Journal of Biology and Nature

Volume 16, Issue 2, Page 9-18, 2024; Article no.JOBAN.12245
ISSN: 2395-5376 (P), ISSN: 2395-5384 (O), (NLM ID: 101679666)

Picrorhiza kurrooa: A Promising Himalayan Medicinal Plant with Therapeutic Research and Conservation Challenges

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Authors' contributions

This work was carried out in collaboration among all authors. Author ER wrote the first draft on therapeutic aspect, Author YYS verified the references, Author IA contributed towards the medical and biotechnology aspect along with graphical representation and the Author BD contributed the conception of the final draft and overall updated/edited with new studies and references with respect to plants status and conservation. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/joban/2024/v16i28789

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12245

Minireview Article

Received: 25/05/2024
Accepted: 22/07/2024
Published: 26/07/2024

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Cite as: *Rana, Ekta, Yogesh Y. Sumthane, Indesh Attri, and Bandana Dhiman. 2024. "Picrorhiza Kurrooa: A Promising Himalayan Medicinal Plant With Therapeutic Research and Conservation Challenges". Journal of Biology and Nature 16 (2):9-18. https://doi.org/10.56557/joban/2024/v16i28789.*

ABSTRACT

Picrorhiza kurrooa is an herbaceous perennial plant that grows in the Himalayas, and for many years it has been used as a medicine in Ayurvedic tradition for its wide range of therapeutic applications which has been mentioned in renowned old texts like Charaka Samhita, Sushurata Samhita, Ashtanga etc. This short review paper highlights the chemical and botanical features of *P. kurrooa* which belongs to family Plantaginaceae with emphasis on its bioactive components such as iridoid glycosides and cucurbitacins. Pharmacological studies have found hepatoprotective, anti-inflammatory and antioxidant activities in *P. kurrooa* aka *'kutki'* locally. In Himalayan region *kutki* is known for its use in traditional systems of medicine by amchies. Molecular mechanisms of action of *P. kurrooa* are under study currently and this paper looks at the mode of actions as well as diseases treated by it especially liver dysfunctions, respiratory conditions, skin problems among others. Among the main obstacles that impede development in PIC research are lack of knowledge regarding active compounds, pharmacokinetic issues and regulatory restrictions. Overcoming these problems may involve multidisciplinary approaches; innovative formulation techniques; cooperation among different stakeholders while conducting clinical trials on patients or preclinical tests involving animals in order to prove its efficacy against disease using globally acceptable standards. Encouragingly, both preclinical and clinical investigations show promising results although standardization should be done along with large-scale trials to affirm safety and effectiveness as a therapy approach for *P. kurrooa*. Progressing in this direction would certainly go a long way in exploiting the full benefits of *P. kurrooa* as a drug since this will also propel novel treatment options for other diseases.

Keywords: Himalayan plants; Kutki; ayurvedic medicine; bioactive constituents; therapeutic; hepatoprotective.

1. INTRODUCTION

Picrorhiza kurrooa Royle ex Benth, commonly known as *Kutki,* is a perennial herbaceous plant that belongs to the family Plantaginaceae (Now Scrophulariaceae-distinct traits) under the order Scrophulariales, earlier order Lamiales-Mint order. It is primarily found in the temperate regions of the Himalayan Mountain range, including India, Pakistan, and Nepal [1]. *P. kurrooa* is considered or used as a substitute of Indian *Karu* (*Gentiana kuroo*) and has a rich history of traditional use in Ayurvedic medicine with significant medicinal properties. Plant has been recalled with synonym names; as *Picrorhiza lindleyana* (Wall.) Steud. And *Veronica lindleyana* Wall [2]. As per previous documented literature, its natural availability lies from 2500 m to above [3,4] at north facing slopes of Western Himalayan [2,5,6]. In Spiti valley having cold desert ecosystem of Trans-Himalayas, for procurement of economic part of *Kutki*-roots/rhizomes, Himalayan local vaids/practitioners aka *amchies* have to trek above 3500 m in the months from June to August signifies the unavailability of wild *kutki* at earlier locations/altitude. Morphological and taxonomical features have been described in detail various by botanists [3,7-15]. In recent years, *Picrorhiza kurrooa*, a perennial Himalayan herbaceous plant has garnered significant attention in the field of pharmacology due to its diverse therapeutic properties. One of the essential aspects of *P. kurrooa* is its chemical composition, which includes a wide range of bioactive constituents. These constituents contribute to its diverse pharmacological activities and therapeutic potential. The major chemical constituents of *P. kurrooa* include iridoid glycosides, cucurbitacins, and monoterpenes [16]. The iridoid glycosides present in *P. kurrooa* include picroside-I, picroside-II or kutkoside (k) especially k-VII [5], in ration of 1:2 (kutkoside & picroside-I) [14] compounds have various biological activities, including hepatoprotective, anti-inflammatory, and antioxidant effects [17]. Another class of compounds found is cucurbitacins, with cucurbitacin-D being the prominent constituent [18]. Cucurbitacins (tetracyclic terpenes) possess potent anti-inflammatory and anticancer properties, making them of significant interest in the field of pharmacology and drug discovery [19]. In addition to iridoid glycosides and cucurbitacins, *P. kurrooa* also contains other bioactive compounds such as apocynin, phenylethanoid glycosides, and lignans [20], Drosin [2] and still researches are carrying out studies with help of chromatographic techniques [21,22,23].

These compounds contribute to the overall therapeutic potential of plants and have been associated with various pharmacological activities such as immunomodulation, antimicrobial effects, cytotoxic, and anti-inflammatory properties [24,2]. The traditional uses of *P. kurrooa* in Ayurvedic medicine are extensive and well-documented. *Kutki* has been used for centuries to treat liver disorders, respiratory infections, fever, and digestive ailments [8]. Its hepatoprotective properties have been attributed due to the presence of picroside-II/kutkoside, which protect liver cells from various toxic insults and promote liver regeneration [25]. Furthermore, *P. kurrooa* has been used in the management of respiratory conditions such as bronchial asthma, chronic bronchitis, and allergic rhinitis [26-34]. The anti-inflammatory and immunomodulatory actions of *P. kurrooa* are believed to contribute to its efficacy in these conditions. Its chemical constituents, including iridoid glycosides, cucurbitacins, and other bioactive compounds, are responsible for its diverse therapeutic properties in medical arena. Its traditional uses in the treatment of liver disorders and respiratory ailments have been supported by scientific studies and represent a valuable natural resource for the development of novel therapeutics and warrants further exploration and research.

2. CURRENT STATE OF RESEARCH: UNDERSTANDING THE MECHANISMS AND APPLICATIONS

Various esearch efforts have been dedicated to unravelling the mechanisms underlying its medicinal effects and exploring its potential applications in various health conditions. As numerous studies and published records mentioned the traditional, ethno-botanical as well as modern medicinal uses of *Kutki* whichhave been explored by various life-science researchers/doctors [16,27,29,31,35,36,37,21,2, 5,14,38,39,23]. This section provides an overview of the current state of research on *Picrorhiza kurrooa*, focusing on the mechanisms of action and its diverse applications in therapeutics.

2.1 Mechanisms of Action

Picrorhiza kurrooa is rich in bioactive compounds such as major iridoid glycosides - picroside I and picroside II (mixture of both kutkin) and minor iridoid glycosides are picroside-III, V, pikuroside, minecoside, 6-feruloylcatalpol another cucurbitacin glycosides (Cucubitacin-B, D, R) [40,14] and apocynin, which contribute to its pharmacological effects. These compounds exert their actions through various molecular mechanisms, targeting multiple pathways within the body. One of the well-studied mechanisms of *P. kurrooa* is its potent antioxidant activity. The presence of picrosides in Kutkin has been shown to scavenge free radicals and inhibit oxidative stress-induced damage in cells and tissues [41]. Additionally, it has been found to modulate the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, further enhancing its antioxidant potential [42]. Moreover, this medicinal plant exhibits anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [43]. Its bioactive constituents have been shown to suppress the nuclear factor-kappa B (NF-κB) signaling pathway, a key regulator of inflammatory responses, thereby attenuating inflammation [44]. Furthermore, plant also demonstrates hepatoprotective effects by enhancing liver function and promoting hepatocyte regeneration. Studies have revealed that its extracts can mitigate liver damage induced by toxins such as carbon tetrachloride (CCl4) by reducing oxidative stress and inflammation in hepatic [45].

2.2 Applications in Therapeutics

The diverse pharmacological activities of *P. kurrooa* have led to its exploration of various therapeutic applications across different medical conditions. One of the primary applications is in the management of liver disorders. Its hepatoprotective properties make it a promising candidate for treating liver diseases such as hepatitis, cirrhosis, and fatty liver disease [19]. Clinical studies have demonstrated the efficacy oimmunf *P. kurrooa* formulations in improving liver function parameters and reducing liver enzyme levels in patients with liver ailments [46]. Additionally, the plant holds potential in the treatment of respiratory conditions such as asthma and bronchitis. Its anti-inflammatory and bronchodilator effects help alleviate respiratory symptoms and improve lung function in patients with these respiratory disorders. The topical application of *P. kurrooa* extracts has been found to reduce skin inflammation and oxidative damage, making it a valuable ingredient in skincare formulations [41] and extracts may help regulate blood sugar levels and improve insulin sensitivity [35].

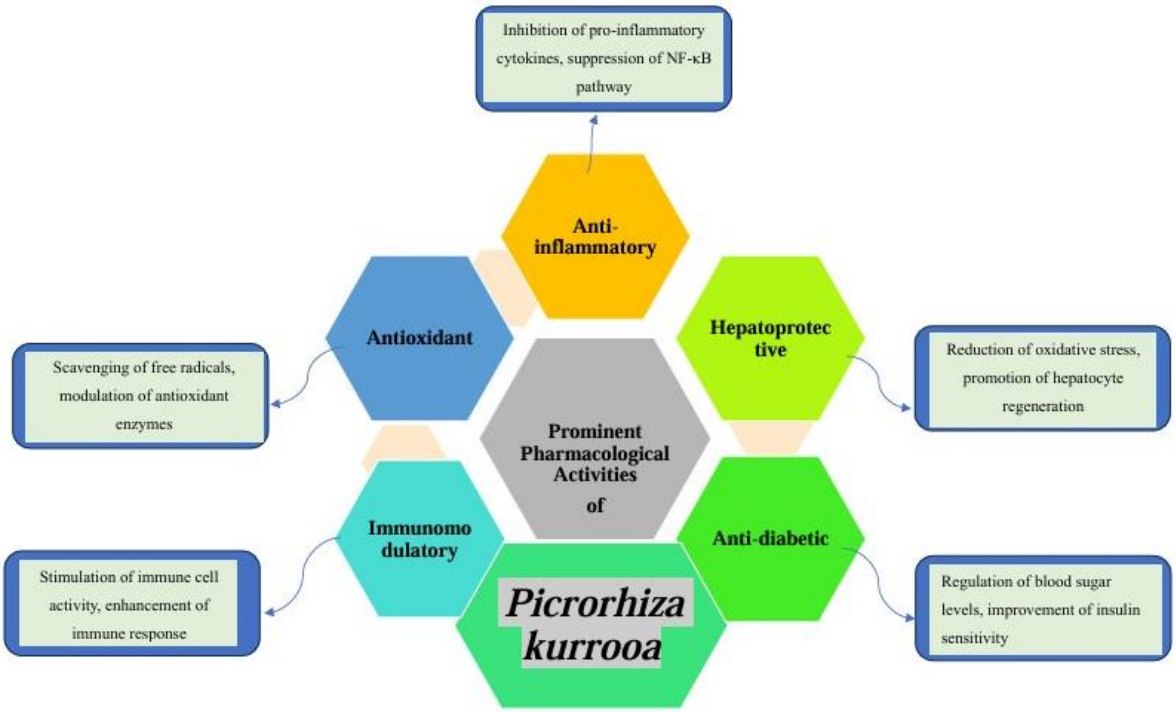

Fig. 1. The diverse pharmacological activities with the mechanism of action of *Picrorhiza kurrooa*

Moreover, it exhibits immunomodulatory effects, enhancing the body's immune response against infections and diseases. As studied by [47], the specific biopolymer fractions- RLJ-NE-205 extracted from rhizomes have activity for immunomodulator. It stimulates the activity of immune cells such as macrophages and lymphocytes, thereby bolstering the immune system's defense mechanisms [17]. The anti-proliftic activities of biopolymeric active compounds found in various Himalayan medicinal plants have been discussed by [48,49]. In addition to its therapeutic applications, Kutki is also being investigated for its potential role for the management of the body's metabolic disorders such as diabetes and obesity.

3. CHALLENGES ON THE HORIZON: ADDRESSING HURDLES IN *Picrorhiza kurrooa* RESEARCH

Being an important perennial Himalayan medicinal plant, *Picrorhiza kurrooa* has been revered for centuries in traditional medicine systems like Ayurveda. With a rich history of traditional medicinal use, modern scientific research has begun to explore its potential applications in treating various health conditions. However, despite its promising attributes, several challenges loom on the horizon, impeding the progress of *P. kurrooa* research for future therapeutic development.

3.1 Limited Understanding of Active Compounds

One of the primary challenges in research is the limited understanding of its active compounds and their mechanisms of action. While traditional medicine attributes its therapeutic effects to compounds like picroside-I and kutkoside, the exact pathways through which they exert their benefits remain elusive [33]. Further investigation is still needed to uncover the pharmacological actions of these compounds and their potential synergistic interactions.

3.2 Pharmacokinetic Challenges

Another significant hurdle is the poor bioavailability and pharmacokinetic properties of *P. kurrooa* compounds. Many bioactive constituents exhibit low solubility and rapid metabolism, leading to reduced efficacy and unpredictable therapeutic outcomes [21].

Overcoming these challenges requires innovative formulation strategies such as nanoencapsulation or prodrug design to enhance stability and improve absorption rates.

3.3 Quality Control and Standardization

Ensuring the consistency and quality of *Picrorhiza kurrooa* extracts poses a significant challenge for researchers and manufacturers. Variability in plant sourcing, growing conditions and extraction methods can lead to differences in chemical composition and therapeutic efficacy [22]. Establishing standardized protocols for cultivation, sustainable harvesting [50] and extraction is crucial to maintain product quality and reliability across studies. Among the above practices standard cultivation w.r.t to the native climatic/environmental conditions and sustainable harvesting from the wild are two important measures to conserve wild or rare medicinal plant species germplasm [51].

3.4 Regulatory Hurdles

Navigating the regulatory landscape presents another obstacle to *P. kurrooa*-based therapeutics. Regulatory agencies require comprehensive safety and efficacy data to approve new drugs or supplements, necessitating rigorous preclinical and clinical studies [52-54]. Meeting these requirements entails significant time, resources, and collaboration between researchers, industry partners, and regulatory authorities.

3.5 Limited Clinical Evidences

Despite promising preclinical findings, its clinical evidence supporting the efficacy of in humans is still inadequate. Most studies have been small-scale or conducted in animal models, making it challenging to extrapolate the results to human populations [55]. Large-scale, well-designed clinical trials are needed to validate the therapeutic benefits of *P. kurrooa* and establish evidence-based treatment guidelines.

3.6 Strategies for Overcoming Challenges

Addressing the hurdles in *P. kurrooa* research requires a multidisciplinary approach and concerted efforts from academia, industry, and regulatory bodies. Collaboration between researchers and traditional medicine practitioners can facilitate knowledge exchange and the integration of traditional wisdom with modern scientific methodologies. Furthermore, investing in advanced analytical techniques such as metabolomics and pharmacogenomics can deepen our understanding of plant's pharmacological properties and potential drug interactions. Innovative formulation strategies, such as nano-emulsions, liposomes, or solid lipid nanoparticles, can enhance the bioavailability and stability of *P. kurrooa* extracts, improving their therapeutic efficacy [56]. Moreover, implementing Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) can ensure the standardized production of high-quality *P. kurrooa* products, bolstering consumer confidence and regulatory compliance. To navigate the regulatory hurdles, stakeholders must engage in proactive dialogue with regulatory agencies to establish clear pathways for the approval of *P. kurrooa*-based therapeutics. Investing in robust clinical trial infrastructure and fostering international collaborations can accelerate the generation of clinical evidence and facilitate market access for *P. kurrooa* products, while it holds immense promise as a source of novel therapeutics, several challenges impede its research and development. By addressing issues related to compound characterization, pharmacokinetics, quality control, regulatory compliance, and clinical evidence, we can overcome these hurdles and unlock its full potential for improving human health.

4. CURRENT PROGRESS AND CHALLENGES IN PRECLINICAL AND CLINICAL STUDIES OF *P. kurrooa* - BASED THERAPEUTICS

In recent years, there has been a growing interest in its potential for modern therapeutics based on both pre-clinical and clinical studies. Preclinical studies play a crucial role in evaluating the safety and efficacy of potential therapeutics before they can be tested in humans. Several preclinical studies have investigated the pharmacological activities of *P. kurrooa* extracts and their bioactive compounds. For instance, a study by [35] demonstrated the hepatoprotective effects of *P. kurrooa* extract in animal models. The study reported a significant reduction in liver damage markers and oxidative stress, suggesting its potential as a hepatoprotective agent. A pre-clinical test by [55], where anti-inflammatory activities of *P. kurroa* extract were witnessed in a rat model of inflammation with significant reduction in inflammatory markers and cytokine level.

Furthermore, a study evaluated the antioxidant activity of *P. kurroa* extract in vitro which revealed a strong antioxidant potential, attributed to the presence of bioactive compounds such as Picroside-I and kutkoside [14].

Moving ahead on clinical studies, provides little valuable insights into the therapeutic potential of *P. kurroa*-based interventions. A randomized controlled trial conducted by [55], they had investigated the efficacy of extract in patients with non-alcoholic fatty liver disease (NAFLD). The study reported improvements in liver function tests, lipid profile, and insulin resistance in the treatment group compared to the placebo group, suggesting the potential of *P. kurroa* in managing NAFLD.

While the progress in preclinical and clinical studies of *P. kurroa*-based therapeutics is promising, several challenges need to be addressed. One of the challenges is the standardization of herbal extracts and formulations. The composition of bioactive compounds in *kutki* can vary depending on numbers of factors such as geographical location, harvesting time, and processing methods. Standardization protocols should be established to ensure consistent quality and efficacy of *P. kurrooa*-based therapeutics. Another challenge is the lack of large-scale clinical trials. Most clinical studies conducted so far have been small-scale and limited in duration. Large-scale clinical trials are essential to establish the safety and efficacy of *P. kurrooa*-based interventions in a diverse population. Additionally, long-term studies are needed to assess the sustainability and potential side effects of prolonged use, the preclinical and clinical studies of *P. kurroa*-based therapeutics have shown promising results in terms of hepatoprotective, anti-inflammatory, and antioxidant activities. However, further research is required to address hidden challenges for its standardization and to conduct large-scale clinical trials.

5. CURRENT STATUS IN THE WILD AND CONVENTIONAL AS WELL AS BIOTECHNOLOGY INTERVENTIONS TOWARD ITS CONSERVATION

Picrorhiza kurroa is classified as an endangered plant in the list of Appendix-I (CITES) which requires stringent management during its extraction or harvesting to safeguard the plant in nature. For wild and endangered Himalayan plant species various conservation measures and strategies can be adopted and listed in previous reviews of Himalayan medicinal plants [51]. To stabilize illegal extraction from wild and preparation of value-added products of *kutki*, LPC (Legal Procurement Certificate) is mandatory to obtain from the respective State wildlife authority, both for the traders and manufacturers.

Efforts are driven using tissue culture-propagation for its mass multiplication from wild to sustain its generation. As *kutki is* the endemic medicinal herb of western Himalaya, requires biotechnological intervention as ex-situ conservation. Various researchers have worked to standardize the propagation through plant tissue culture. Some pioneer plant biotechnologists such as [12], had standardized regeneration protocol for wild *kutki* using middle portions of ex-vitro leaf cultures with 2.32 µM kinetin (Kn) which has the highest regeneration rate of about 94.33% which was noticed low in shoots (38.0 %). In another study, nodal segments were cultured on Murashige and Skoog (MS) medium with various kinetin (Kn) and indole-3-acetic acid (IAA) concentrations to enhance multiple shooting. Optimal shoot bud induction occurred in MS medium with 6 µM Kn and 10 µM IAA, resulting in 66.8% of cultures producing an average of 4.2 ± 0.4 shoots per culture. Subsequent subcultures on similar media led to extensive multiple shoot proliferation and callus regeneration [13]. There was prominent effect of growth hormones on the shoot regeneration as higher BAP concentrations (2.5–5.0 µM) caused hyperhydric (vitrified) shoots in subsequent subcultures. Reducing cytokinin levels restored normal shoot regeneration from the base of vitrified shoots [57]. Herbs are hardened at incubator for first two weeks, thereafter transplanted to adapt to that environment. With a futuristic vision toward ex-situ conservation and to develop standard agro-techniques [37] for mass multiplication, the renowned institute CSIR-IIM has developed a germplasm base from seven different assessions [37] from different altitudes of Kashmir.

The next level of *in vitro* propagation of *P. kurrooa* was through callus culture which was maintained for 64 weeks on hormone-free MS media with incubated at 4-10°C in the dark (Lal & Ahuja, 1995). Later, callus cultures from various *P. kurrooa* explants, i.e., leaf discs, nodal segments, and root segments, were established on MS medium with 2,4-D (2 mg/l) and IBA (0.5

mg/l). Root segments showed the highest callus induction (70%), followed by leaf discs (56.3%) and nodal segments (38.3%). These callus cultures were then differentiated into multiple shoots on MS medium when subjected to varying concentrations and combinations of BA, Kn, and IBA [58].

6. CONCLUSION

Picrorhiza kurrooa- aka Kutki is a widely recognized herb in Ayurvedic medicine and has an impressive botanical history concerning great therapeutic potential. With its potential for therapeutics, wild *Kutki* herbs have been already extended to the verge of extinction in their native habitat. Hence priority is to sustain adaptive conservation measures (both in-situ & ex-situ) along with awaring the native denizen as well as *amchis* about the unique importances/uses of *kutki* for modern pharmacy and its cultivation techniques. *Kutki* herb is characterized by iridoid glycosides and cucurbitacins, which define its chemical composition and cover the full range of beneficial pharmacological activities such as anti-inflammatory effects, antioxidant properties, and liver protection. The significant scientific proofs of traditional applications of '*kutki'* has been addressed in liver problems and breathing issues that set their place in the modern healthcare system. However, clinical trials on humans are somewhat complex to handle. Poor bioavailability and pharmacokinetic properties hinder therapeutic optimization while limited understanding regarding the active compounds of this plant complicates mechanistic elucidation. Standardization issues including regulatory compliance and quality control are equally critical for ensuring consistent product efficacy. In addition, the paucity of clinical evidence necessitates large-scale trials to provide conclusive proof of the therapeutic benefits of *P. kurrooa* and determine any long-term effects. To overcome these barriers, collaboration is necessary, along with the blending of conventional knowledge and new scientific techniques. New ways of making drugs like nanotechnology provide answers to better absorption into the body and durability. Moreover, this will guarantee consistent quality of medicine together with its compliance for regulatory requirements which in turn will strengthen product quality as well as adherence to regulation. By interacting proactively with regulators can assist companies in streamlining their *P. kurrooa*-based therapeutic products' approval process hence allowing them to enter the market faster than their competitors. Compound characterization, pharmacokinetics and clinical validation are key areas that deserve greater attention to ensure that *P. kurrooa* realizes its full potential in terms of therapy development and becomes an invaluable resource in research for finding new remedies for various disorders.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

We declare No any generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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