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Original Research Article

Natural treasures from *Picrorhiza kurrooa*: a computational exploration of drug-like properties and bioactivity of kutkin, cucurbitacin, apocynin and lupanine

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ABSTRACT

Background: To analyse and predict the basic pharmacokinetic and toxicological properties of four compounds of interest found in *Picrorhiza kurroa* (Kutkin, cucurbitacin, apocynin and lupanine) using computational bioinformatics tools.
Methods: The chemical structures and molecular properties of the compounds were obtained from authentic sources and processed for data profiling. 2D structures were converted to 3D structures using ChemSketch software and PHASE module. *In silico* screening of the 3D structures was performed using bioinformatics prediction software to assess drug-likeness, absorption, blood-brain barrier penetration, enzyme interaction potential, skin penetration, and acute oral toxicity.
Results: Kutkin exhibited poor drug-likeness and low oral absorption, while the other three compounds showed promising drug-like properties and good oral absorption. Cucurbitacin and lupanine were predicted to cross the blood-brain barrier, while Kutkin and Apocynin were not. None of the compounds were substrates for P-glycoprotein, but Kutkin and cucurbitacin were substrates for CYP3A4. All four compounds had low skin penetration. Acute oral toxicity varied, with cucurbitacin classified as highly toxic and the others as slightly toxic.
Conclusions: Cucurbitacin, apocynin, and lupanine have potential for further development as therapeutic agents due to their favorable drug-like properties and good absorption. Kutkin's poor drug-likeness and low absorption make it less suitable for oral drug development. This information provides valuable insights for further research on the medicinal properties of *Picrorhiza kurroa* and the development of new drugs based on its active compounds.

Keywords: *Picrorhiza kurroa, In-silico,* Bioinformatics, Pharmacokinetics, Toxicology

INTRODUCTION

The reasons responsible for the rising prevalence of the processes leading to various illnesses contribute to the steadily growing life expectancy, contemporary lifestyles, and environmental circumstances. Multiple ailments have been the reason behind increased death rate worldwide. Over the years, seeking new treatments that may cure chronic diseases and other infectious disorders has become a necessary. The study is to mainly focus on identifying natural compounds with a lower potential for adverse effects than synthetic pharmaceuticals because of the significant adverse effects caused by synthetic medications.

Picrorhiza in Greek language, means “bitter” (picros) and

"roots" (rhiza). *Picrorhiza* is a genus comprising two species (*Picrorhiza kurroa* Royle ex Benth and *Picrorhiza scrophulariiflora* Pennell) of the family *Plantaginaceae*. These species are found in nature at sites such as cliffs, crevices, and mountainsides. *Picrorhiza kurroa* Royle ex Benth is mainly found at an altitude of 3000-5000 m above sea level in the Himalayan ranges. It has been used as an herbal medicine for many ailments, ranging from dyspepsia to hepatitis. Bitter extracts from the dried rhizomes of this plant has been used as a purgative, brain tonic, stomachic, and antiperiodic to treat dyspepsia in traditional and modern medicine.[2-4]

Many herbs are known for their cooling, heating, or neutral properties. *P. kurroa* has a cooling effect and is used as an antipyretic, laxative, anthelmintic antiasthmatic, and cardiotonic.[5] *P. kurroa* has been used to treat respiratory diseases, allergies, inflammatory conditions, fever, asthma, diarrhea, chronic scorpion stings, and liver-related diseases.[6-10] Hepatoprotective effects against aflatoxin, carbon tetrachloride, alcohol, paracetamol, isoniazid, rifampicin, *Plasmodium berghei*, and amanita mushrooms poisoning have been reported.[11-15] *P. kurroa* can be used to treat jaundice.[16] Preclinical studies done with ethanolic extract contains 50-60% of two iridoid glycohepatosides in the ratio of 1:1.5, picroside-I, and kutkoside, which have been proven to have powerful hepatoprotective effects against miscellaneous hepatotoxins, including alcohol. The active constituents of rhizome and roots of kutki consists of kutkoside and iridoid glycosides (IGs) (picroside I and II).[17]

P. kurroa, an endangered medicinally significant plant with diverse pharmacological activities and no major adverse effects have led researchers to develop practical techniques for its in vitro mass multiplication.[2-4] The international union for conservation of nature (IUCN) reported that this species should be protected under the rare endangered species (RET) category. To preserve this plant practical actions, such as in situ and ex situ conservation, are required.[18]

Recently, *P. kurroa* has gained the interest of researchers to identify the active ingredients of this plant and the mechanisms by which they exert their effects. The following compound's are selected for pharmacokinetic and toxicological profiling using bio informatics tools Kutkin: Diterpene glycosides, close cousins of picroside, share promising hepatoprotective and anti-inflammatory properties, cucurbitacin: These triterpenoid saponins boast a diverse range of activities, including antitumor, antiviral, and antidiabetic properties. Apocynin: This natural phenolic compound exhibits potent antioxidant and anti-inflammatory effects and lupanine and other alkaloids: These nitrogen-containing compounds contribute to the overall therapeutic arsenal of *Picrorhiza kurroa*, showcasing antimicrobial, antispasmodic, and analgesic properties. Furthermore, this review examines the ethnopharmacological uses, biological and chemical properties, clinical evidence, and toxicology of *P. kurroa*. This information will be a source for future fundamental and clinical studies. Hence based on the review, COGSCIENTIA

Aims and objectives

The current study aims to analyze and predict basic pharmacokinetic and toxicological aspects of compounds of interest from *Picrorhiza kurroa* i.e. Kutkin, cucurbitacin, apocynin and lupanine. The objectives of the present study are to analyse quantitative structure-activity relationship (QSAR) between structural properties of these chemical compounds and their biological activities using standardized computational bioinformatics tools.

METHODS

The computational hardware with HP PC 32-b0390-2022 Model installed with software Win-11 and java enabled with updated plugins with data processing workstation will be used for processing. The chemical structure and molecular properties of compounds of interest derived from *Picrorhiza kurroa* are procured from authentic sources and processed out for data profiling.

Data collection

The two dimensiona (2D) chemical structure of compounds derived from *Picrorhiza kurroa* will be collected through extensive review from indexed published journals and also form other standardized validated data banks such as Chembank, PubChem and ChemPDB. The ChemSketch software will be used to develop the three-dimensional structures, followed by software PHASE module to convert the structures from 2D to three dimensional (3D) structures.[19]

Screening

The *in silico* screening of 3D structure of compounds for Pharmacokinetic properties, Drug likeness, and Toxicological Prediction will be explored virtually using bioinformatics prediction software platform.[20] The Virtual screening workflow protocol from software admet SAR will then be acquired and results will be analysed for *in silico* data of compounds for drug like property data, pharmacokinetic data and toxicological data.[21]

Statistical methods and calculation

Mol Soft L.L.C. San Diego, CA, USA an interactive calculator applet employed for molecular properties and analysis of drug-likeness score.[22] The study site is department of pharmacology, Govt. Erode medical college and hospital, Perundurai, Erode. Conducted during December 2023 to January 2024, self-funded. No animals and humans are involved in the study and entire research is conducted over *in silico* computational bioinformatics tools, hence considered under the category for exemption from institutional ethics committee approval.

RESULTS

The 2D-3D structures of kutkin, cucurbitacin, apocynin and lupanine from *P. Kurrooa* are viable to screen molecular and pharmacokinetic properties.

The results from physiochemical properties of compounds from *Picrorhiza Kurrooa* exhibited kutkin with the highest hydrogen bond acceptor site with twelve and hydrogen bond donor site of six.

Table 1: *Picrorhiza Kurrooa* compounds 2D and 3D structure.

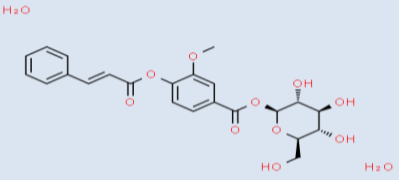
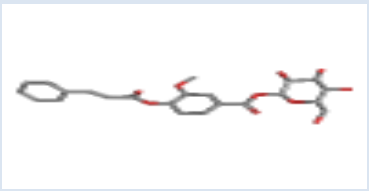
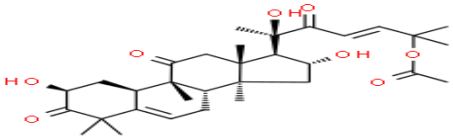
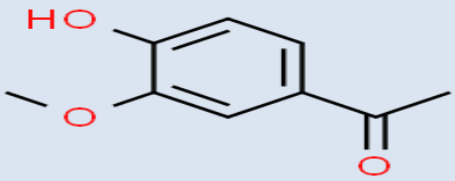
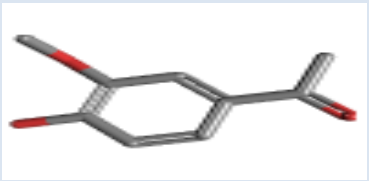
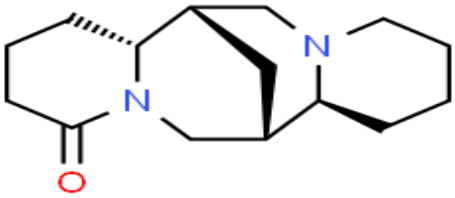
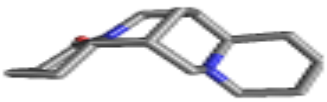
S. no	Compounds	2D structure	3 D structure
1.	Kutkin		
2.	Cucurbitacins		
3.	Apocynin		
4.	Lupanine		

Table 2: *Picrorhiza Kurrooa* compounds physiochemical properties.

Physiochemical properties	Kutkin	Cucurbitacin	Apocynin	Lupanine
Chemical formula	$C_{23}H_{28}O_{12}$	$C_{30}H_{42}O_6$	$C_9H_{10}O_3$	$C1_5H_{24}N_2O$
Molecular weight	496.5 g/mol	498.6 g/mol	166.17 g/mol	248.36 g/mol
Num. H bond acceptors	12	6	3	2
Num. H bond donors	6	2	1	0

H-Hydrogen

Table 3: Pharmacokinetic predicted profile of *Picrorhiza Kurrooa* compounds.

Pharmacokinetic parameters	Kutkin, p score	Cucurbitacin, p score	Apocynin, p score	Lupanine, p score
H. I. A	-0.6823	+0.9951	+0.9954	+0.9870
BBB	-0.7000	+0.7250	-0.5500	+0.9750
P-gp substrate	-0.7223	-0.5662	-0.9427	-0.7303
CYP450 3A4 inhibitor	-0.7859	-0.7946	-0.9210	-0.9559
CYP450 3A4 inducer	+0.5990	+0.6847	-0.6825	-0.5282
Log K_P	-8.97 cm/s	-7.05 cm/s	-6.95 cm/s	-6.69 cm/s
Drug likeness (Lipinski score)	No; 2 violations	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Bioavailability score	0.17	0.55	0.55	0.55
Plasma protein binding	100%	100%	100%	100%
Acute oral toxicity (c)	III 0.7427	I 0.3348	III 0.9007	III 0.8068

P-gp-P Glycoprotein, P-Probabilty, CYP-Cytochrome, H.I.A-Human intestinal absorbtion, BBB-Blood brain barrier, log kp -Human skin permeability coefficients, T1/2-plasma half life

The pharmacokinetic parameters of kutkin predicted with poor intestinal absorption score of -0.68 (less than 30%) and low bioavailability score of 0.17. Compounds cucurbitacin and lupanine had positive score of +0.7250 and +0.9750 over ability to cross blood brain barrier. Cucurbitacin is predicted and graded as highly toxic for acute oral toxicity

DISCUSSION

The physiochemical properties of kutkin, cucurbitacin, apocynin and lupanine from *Picrorhiza Kurrooa* evaluation and analysis explores and predicts the possibilities of various physio-chemical properties of each bio molecule that it interacts with ability to prompt biological effect. The Lipinski analysis utilizes physicochemical properties to predict the drug-likeness of an oral therapeutic agent.[23] Based on results from physiochemical properties, kutkin violated two aspect with respect to hydrogen bond donor 6 and acceptor site 12 and poor bioavailability score of 0.17. This quality of the compound eliminates from drug likeness for oral drug development. The rest of the compounds cucurbitacin, apocynin and lupanine are predicted to have favorable drug likeness score and bioavailability of 0.55 (Table 3) without any violations as per Lipinski rule of five.[23]

The absorption kinetics of compounds cucurbitacin, apocynin and lupanine with a score of +0.9951, +0.9954 and +0.9870 H.I.A values respectively (Table 3) is predicted to have more 30% absorption on oral administration and compound kutkin predicted to have negative H.I.A absorption score.[24] The compounds cucurbitacin and lupanine is predicted to cross blood brain barrier whereas compounds Kutkin and apocynin projected negative results on crossing blood brain barrier. P-glycoprotein functions as a biological barrier and the compounds are not a substrate for P-gp and predicted with negative scoring and similar results with CYP3A4 inhibition whereas kutkin and cucurbitacin is predicted to be substrates of CYP3A4 (Table 3). The probability of skin penetration of the compounds kutkin, cucurbitacin, apocynin and lupanine are -8.97 cm/s, -7.05 cm/s, -6.95 cm/s and -6.69 cm/s respectively, which is analyzed as low skin penetrability i.e. The more negative the log Kp the less skin permeant the molecule.[25]

Acute oral toxicity testing is predicted for possible four categories, toxicity category I is highly toxic and severely irritating, toxicity category II is moderately toxic and moderately irritating, toxicity category III is slightly toxic and slightly irritating, toxicity category IV is practically non-toxic and not an irritant. The compounds kutkin, apocynin and lupanine are placed in category III with LD50 values greater than 500 mg/kg but less than 5000 mg/kg. The compound cucurbitacin is placed in category I with LD50 values up to and including 50 mg/kg. The limitations of the study are it mainly provides predictions about the properties of the biomolecules and lacks detailed information about the pharmacodynamics by which these biomolecules exert their biological effects. while the study offers valuable insights into the potential of these biomolecules, it's crucial to acknowledge its limitations and consider further research to fully understand their therapeutic potential and ensure their safety and efficacy.

CONCLUSION

Cucurbitacin, apocynin, and lupanine present promising drug-like potential with good bioavailability, favorable absorption kinetics, and acceptable toxicity profiles. They also hold the possibility of crossing the blood-brain barrier, making them potentially suitable for targeting neurological conditions. Further investigation is essential to validate the *in-silico* predictions through in vitro and in vivo studies. This will help confirm their safety, efficacy, and specific therapeutic applications.

Kutkin, while interesting, does not demonstrate favorable drug-like properties due to violations of Lipinski's rule of five and poor bioavailability. It also exhibits negative absorption predictions and potential interactions with metabolic enzymes, suggesting limited oral drug development potential. Overall, this study unveils the potential of cucurbitacin, apocynin, and lupanine as candidate drugs extracted from *Picrorhiza Kurrooa*. However, continued research is crucial to unlock their full therapeutic potential and ensure their safe and effective use in medicine.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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