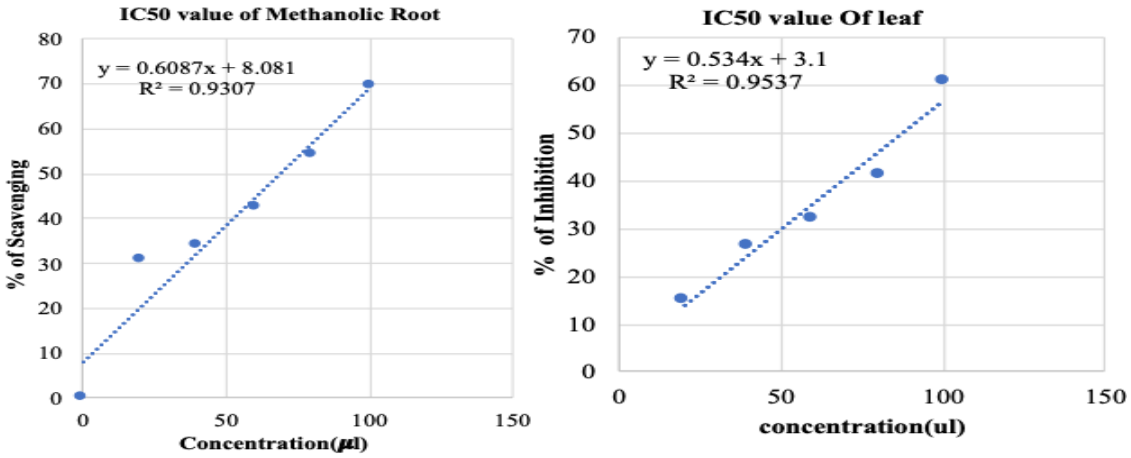

Figure 9 IC50 value of Methanolic Root

4.0 Conclusion

This study evaluated phytochemical screening, antimicrobial activity, and antioxidant activity of *G. kurroo* royle. This medicinal plant samples were collected from village shaglahan, distt. Sirmour, Himachal Pradesh. The phytochemical screening revealed that *G. kurroo's* methanolic root extract contains higher concentration of phytochemical as compared to methanolic leaf extract. The extracts of roots and leaves of *G. kurroo* possessed relatively higher antibacterial activity against Gram positive bacteria than the Gram-negative bacteria, while the antioxidant activity of *G. kurroo* revealed that the methanolic extracts of root as compare to the methanolic extract of leaves showed comparatively high antioxidant activity and this is due to the presence of high phenol and flavonoid content in methanoic root. The results suggested that *G. kurroo* is a potential source of

antibacterial and antioxidant molecules. The leaves and roots of the plant can be used as natural antioxidants and preservatives in food and non-food systems.

Acknowledgment

All authors are thankful to the Chairperson, Shoolini Institute of Life Sciences and Business Management, Solan for providing the necessary facilities.

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