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Exploration of Leaves of *Picrorhiza kurroa* for their Medicinal Potential by Phytochemical Analysis and Antibacterial Activity

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ABSTRACT

Picrorhiza kurroa Royle ex Benth is a high-altitude medicinal herb belonging to the family Scrophulariaceae, commonly called Kutki. It secretes various active constituents which impart pharmaceutical value to the plant. The roots and rhizomes of this plant are used for their health benefits since ancient times. Looking at the endangered status of the plant species, the present study was conducted on the leaves of *Picrorhiza kurroa*. The leaf samples were collected from two sites, Pothivasa (2200 masl) and Tungnath (3600 masl), Rudraprayag, Uttarakhand India. The leaf extracts were prepared using different solvents like petroleum ether, chloroform, acetone, ethanol and methanol and screened for phytochemicals. Quantitative estimation of a few phytochemicals found in the leaf extracts of *P. kurroa* from Pothivasa (PVLE) and Tungnath (TNLE) was performed. The leaf extracts were further tested for their antibacterial potential. The qualitative screening of all the leaf extracts showed that the maximum number of phytochemicals were present in the methanolic leaf extract of *P. kurroa* from Pothivasa (Met-PVLE) and the methanolic leaf extract of *P. kurroa* from Tungnath (Met- TNLE). The quantitative analysis showed that the alkaloid content was 20.23±1.2% in PVLE and 23.73±1.69% in TNLE. The flavonoid content was 8.54±0.43% in PVLE and 17.43±1.72% in TNLE, and Terpenoid content was 18.6±1.02% and 6.1±0.26% in PVLE and TNLE samples, respectively. The saponin content was 12.4±0.05% in PVLE and 16.6±0.45% in TNLE. The total phenolic content (Gallic acid equivalent mg/g) was measured to be 45.99±2.19 mg/g in PVLE and 80.77±2.65 mg/g in TNLE. This study confirms that the leaves of *Picrorhiza kurroa* contain many vital phytochemicals, which implies that along with the roots and rhizomes, leaves of *P. kurroa* also impart medicinal value to this plant. Met-PVLE and Met-TNLE showed significant antibacterial activity against *Escherichia coli, Enterobacter* sp., *Acinetobacter* sp., and *Pseudomonas* sp. Further studies can be performed to identify and isolate bioactive compounds from leaf samples of *P. kurroa.*

Keywords *Picrorhiza kurroa,* Leaf sample, Medicinal herb, Phytochemicals, Antibacterial activity.

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INTRODUCTION

The Himalayas are the abode of a myriad of flora and fauna. Various plants grown here have prodigious medicinal and other valuable properties. The Garhwal Himalayas sustain the growth of a plethora of valuable plants (Dhar *et al.* 2000). These plants synthesize secondary metabolites, thus, inferring therapeutic value. Moreover, they exhibit great potential for containing novel biomolecules and other active components (Borokini and Omotayo 2012).

Phytochemicals are constitutive metabolites produced by plants and are important for their survival and proper functioning. Based on their role in plant metabolism, phytochemicals are divided into primary and secondary metabolites. Carbohydrates, amino acids, proteins, lipids, purines, and pyrimidines of nucleic acids are examples of primary metabolites that are essential for plant life. Contrarily, secondary metabolites are the final plant chemicals produced by cells through metabolic pathways that are derived from the fundamental metabolic pathways (Bone and Mills 2013, Hussein and El-Anssary 2019). They help defend plants from competition, infections, and predators. Plants have phytochemicals in their stems, leaves, roots, seeds, fruits, and flowers, among other plant sections. However, the outer layers of plant tissues contain significant amounts of numerous phytochemicals, particularly color pigments. These phytochemicals, alone or in combination, have a remarkable curative property to treat various diseases. Previous investigations have reported that phytochemicals lead to a reduction in the risk of some diseases such as coronary heart diseases, diabetes, inflammation, microbial, parasitic, and viral infections, osteoporosis, psychotic conditions, spasms, ulcers, liver disorders, high blood pressure, as well as reducing the synthesis or absorption of cholesterol (Prakash and Kumar 2011, Saxena *et al.* 2013).

Picrorhiza kurroa Royle (Kutki) is a momentous Himalayan medicinal herb that belongs to the family Scrophulariaceae, distributed in the sub-alpine and alpine regions of the Northwestern Himalayas between 2700- 4500 m asl (Bhattacharjee *et al.* 2013). It is distributed in the Western Himalayas in Jammu and Kashmir, Himachal Pradesh, and Uttarakhand (Kaul and Kaul 1996). Its odor is unpleasant, and its taste is bitter. It secretes many active constituents like apocynin, cucurbitacins, drosin, kutkosides, and picrosides I, II, III, V, which infer medicinal properties to the plant (Sah and Varshney 2013, Krupashree *et al.* 2014). It helps cure various diseases such as abdominal pain, stomach disorders, anaemia, jaundice, inflammation, and leishmaniasis. It is also used as a blood purifier, antitumor, anthelminthic, cardiotonic, carminative, expectorant (Debnath *et al.* 2020).

The present study was performed to identify the bioactive compounds found in the leaves of *Picrorhiza kurroa* collected from high altitudes in the Garhwal Himalayas, Uttarakhand. Qualitative and quantitative analysis of the phytochemicals as well as their anti-microbial potential, was evaluated. Previously, the roots and rhizomes of *Picrorhiza kurroa* were widely investigated and employed to achieve various health benefits. However, it is an endangered plant, and continuous use of roots and rhizomes may harm its existence. Thus, this study was designed to study phytochemical composition and assess the medicinal potential of the leaves of this plant. The use of the leaves of this plant will not only prevent the over-exploitation of the plant but will also enhance the overall utility of the plant for the pharma industry. Besides, the current study was performed on two populations of *P. kurroa* from different altitudes. This study will help us to understand the effect of altitudes on the medicinal potential and plants' response to stress conditions at higher altitudes.

MATERIALS AND METHODS

Sampling

The biological replicates of the leaf sample of *Picrorhiza kurroa* plants were collected from HAPPRC (High Altitude Plant Physiology Research Center), HNBGU located at Pothivasa (30°28′N lat; 79°16′E long, 2200 masl) and Tungnath (30°14′N lat; 79°13′E long, 3600 masl) in Rudraprayag district, Uttarakhand, India (Fig. 1). The temperature observed in Pothivasa at the time of sampling was 13°C, and at Tungnath was 4°C, and the altitudinal difference was about 1400 m.

Fig. 1. *Picrorhiza kurroa* growing in Pothivasa.

Sample preparation

Leaf samples from both the study locations were washed, air-dried, and pulverized for further experimental purposes. The extracts were prepared from different solvents in an increasing polarity order, viz., Petroleum ether, Chloroform, Acetone, Ethanol, and Methanol. For this, 10 g crushed leaves were dissolved in 50 ml solvent and were kept in an orbital shaker at 50 rpm for 24 hrs. Whatmann's filter paper No. 41 was used to filter the extract. The filtrate obtained was used as the extract and kept at 4°C until future usage.

Phytochemical screening

Preliminary qualitative screening of all the leaf extracts of *P. kurroa* from Pothivasa (PVLE) and Tungnath (TNLE) was done to detect the presence of different bioactive compounds viz., alkaloids, flavonoids, anthocyanins, saponins, steroids, tannins, terpenoids, fatty acids, anthocyanins, leucoanthocyanins, coumarins, phenol, xanthoproteins, quinones, oxylate, glycosides, carboxylic acids, proteins, phlobatannins, resins, and carbohydrates. The screening was performed in accordance with the methods described by Banu and Catherine (2015) with minor modifications.

Quantitative determination of phytochemicals

Alkaloids Alkaloids were determined as per the method given by Harborne (1973), using acetic acid and concentrated ammonium hydroxide, and the percentage of alkaloids was expressed mathematically as-

$$\% \text{ Alkaloid} = (\text{Weight of alkaloid}/\text{Weight of sample}) \times 100$$

Flavonoids: The protocol by Boham and Kocipai (1974) was used to determine the concentration of flavonoids. At room temperature, a leaf sample was extracted using 80% aqueous methanol, and the filtrate was allowed to dry out over a water bath and then weighed. The percentage of flavonoid was calculated as:

$$\% \text{ Flavonoid} = (\text{Weight of flavonoid}/\text{Weight of sample}) \times 100$$

Terpenoids : Total Terpenoids content was measured by following the method by Ferguson (1956). Ethanolic leaf extract was prepared and then it was extracted with 10 mL petroleum ether. Total terpenoids were measured, and the percentage of total terpenoid content was expressed as:

$$\% \text{ Terpenoid} = \{(\text{Final weight of sample} - \text{Initial weight of sample})/\text{Weight of sample}\} \times 100$$

Saponin : Saponin determination was performed according to the Obadoni and Ochuko (2001) method. In this, ethanolic leaf extract was purified using diethyl ether, and to the aqueous layer obtained, n- butanol was added and washed with 5% aq. Sodium chloride. The remaining solution was evaporated, dried and weighed. The resultant product is the saponin which is calculated as:

$$\% \text{ Saponin} = (\text{Weight of saponin}/\text{Weight of sample}) \times 100$$

Total phenols : Total phenolic content was determined using spectrophotometer according to the protocol given by Saeidnia *et al.* (2011). To the methanolic leaves extract and gallic acid standard solutions, 1 ml Folin Ciocalteu reagent and 0.8 ml Sodium carbonate were added, and the total volume was made up to 10 ml using distilled water and were incubated at room temperature for 30 minutes. The absorbance was taken at 725 nm, and the total phenol concentration was calculated using the standard curve.

Anti-bacterial activity

Anti-bacterial activity of methanolic extract of

leaves of *P. kurroa* from Pothivasa (Met-PVLE) and Tungnath (Met-TNLE) was performed by the Agar well diffusion method. First, 20 ml of nutrient agar was poured into autoclaved petri plates, and different clinical microbial strains, i.e., *Escherichia coli, Staphylococcus* sp., *Enterobacter* sp., *Acinetobacter* sp., and *Pseudomonas* sp., were inoculated using the spread plate method. Then, 50 µl each of negative control, Met-PVLE and Met-TNLE was poured into the wells made in the nutrient agar plate and incubated at 37°C for 24 hrs, and the zone of inhibition was measured. Tetracycline 30 mcg discs were used as the positive control, and Dimethyl Sulfoxide (DMSO) as the negative control.

Statistical analysis

All the experiments were performed in triplicate and expressed as mean standard deviation. The linear regression coefficient (R^2) for the quantification of phenolic content was analyzed by Microsoft Office Professional Plus 16.

RESULTS AND DISCUSSION

Phytochemical screening

Picrorhiza kurroa is a well-known herb with a rich history of use in the Ayurvedic medicine system. Phytochemicals are bioactive compounds which exhibit several therapeutic properties. The preliminary screening of phytochemicals in the leaves of *Picrorhiza kurroa* was done to investigate the presence of different important phytochemicals in the extracts prepared from solvents of different polarities. The screening showed a positive correlation with the polarity, i.e., the more the polarity of the extraction solvent, the more the number of phytochemicals present in the solution. The maximum phytochemicals were found in Methanol, followed by Ethanol, then Acetone, Chloroform, and Petroleum ether. The phytochemical screening showed that among all the extracts, methanolic extract of leaves of *Picrorhiza kurroa* had maximum phytochemicals and leaves extract prepared from petroleum ether had minimum phytochemicals as shown in Tables 1-2.

The study by Rathee *et al.* (2016) on the methanolic extract of rhizomes of *Picrorhiza kurroa* showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins/ amino acids, unsaturated sterols/triterpenes, phenolic compounds, polyphenols, saponins, and tannins. Many other researches have also reported the presence of these phytochemicals

Table 1. Phytochemical screening of *Picrorhiza kurroa* leaves' extract from samples from Pothivasa (PVLE) ('++' represents very significantly present, '+' represents present, '-'represents Absent).

Sl. No.	Phytochemical	Petroleum ether	Chloroform	Acetone	Ethanol	Methanol
1	Alkaloids	+	-	+	+	+
2	Flavonoids	-	-	-	+	+
3	Saponin	++	+	-	++	-
4	Steroids	-	+	+	++	+
5	Tannins	-	-	-	-	-
6	Terpenoids	-	-	-	+	++
7	Fatty acids	-	-	-	-	+
8	Anthocyanins	-	+	+	-	+
9	Leucoanthocyanins	+	++	+	+	-
10	Coumarins	-	+	-	++	-
11	Phenol	-	-	+	-	++
12	Xanthoprotein	-	-	++	+	++
13	Quinone	-	+	+	+	+
14	Oxylate	-	-	-	++	+
15	Glycoside	-	-	-	++	+
16	Carboxylic acid	-	+	-	+	+
17	Protein	-	-	+	+	+
18	Phlobatannin	-	-	-	-	-
19	Resin	-	+	++	-	+
20	Carbohydrates	-	-	-	+	+

Table 2. Phytochemical screening of *Picrorhiza kurroa* leaves' extract from samples from Tungnath (TNLE) ('++' represents very significantly present, '+' represents present, '-'represents absent).

Sl. No.	Phytochemical	Petroleum ether	Chloroform	Acetone	Ethanol	Methanol
1	Alkaloids	+	-	+	+	+
2	Flavonoids	-	-	+	+	+
3	Saponin	++	++	-	+	-
4	Steroids	-	+	+	++	+
5	Tannins	-	-	-	-	-
6	Terpenoids	-	-	+	++	++
7	Fatty acids	-	-	-	-	+
8	Anthocyanins	-	++	+	-	+
9	Leucoanthocyanins	+	+	+	++	-
10	Coumarins	-	-	+	++	-
11	Phenol	-	-	++	+	++
12	Xanthoprotein	-	-	++	-	++
13	Quinone	-	+	+	-	+
14	Oxylate	-	-	-	++	+
15	Glycoside	-	-	-	++	+
16	Carboxylic acid	-	+	-	+	+
17	Protein	-	-	+	+	+
18	Phlobatannin	-	-	-	-	-
19	Resin	-	+	+	-	+
20	Carbohydrates	-	-	-	+	+

(Deb *et al.* 2018, Sharma *et al.* 2018, Thakur *et al.* 2018). Further, the presence of these phytochemicals in the plants of the family Scrophulariaceace was confirmed by the study on the leaves of *Buddleja asiatica* (Nafees *et al.* 2021). Current study in the leaves of *P. kurroa* also confirms the presence of various phytochemicals like alkaloids, flavonoids, terpenoids, phenols.

Phytochemical quantification

Based on the findings by phytochemical screening, a few important phytochemicals, viz., alkaloids, flavonoids, terpenoids, saponins and phenols, were quantified. The total alkaloid content in the Pothivasa leaves' sample (PVLE) was found to be 20.23±1.2%, and that in the Tungnath leaves' sample (TNLE) was found to be 23.73±1.69%. The alkaloids in the plant signify its pharmaceutical role as an analgesic, anaesthetic, anticancer, antifungal, anti-inflammatory, antimicrobial, neuropharmacological and other activities. The flavonoid content was investigated to be 8.54±0.43% and 17.43±1.72% in PVLE and TNLE, respectively. Flavonoids have anti-allergic, anti-inflammatory, antimicrobial, antioxidant, vascular, estrogenic, and cytotoxic antitumor properties (Harborne and Williams 2000). Total terpenoid content found in the leaf samples of both the sites, Pothivasa and Tungnath, is 18.6±1.02% and 6.1±0.26%, respectively. Terpenoids are credited for analgesic and anti-inflammatory activities. Steroids in the plant possess potent anti-inflammatory effects (Podolak *et al*. 2010, Hussain *et al*. 2019).The saponin content measured was 12.4±0.05% in PVLE and 16.6±0.45% in TNLE. The above results are represented in Fig. 2. Saponin has antibacterial, anticancer, antifungal, anti-inflammatory, antiparasitic and antiviral activities (Podolak

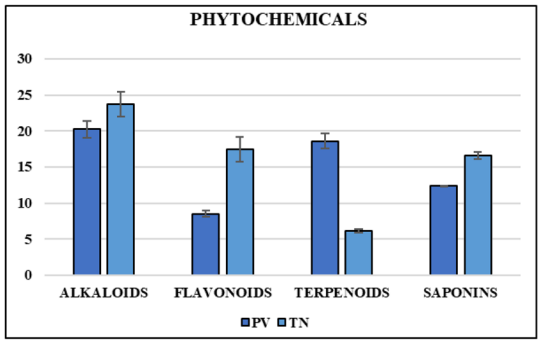

Fig. 2. Phytochemical quantification of *P. kurroa* leaves' extract from samples collected from Pothivasa and Tungnath.

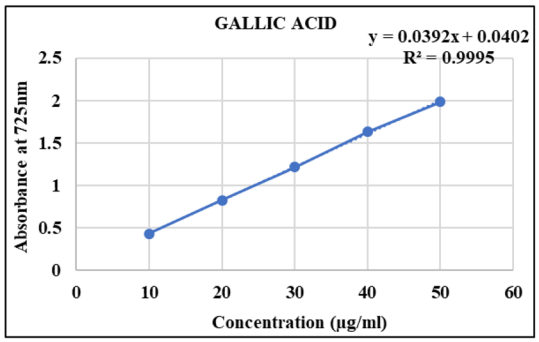

Fig. 3. Absorbance (OD) of gallic acid (Standard phenol) at different concentrations.

et al. 2010, Hussain et al. 2019).The total phenolics content (Gallic acid equivalents, mg/g) in PVLE and TNLE were estimated using the calibration curve (y= 0.0392x + 0.0402, R^2= 0.9995) (Fig. 3), and it was calculated to be 45.99±2.19 mg GAE/g dry weight and 80.77±2.65 mg GAE/g dry weight, respectively. Phenols exhibit antioxidant and antitumor properties. The present, the study showed that the quantity of most of all the studied phytochemicals increased at higher altitude, i.e., Tungnath, with the exception of saponin, which was higher in Pothivasa. The study by Rana *et al.* (2020) in *Coleus forskohlii* also found the higher content of flavonoids, terpenoids and phenols at higher altitude. The increase in the concentration of phytochemicals with the elevation might be related to their role in stress tolerance.

Hence, the presence of these phytochemicals in the leaves of *Picrorhiza kurroa* shows that the leaves also have therapeutic potential and it can also be explored for medical purposes besides the more popular parts; roots and rhizomes of *P. kurroa*.

Anti-bacterial activity

This study observed the effect of methanolic leaf extracts of *P. kurroa* (Met-PVLE and Met-TNLE) against five different bacterial strains (*Escherichia coli, Enterobacter* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp.) by measuring the zone of inhibition. Met-PVLE inhibits the growth of *Acinetobacter* sp., *Enterobacter* sp., and *Pseudomonas* sp. Met-TNLE showed a zone of inhibition against *E. coli., Enterobacter* sp., and *Acinetobacter* sp. Both the extracts showed better inhibition against *Acinetobacter* sp. in comparison to the antibiotic Tetracycline. No inhibition was observed in *Staphylococcus* sp. as shown in Table 3.

The phytochemicals present in the plant contributes to its antimicrobial potential. The alkaloids exhibit this potential as it can inhibit various gram-negative bacteria, gram-positive bacteria, and fungi. Its potential is comparable to the commercially available antibiotics (Yan *et al*. 2021). The flavonoids are also known to possess the antimicrobial properties. So, its presence in this plant contributes in providing the antibacterial potential to the plant. Similar results were seen in the methanolic extract of *Achillea millefolium, Bergenia ciliata,* and *Aloe vera* that contained high amounts of flavonoids (Mehmood *et al*. 2022). The biosynthesis of nucleic acid and other metabolic processes in bacteria have been shown to be inhibited by flavonoids (Donadio *et al.* 2021). The impact of flavonoids on the permeability of cellular membranes is another factor contributing to their potent antibacterial activity (Biharee *et al.* 2020). In addition to flavonoids, phenolic substances are crucial in preventing the growth of bacteria. Phenols' C3 side chains reduce the amount of oxidation required for their antibacterial action (Baba and Malik 2015).

Therefore, the present study on *Picrorhiza kurroa* leaves from Pothivasa and Tungnath, Uttarakhand, revealed the presence of a plethora of metabolites which exhibit numerous medicinal properties, and a maximum of these were present in the methanolic

Table 3. Anti-bacterial activity of Met-PVLE and Met-TNLE by measuring the zone of inhibition (mm) (-ve: Negative, +ve: Positive).

	-ve control (DMSO)	+ve control (Tetracycline)	Met-PVLE	Met-TNLE
Escherichia coli	0.0	0.0	0.0	1.83±0.62
Acinetobacter sp.	0.0	2.0±0.01	2.5±0.71	3.0±0.0
Enterobacter sp.	0.0	19.0±0.0	5.25±0.35	4±1.41
Pseudomonas sp.	0.0	2.0±0.0	5.75±1.06	0.0
Staphylococcus sp.	0.0	0.0	0.0	0.0

extracts of leaves. Their presence is also significant for the stress metabolism of the plant, which helps the plant adapt to lower temperatures or cold stress. The methanolic leaves extract also shows anti-bacterial properties against *Escherichia coli*, *Enterobacter* sp., *Acinetobacter* sp., and *Pseudomonas* sp. The study shows that the leaves of *P. kurroa* have high medicinal potential, and further studies can be done to identify and isolate the specific bioactive compounds present in the plant. This study will help conserve the plant in its natural environment, as *P. kurroa* is an endangered plant.

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REFERENCES

Baba SA, Malik SA (2015) Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J Taibah Univ Sci.*9(4):449–454.

Banu SK, Catherine L (2015) General techniques involved in phytochemical analysis. *Int J Adv Res Chem Sci* 2: 25-32.

Bhattacharjee S, Bhattacharya S, Jana S, Baghel DS (2013) A review on medicinally important species of *Picrorhiza*. *Int J Pharmaceut. Res Biol Sc*i 2(4): 1–16.

Biharee A, Sharma A, Kumar A, Jaitak V (2020) Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. *Fitoterapia* 146:104720.

Boham BA, Kocipai AC (1974) Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium. Pacific Sci* 48: 458-463.

Bone K, Mills S (2013) Principles and Practice of Phytotherapy. Principles of herbal pharmacology. pp 17–82.

Borokini TI, Omotayo FO (2012) Phytochemical and ethnobotan ical study of some selected medicinal plants from Nigeria. *J Med Pl Res* 6 (7): 1106-1118.

Deb K, Kaur A, Ambwani S, Ambwani TK (2018) Preliminary phytochemical analyses of hydromethanolic leaf extract of *Melia azedarac*h L. *J Med Pl Stud* 6: 4–8.

Debnath P, Rathore S, Walia S, Kumar M, Devi R, Kumar R (2020) *Picrorhiza kurroa*: A promising traditional therapeutic herb from higher altitude of western Himalayas. *J Herbal Med* 23:100358.

Dhar U, Rawal RS, Upreti J (2000) Setting priorities for conservation of medicinal plants – A case study in the Indian Himalaya. *Biol Cons* 95: 57-65.

Donadio G, Mensitieri F, Santoro V, Parisi V, Bellone M, De Tommasi N, Izzo V, Piaz FD (2021) Interactions with microbial proteins driving the antibacterial activity of flavonoids. *Pharmaceutics* 13:660.

Ferguson NM (1956) A Text book of Pharmacognosy. MacMillan Company, New Delhi, pp 191.

Harborne JB (1973) Phytochemical methods. Chapman and Hall, Ltd, pp 49-188.

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. *Phytochemistry* 55 (6): 481-504.

Hussain M, Debnath B, Qasim M, Bamisile BS, Islam W, Hameed MS, Wang L, Qiu D (2019) Role of saponins in plant defense against specialist herbivores. *Molecules* 24(11): 2067.

Hussein R, El-Anssary A (2019) Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal Med* 1: 13.

Kaul MK, Kaul K (1996) Studies on medico-ethnobotany, diversity, domestication and utilization of *Picrorhiza kurrooa*, In Supplement to cultivation and utilization of medicinal plants, Jammu. CSIR, pp 333-348.

Krupashree K, Kumar KH, Rachitha P, Jayashree GV, Khanum F (2014) Chemical composition, antioxidant and macromole cule damage protective effects of *Picrorhiza kurroa* Royle-ex Benth. *S. Afr J Bot* 94: 249–254.

Mehmood A, Javid S, Khan MF (2022) *In vitro* total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chem* 16 : 64.

Nafees M, Barkatullah, Ullah S, Ikram N (2021) Phytochemical and pharmacognostic studies of *Buddleja asiatica* leaves. *Microscopy Res Technique* 85(2): 510-520.

Obadoni BO, Ochuko PO (2001) Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global J Pure Appl Sci* 8:203-208.

Podolak I, Galanty A, Sobolewska D (2010) Saponins as cytotoxic agents: A review. *Phytochem Rev* 6 : 425–474.

Prakash D, Kumar N (2011) Cost effective natural antioxidants. In Nutrients, dietary supplements, and nutriceuticals. Humana Press, pp 163-187.

Rana PS, Saklani P, Chandel C (2020) Influence of Altitude on secondary metabolites and antioxidant activity of *Coleus forskohlii* root extracts. *Res J Med Pl* 4 (2): 43-52

Rathee D, Rathee P, Rathee S, Rathee D (2016) Phytochemical screening and antimicrobial activity of *Picrorhiza kurroa,* an Indian traditional plant used to treat chronic diarrhea. *Arabian J Chem* 9(2): S1307-S1313.

Saeidnia S, Gohari AR, Mokhber-Dezfuli N, Kiuchi F (2011) A review on phytochemistry and medicinal properties of the genus *Achillea. DARU J Fac Pharm Tehran Univ Med Sci* 19(3):173.

Sah JN, Varshney VK (2013) Chemical constituents of *Picrorhiza* genus: A review. *Am J Essent Oils Nat Prod* 1(2): 22–37.

Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013) Phytochemistry of medicinal plants. *J Pharmacog Phytochem* 1 : In prees.

Sharma R, Deb K, Ambwani TK, Ambwani S (2018) Preliminary phytochemical screening and antioxidative potential of *Picrorhiza kurroa* Royale ex. Benth, *Bull Environ Pharmacol Life Sci* 7:134–139.

Thakur AV, Ambwani S, Ambwani TK (2018) Preliminary phytochemical screening and GC-MS analysis of leaf extract of *Acacia catechu* (Lf.) Willd. *Int J Herb Med* 6: 81–85.

Yan Y, Li X, Zhang C, Lv L, Gao B, Li M (2021) Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics (Basel)* 10(3) : 318.