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REVIEW ARTICLE

Biosynthesis and therapeutic implications of iridoid glycosides from *Picrorhiza* genus: the road ahead

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Abstract *Picrorhiza* genus is emerging as an important paradigm for herbal drug formulations due to its versatile iridoid glycosides exhibition and robustness in the treatment of diverse infections including hepatic amoebiasis, cancer, malaria, ulcerative colitis and cerebral ischemia reperfusion injury. Owing to the superiority of these bioactivities, iridoid glycosides from *Picrorhiza* have become a hot research area over the years. A metabolic pathway for the formation of iridoid glycosides has been proposed. However, some enzymes and genes of this route are still unidentified and demand the enumeration of facilitating pathways contributing to the biosynthesis of iridoid glycosides. This review summarizes the current knowledge of all naturally occurring iridoid glycosides from *Picrorhiza*, their biosynthesis and pharmacological capabilities which could provide the insight into metabolic regulation and the basis for the development of new drugs.

Keywords Anti-inflammatory · Antioxidant · Kutkin · *Picrorhiza* · Picrosides

Abbreviations

DXPS	1-Deoxy-D-xylulose-5-phosphate synthase
DXPR	1-Deoxy-D-xylulose-5-phosphate reductoisomerase
ISPD	4-Diphosphocytidyl-2C-methyl-D-erythritol synthase
ISPE	4-Diphosphocytidyl-2C-methyl-D-erythritol kinase
MECPS	2-C-methyl-D-erythritol 2,4-cyclopyrophosphate synthase
HDS	1-Hydroxy-2-methyl 2-(E)-butenyl-4-pyrophosphate synthase

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Introduction

Over the years, common liver disorders such as chronic hepatitis and fatty liver face problems in treatment with colchicines, interferon and corticosteroids due to their reflective side-effect incidences (Luper 1998). Hence, plants have been explored to treat liver disorders. The iridoid glycosides derived from *Picrorhiza* genus showed extensive promises against liver disorders and this article has reviewed discoveries on this class of natural products published over the last 25 years. This provides the platform for the researchers to identify the research gaps in the biosynthesis of picrosides along with the knowledge of therapeutic implications which could present good alternative to the available ones in the development of new drugs in future. Some of the recent reviews, however, have also documented the pharmacological properties of iridoid glycosides from *Picrorhiza* species but they did not catalogue the pharmacological properties of individual iridoid glycosides (Bhattacharjee et al. 2013; Mondal et al. 2013; Sah and Varshney 2013). Moreover, we have also stressed at the biosynthetic point of iridoid glycosides to infer critical evaluation, which in turn suggests further types of investigations.

Picrorhiza genus belongs to family Plantaginaceae (formerly known as Scrophulariaceae) and comprises of two species, *Picrorhiza kurroa* Royle ex Benth and *Picrorhiza scrophulariiflora* Pennell, which grow in the Himalayan Mountains (Bhandari et al. 2008). *P. kurroa*, commonly known as Kutki, Karru and Indian gentian is distributed in the north-western Himalayas at an elevation of 3000–5000 m (Kumar et al. 2015a) while *P. scrophulariiflora*, commonly known as Nepalese kutki, is found in the alpine zone of Sikkim, Nepal and China at an elevation of 3000–4000 m (Bantawa et al. 2011). The plants are perennial herbs and self-regenerating but their unregulated over-harvesting and lack of organized cultivation has listed them as 'endangered' by 'The International Union for Conservation of Nature and Natural Resources (IUCN)' (Nayar and Sastri 1990; Bantawa et al. 2011). Recently, a new species of *Picrorhiza* genus i.e. *P. tungnathii* has been identified which is distributed at an elevation of 3400–4550 m in the Himalayan regions of Himachal Pradesh and Uttarakhand state, India. The plants are locally known by the same names as of *P. kurroa* i.e. kutki and karvi. The current conservation status is unknown as the exact population of this species is still to be examined due to its misidentification with *P. kurroa* (Pusalkar 2014).

The use of *Picrorhiza* species in the Ayurvedic system of medicine is well known and is official in "The Indian Pharmacopoeia" and "Indian Herbal Pharmacopoeia" Handa et al. (1998). The pharmaceutical activities are attributed to iridoid glycosides, the active constituents of these plants (Viljoen et al. 2012). So far, only 22 different iridoid glycosides have been reported from *Picrorhiza* plants. Out of these, only 7 iridoid glycosides are present solely in *P. kurroa* viz. kutkin, kutkoside, picroside V (P-V), boschnaloside, Bartsioside, mussaenosidic acid and pikuroside (Basu et al. 1970; Jia et al. 1999; Mandal and Mukhopadhyay 2004; Bhandari et al. 2010; Kumar et al. 2013) as shown in Fig. 1, while 6 iridoid glycosides are solely present in *P. scrophulariiflora* viz. amphicoside, piscroside A, piscroside B, verminoside, specioside and catalposide (Daqi et al. 1993; Li et al. 1998; Huang et al. 2006) (Fig. 2). On the other hand, 9 iridoid glycosides are present both in *P. kurroa* and *P. scrophulariiflora* viz. picroside I (P-I), picroside II (P-II), picroside III (P-III), picroside IV (P-IV), veronicoside, minecoside, 6-feruloyl-catalpol, catalpol and aucubin (Stuppner and Wagner 1989; Li et al. 1998; Mandal and Mukhopadhyay 2004; Huang et al. 2006; Bhandari et al. 2010; Kumar et al. 2013; Patil et al. 2013; Sah and Varshney 2013) (Fig. 3). These iridoid glycosides demonstrate an assortment of bioactivities such as antiinflammatory, antitumor, antioxidative, antiasthma, choleretic, immunostimulatory and hepatoprotective which are used to treat the liver disorders (Viljoen et al. 2012). Among these iridoid glycosides, 'Kutkin' also known as 'Picroliv' obtained from 3 to 4 years old roots and rhizomes of *Picrorhiza kurroa*, was launched as herbal drug formulation which contained 60 % of P-I and Kutkoside in 1:1.5 ratio (Dwivedi et al. 1992; Verma et al. 2009). Moreover, other drug formulations containing P-I and P-II are: Katuki (1.29 % P-I and 1.16 % P-II); Arogya (1.01 % P-I and 0.55 % P-II); Livocare (4.17 % P-I and 3.25 % P-II) and Livplus (0.07 % P-I and 0.01 % P-II) (Pandit et al. 2013a). Mainly the roots and rhizomes tissues of *Picrorhiza* species are used for the herbal drug formulations. In *P. kurroa*, shoots and roots accumulate P-I and P-II, respectively whereas rhizomes accumulate both P-I and P-II (Pandit et al. 2013a).

So, keeping in view the diverse therapeutic potential of *Picrorhiza* genus and threat to its extinction; the need to completely dig out the biosynthetic route and therapeutic activities of its active constituent's demands attention. In this review, we have summarized the iridoid glycosides from *Picrorhiza* genus, including their biosynthesis and pharmacological activities which were the focal point to describe.

Biosynthesis of iridoid glycosides

Picrosides have attracted prime attention among all the iridoid glycosides present in *Picrorhiza* genus and thus, it is necessary to understand how they are synthesized in nature. Picrosides are monoterpenes (C10) attached with a glucose molecule. The biosynthesis of picrosides proceeds via geranyl pyrophosphate (GPP), which is a monoterpene synthesized by the fusion of active isoprene units i.e. isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP), the two universal C5 building blocks. The production of these building blocks can be achieved by two routes: the mevalonate pathway (MVA) and the methylerythritol phosphate (MEP) pathway (Laule et al. 2003).

The mevalonate pathway, first discovered in 1950s, consists of six steps that start from acetyl CoA and proceeds through the intermediate mevalonic acid to produce IPP which is isomerised to DMAPP in the presence of an IPP isomerase (Lange et al. 2000). On the other hand, an alternative route to IPP i.e. MEP pathway was discovered in 1990s (Rohmer et al. 1993), that starts from the condensation of glyceraldehyde-3-phosphate and pyruvate to produce DXP as the first intermediate. The key evidence for the involvement of MVA and MEP pathways in biosynthesis of picrosides was provided by the gene expression analyses performed under differential conditions of picrosides accumulation in *P. kurroa* (Pandit et al. 2013b). Moreover, the gene expression patterns *vis-à-vis* P-I content were also observed in *P. kurroa* shoots at

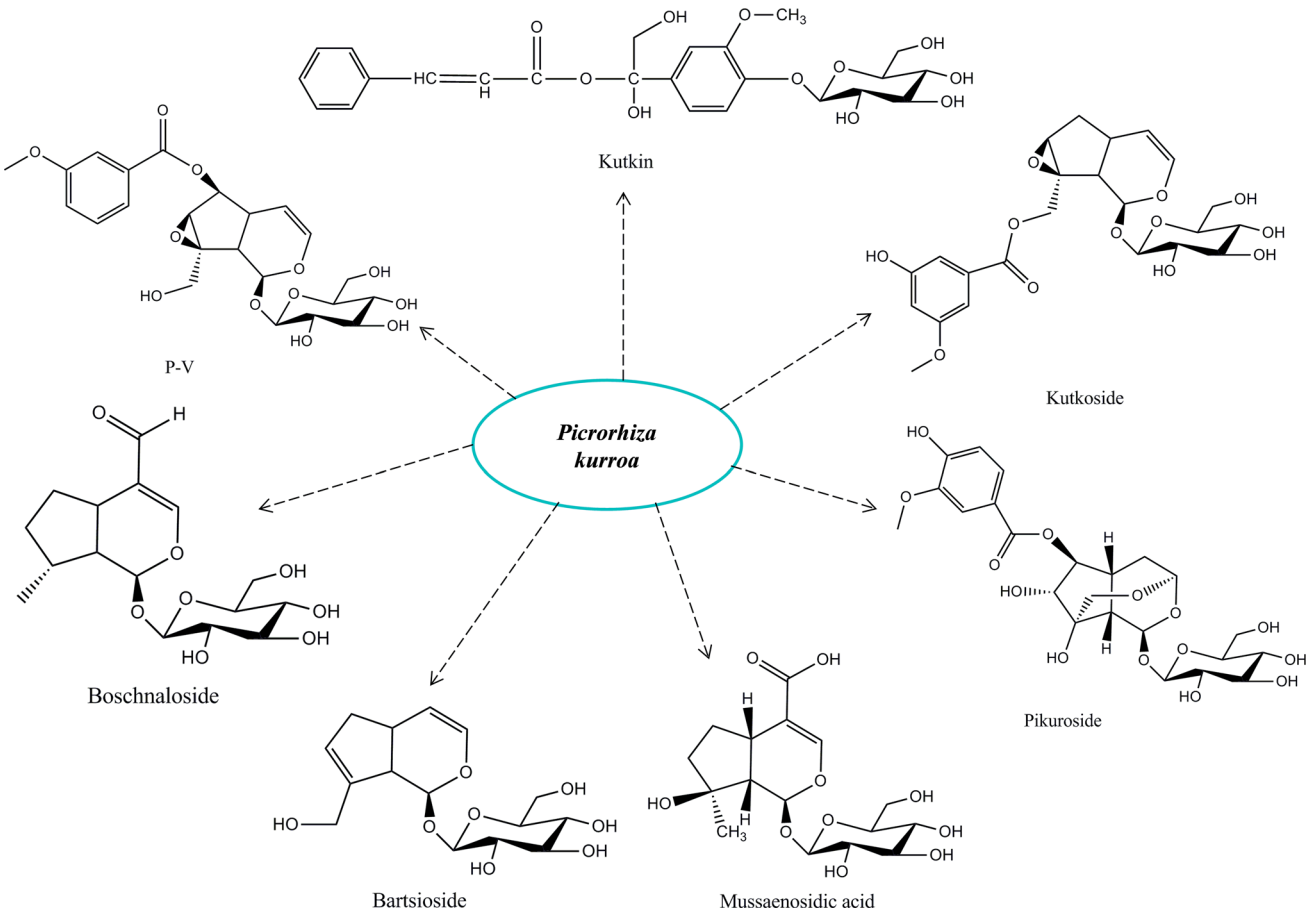


Fig. 1 Chemical structure of iridoid glycosides present in *P. kurroa*

different time intervals of plant growth at 15 °C under tissue culture conditions. The study revealed contribution of MVA/MEP pathway genes (DXPS, ISPD, HMGR and PMK) for the first 20 days of plant growth with the major role of MEP pathway as compared to the MVA pathway. Conversely, geraniol-10-hydroxylase (G10H) and DAHPS contributed to P-I biosynthesis at 30 days of plant growth (Kumar et al. 2015b). Further, application of hydrogen peroxide and abscisic acid up-regulated the genes, *PkDXS*, *PkHDR*, *PkCOMT* and *PkIPPI* in congruence with picrosides accumulation in *P. kurroa* (Singh et al. 2013). These analyses provide clues that up-regulation of above mentioned genes might elevate the levels of GPP but they did not positively identify the activity of enzymes which are being encoded by these genes. Functional validation of above selected genes by either silencing or overexpression can make a strong contribution towards elucidating their functions in picrosides biosynthesis.

The picrosides biosynthesis beyond GPP has not been yet fully elucidated. Recently, a pathway for picrosides biosynthesis has been proposed in *P. kurroa* which reported that all picrosides viz. P-I, P-II, P-III, P-IV, verminoside and specioside are derived from esterification of catalpol produced via Iridoid terpene biosynthetic route and aromatic acids from shikimate/phenylpropanoid pathway as shown in Fig. 4. The detection of intermediate metabolites of catalpol biosynthetic pathway (boschnaloside, bartsioside, mussaenosidic acid, aucubin and catalpol) in *P. kurroa* using LC/ESI–MS/MS method indicated that the picrosides biosynthesis proceeds via the proposed biosynthetic route (Kumar et al. 2013). Nevertheless, enzymes involved in the picrosides biosynthesis are still largely unresolved. The biosynthesis of picrosides initially involves geraniol synthase (GES) which catalyses the dephosphorylation of GPP to form geraniol. The geraniol is then hydroxylated to form 10-hydroxygeraniol by G10H. This NADPH-dependent cytochrome P450 has been heterologously expressed in yeast and shown to catalyse the first committed step of iridoid terpene biosynthesis in *Catharanthus roseus* (Collu et al. 2001). A reversible dehydrogenation and subsequent oxidation of 10-hydroxygeraniol leads to formation of 10-oxogeranial by 10-hydroxygeraniol oxidoreductase. The reduction of 10-oxogeranial in a NADH/NADPH dependent manner with a subsequent cyclization step leads to formation of iridodial by iridoid synthase (Geu-Flores et al. 2012). The

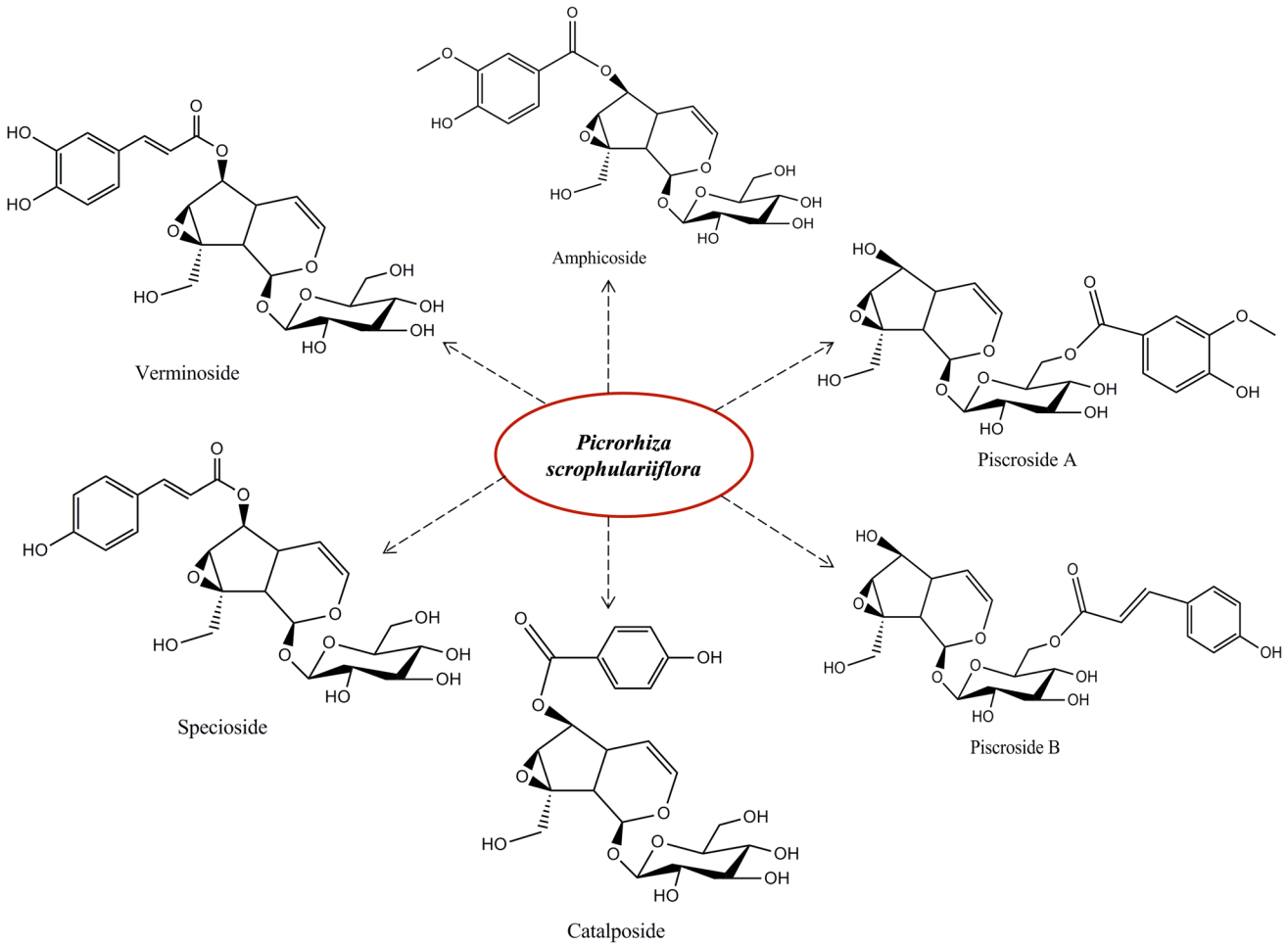


Fig. 2 Chemical structure of iridoid glycosides present in *P. scrophulariiflora*

next enzyme, which is responsible for constructing the iridotrial and 7-deoxyloganetic acid from an iridodial intermediate, is an iridoid oxidase. Miettinen et al. (2014) functionally characterized iridoid oxidase from *C. roseus* and reconstituted it in *Nicotiana benthamiana* which revealed its essentiality for iridoid biosynthetic pathway. A glucosylation step follows the formation of iridotrial and 7-deoxyloganetic acid which is catalysed by UDP-glucosyltransferase. Bhat et al. (2013) identified an UDP-glucosyltransferase (UGT94F2) which showed positive relationship with picroside content in different tissues of *P. kurroa* upon elicitor treatment and seemed to be involved in the conversion of 7-deoxyloganetin to epi-deoxyloganic acid. This investigation adds complication in picrosides biosynthesis by providing an alternative route to the biosynthesis of picrosides which involves 7-deoxyloganetin instead of boschnaloside as an intermediate in the formation of epi-deoxyloganic acid. The correct identity of the step involved or the possibility of involvement of both routes in the biosynthesis of picrosides must be fed with more evidences. However, docking of enzymes with various substrates helps in the prediction of preferred substrate for the enzyme, albeit does not guarantee that the substrate has been correctly identified. Thus, a meticulous analysis of the above enzyme which appears to be an UDP-glucosyltransferase is needed as it is still not well characterized at the enzymatic level. An enzymatic reaction can be considered to be 'proven' only when the enzyme responsible for the catalysis of the given step catalyses the desired reaction (Fridman and Pichersky 2005).

The next steps of picrosides biosynthesis involve hydroxylation, dehydration, decarboxylation, epoxidation and esterification reactions (Fig. 4). These steps are still not characterized at enzymatic level. On the basis of biochemical reactions, it could be hypothesised that cytochrome p450s along with decarboxylase, dehydratase and acyltransferase might catalyse the desired steps. The tedious purification of cytochrome p450s from crude plant extracts to homogeneity followed by protein sequencing requires standardized enzymatic assay which would be hampered by very low levels of secondary metabolic enzymes which are even five to six order lower in

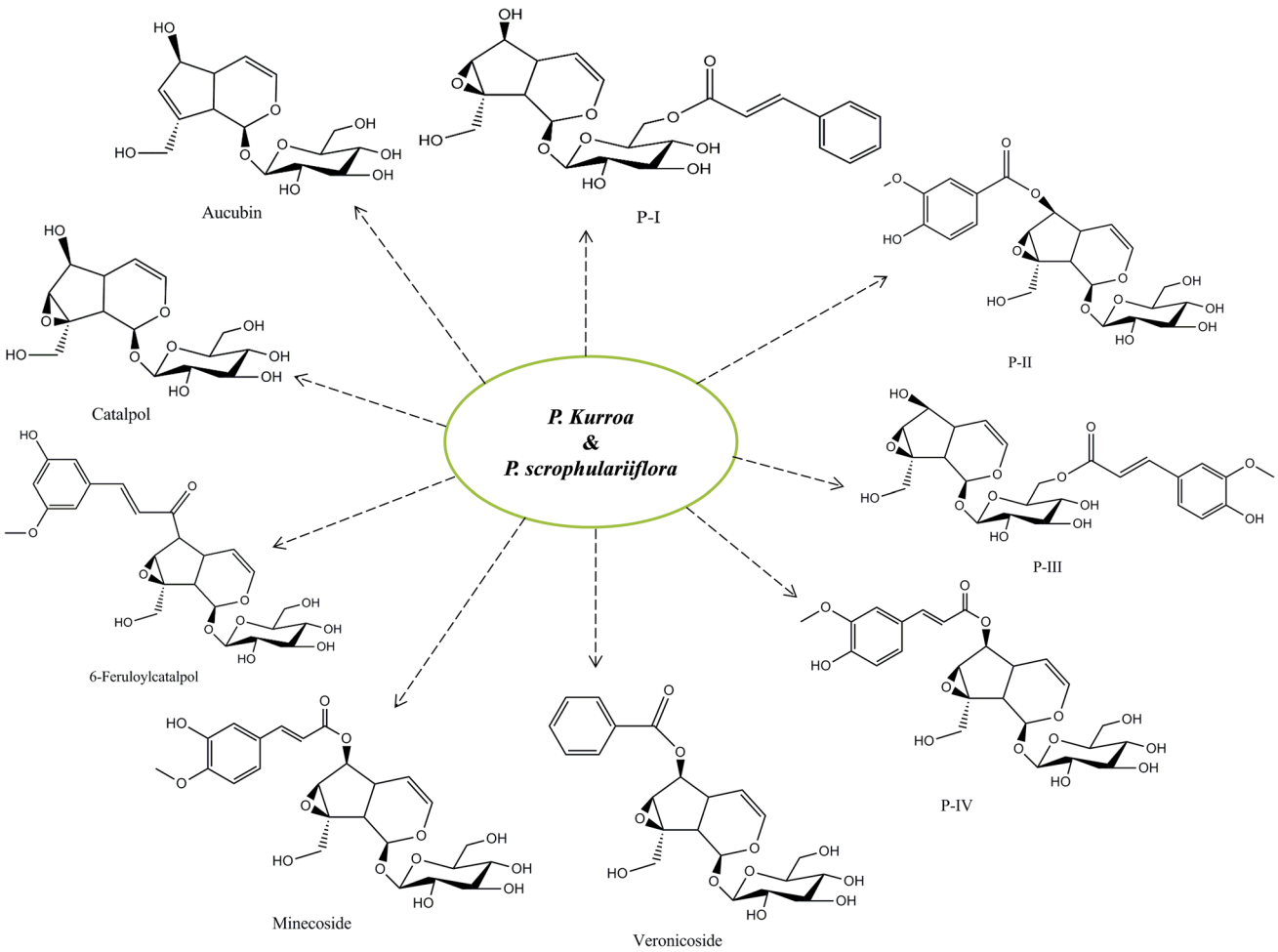


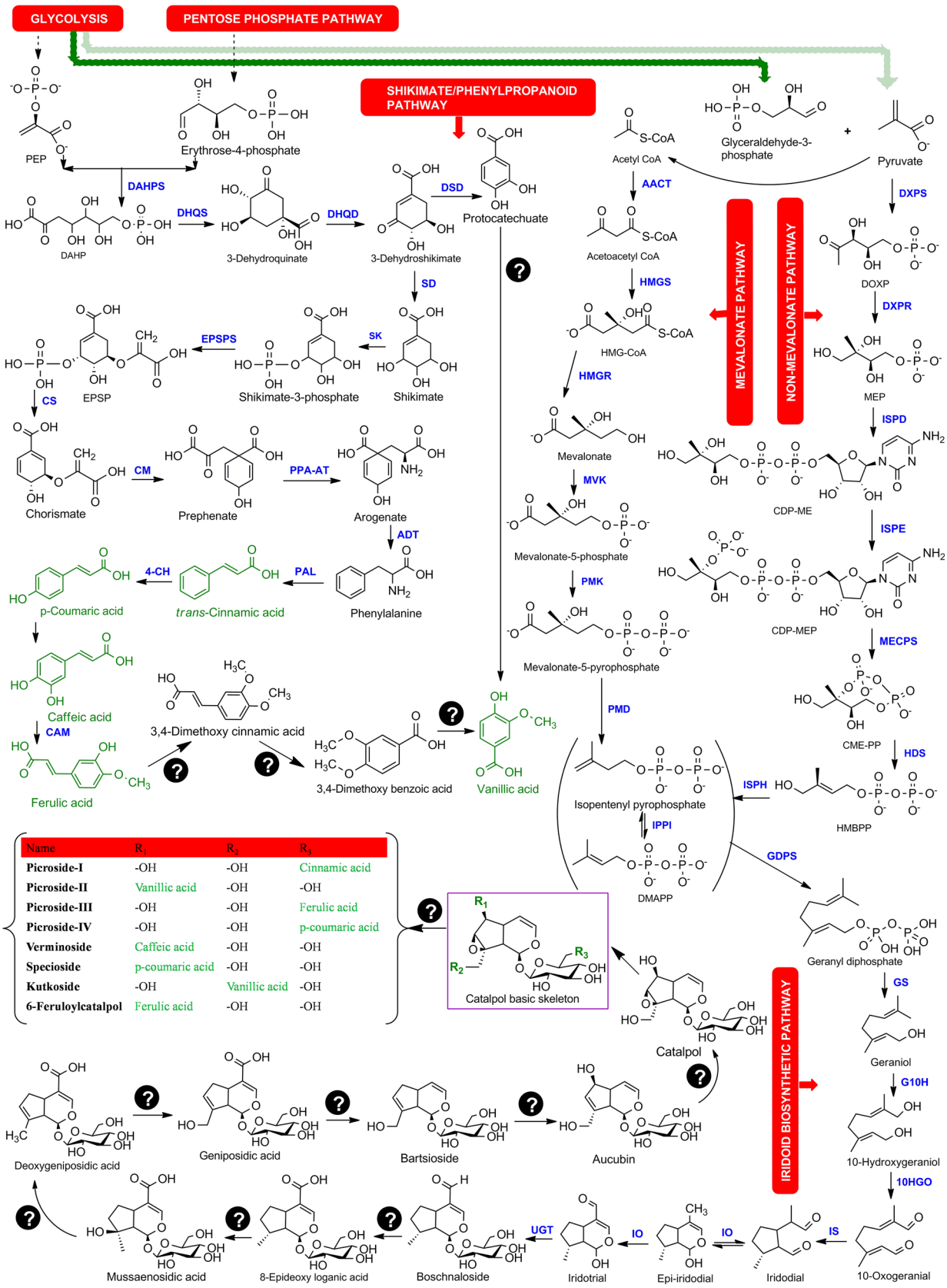
Fig. 3 Chemical structure of iridoid glycosides present in both *P. kurroa* and *P. scrophulariiflora*

magnitude as compared to their respective substrates in planta (Weitzel and Simonsen 2015). Further, accessibility to the intermediates of picrosides biosynthetic pathway is limited. Some of the intermediate metabolites are available but others require chemical synthesis which can be costly and laborious.

To circumvent this problem, current research efforts for the identification of missing enzymes have been focused on the use of transcriptomic data to isolate full length cDNAs by random approach and heterologously expressed in *Escherichia coli* or yeast to reveal their substrate specificity. Bhat et al. (2014) heterologously expressed a NADPH-cytochrome P450 reductase in *E. coli*, which was isolated from *P. kurroa* and dedicated this enzyme to picroside biosynthesis due to its positive correlation with picroside content in samples collected at varied altitudes. Cytochrome P450 reductases are known to serve as electron donor to cytochrome P450s which are supposed to be major enzymes that catalyze the picroside biosynthesis in *P. kurroa*.

This approach seems to be more efficient in the identification of missing enzymes in biosynthetic pathways but must be ensured with in depth biochemical characterization of all the isolated enzymes.

Considerable efforts have also been focused on the identification of proteins involved in picrosides biosynthesis in *P. kurroa* by using differential proteomics study under metabolite accumulating and non-accumulating conditions. Sud et al. (2013, 2014) identified eight differentially expressed proteins in *P. kurroa* shoot samples while revealed 21 differentially expressed proteins from stolon and roots of *P. kurroa* using MALDI-TOF MS followed by MASCOT database search. This subject still needs further attention and could be supplemented with temporal patterns of proteins expression *vis-à-vis* target metabolite accumulation in order to give a strong indication of involvement of these genes in picrosides biosynthesis. Additionally, the researchers would also endeavour different subcellular organelle extracts in order to identify the different cytochrome P450s in *P. kurroa*. Owing to the


GLYCOLYSIS
PENTOSE PHOSPHATE PATHWAY
SHIKIMATE/PHENYLPROPANOID PATHWAY
PEP
Erythrose-4-phosphate
DAHPS
DAHP
DHQS
3-Dehydroquinate
DHQD
3-Dehydroshikimate
DSD
Protocatechuate
SD
Shikimate
SK
Shikimate-3-phosphate
EPSPS
EPSP
CS
Chorismate
CM
Prephenate
PPA-AT
Arogenate
ADT
Phenylalanine
PAL
trans-Cinnamic acid
4-CH
p-Coumaric acid
Caffeic acid
CAM
Ferulic acid
3,4-Dimethoxy cinnamic acid
3,4-Dimethoxy benzoic acid
Vanillic acid
Acetyl CoA
AACT
Acetoacetyl CoA
HMGS
HMG-CoA
HMGR
Mevalonate
MVK
Mevalonate-5-phosphate
PMK
Mevalonate-5-pyrophosphate
PMD
Glyceraldehyde-3-phosphate
Pyruvate
DXPS
DOXP
DXPR
MEP
MEVALONATE PATHWAY
NON-MEVALONATE PATHWAY
ISPD
CDP-ME
ISPE
CDP-MEP
MECPS
CME-PP
HDS
HMBPP
ISPH
Isopentenyl pyrophosphate
IPPI
DMAPP
GDPS
Geranyl diphosphate
GS
Geraniol
G10H
10-Hydroxygeraniol
10HGO
10-Oxogeranial
IRIDOID BIOSYNTHETIC PATHWAY
IS
Iridodial
IO
Epi-iridodial
IO
Iridotrial
UGT
Boschnaloside
8-Epideoxy loganic acid
Mussaenosidic acid
Deoxygeniposidic acid
Geniposidic acid
Bartsioside
Aucubin
Catalpol
Catalpol basic skeleton
Name R1 R2 R3
Picroside-I -OH -OH Cinnamic acid
Picroside-II Vanillic acid -OH -OH
Picroside-III -OH -OH Ferulic acid
Picroside-IV -OH -OH p-coumaric acid
Verminoside Caffeic acid -OH -OH
Specioside p-coumaric acid -OH -OH
Kutkoside -OH Vanillic acid -OH
6-Feruloylcatalpol Ferulic acid -OH -OH

◀**Fig. 4** Metabolic pathway showing biosynthesis of iridoid glycosides in *P. kurroa*. The metabolic network was reconstructed including mevalonate/non-mevalonate, shikimate/phenylpropanoid and iridoid biosynthetic pathways. *Solid* and *dotted arrows* indicate the single and multiple steps, respectively. *Question marks* indicate enzymatic steps with no available information

limited database of plant proteins along with the low sequence similarity of cytochrome P450s, searching of strictly conserved regions for cytochrome P450s in the hypothetical proteins i.e. the four-helix form (D, E, I and L), could provide further elucidation of cytochrome P450s in *P. kurroa* (Weitzel and Simonsen 2015). Further, integration of proteomics with transcriptomics could lead to a rapid elucidation of cytochrome P450s involved in the biosynthesis of picrosides but it must be clear that only enzymatic activity can unambiguously assign the function to protein. Interestingly, the effect of primary metabolism on the accumulation of secondary metabolites was observed by correlating the catalytic activities of the rate limiting enzymes of primary metabolic pathways viz. hexokinase, pyruvate kinase, isocitrate dehydrogenase, malate dehydrogenase and NADP-malic enzyme with their gene expression under differential conditions of picrosides accumulation in *P. kurroa* (Kumar et al. 2016). This provides broader targets for metabolic engineering in order to attain enhanced secondary metabolite production.

In summary, the enzymes for six of the fifteen steps involved in the biosynthesis of picrosides in *P. kurroa*, have been identified. Despite this impressive progress in the elucidation of biosynthetic pathway for picrosides in *P. kurroa*, a lot of work is still required to identify the missing enzymes along with the key enzymes involved in picrosides biosynthesis in order to control the flux of intermediates through the pathway that will eventually benefit the biotechnological production of picrosides.

Pharmacological activities of iridoid glycosides

In this section, we have presented an exhaustive compilation of the pharmacological properties of *Picrorhiza* extract along with its all iridoid glycosides in order to look into their therapeutic potential (Table 1).

Picrorhiza plant extracts

Picrorhiza genus has been used in the treatment of various disorders as recorded by a wide range of bioactivities. The dried rhizome of *P. scrophulariiflora* was traditionally used to remove damp-heat and to relieve consumptive fever (Pharmacopeia 1995). Further, the extract of *P. kurroa* rhizome at a dose of 20 mg/kg body weight exhibited antioxidant properties which increased the rate of gastric ulcer healing in rats (Ray et al. 2002). The extract enhanced in vivo free radical scavenging action by restoring the superoxide dismutase and catalase enzyme's activities along with inhibition of lipid peroxidation. A detailed analysis of mode of action revealed that healing of gastric ulcer in rats was also associated with increase in mucus production, prostaglandin E_2 levels, expressions of cyclooxygenase isoforms (COX-1 and COX-2), and growth promoting factors viz. vascular endothelial growth factor (VEGF) and epidermal growth factor, EGF (Banerjee et al. 2008). Further, the root extract of *P. kurroa* at a dose of 200 mg/kg body weight, exhibited cardioprotective effect by restoring myocardial superoxide dismutase, catalase, and glutathione peroxidase enzymes which resulted in the attenuation of isoproterenol-induced oxidative stress (Nandave et al. 2013). Continuing investigation on antioxidant activity of *P. kurroa* extracts revealed that butanol and ethyl acetate extracts were more efficient in scavenging of free radicals as compared to ethanol extract (Kant et al. 2013). On the other hand, Rajkumar et al. (2011) observed that both methanolic and aqueous extracts of *P. kurroa* rhizome exhibited antioxidant potential by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) and inhibiting lipid peroxidation.

Picrorhiza kurroa extract was also evaluated for anti-carcinogenic activity which showed significant inhibition of N-nitrosodiethylamine (NDEA) induced hepatocarcinogenesis by reducing the levels of glutathione S-transferase (GST), aniline hydroxylase (AH) and gamma-glutamyl transpeptidase (GGT) in a dose dependent manner (Jeena et al. 1999). Similarly, administration of *P. kurroa* extract significantly inhibited sarcoma induced by 20-methyl-cholanthrene (20 MC) in mice along with a significant increase in the life span of mice bearing transplanted tumour and thus exhibited both anti-tumour and anticarcinogenic activities (Joy et al. 2000).

The antimalarial potential of *P. kurroa* extracts was observed by significant inhibition of *Plasmodium bergheii* (Singh and Banyal 2011). Further, the examination of *P. scrophulariiflora* extract along with its ten isolated pure compounds, showed that 10 mg/mL concentration of crude extract inhibited 95 % *P. falciparum* 3D7 malaria parasites and the compound 10 solely accounted for the antimalarial activity (IC_{95} value of 23.5 μM) (Wang et al. 2013a, b).

The investigations on antimicrobial potential of *Picrorhiza* revealed that methanolic extract of *P. kurroa* rhizomes exhibited a significant activity against *E. coli*, *B. subtilis* and *S. aureus*, bacterial strains when compared with a standard drug Ciprofloxacin (Sharma and Kumar 2012).

The immunostimulatory activity was observed in experimental animals upon treatment with 50 % ethanolic

Table 1 Pharmacological properties of iridoid glycosides from *Picrorhiza*

S. No.	Bioactivities	Conc. of active compound	Model used	Iridoid glycoside	Reference
1	Anti-leishmanial	10 mg/kg for 12 days, oral	In vivo	Kutkin	Sane et al. 2011
2	Neuritogenic	60 μM	In vitro	P-I; P-II	Li et al. 2000; 2002
3	Anticancer	5 μM and 10 μM	In vitro	P-I; kutkoside; kutkin	Rathee et al. 2013
4	Immunostimulant	10 mg/kg for 7 days, oral	In vivo	Kutkin	Puri et al. 1992
5	Neuroprotective	10 mg/kg, 250 μl intravenously at the end of ischemic 2 h before reperfusion 22 h	In vivo	P-II	Li et al. 2010; Guo et al. 2010
		20 mg/kg, intraperitoneally at ischemia 1.5 h	In vivo		Pei et al. 2012
		10–20 mg/kg, intraperitoneally at ischemia 1.5–2 h	In vivo		Zhao et al. 2014; 2013; Yang et al. 2014
6	Hepatoprotective	5, 10, 20 mg/kg for 7 days, intragastrically	In vivo	P-II	Gao and Zhou 2005a, b
		0.005, 0.01, and 0.02 mmol/L	In vitro		
		12 mg/kg for 7 days, oral	In vivo	Kutkin	Dwivedi et al. 1992
		6 and 12 mg/kg for 7 or 8 days, oral	In vivo		Ansari et al. 1991
		12 mg/kg/day for 15 days, oral	In vivo		Rastogi et al. 1996
		25 mg/kg/day for 15 days, oral	In vivo		Rastogi et al. 2000
		12 mg/kg for 7 days, oral	In vivo	P-I	Dwivedi et al. 1992
		12 mg/kg for 7 days, oral	In vivo	Kutkoside	Dwivedi et al. 1992
7	Protection against hypoxia/ reoxygenation injuries	50–200 μg/ml for 48 h	In vitro	P-II	Meng et al. 2012a; b
		0–50 μg/ml for 24 h	In vitro	Kutkin	Gaddipati et al. 1999
8	Hepatic amoebiasis	12 mg/kg for 7 days, oral	In vivo	Kutkin	Singh et al. 2005
9	Prevention of myocardial ischemia reperfusion injury	1, 10 and 100 μM	In vitro	P-II	Wu et al. 2014
10	Hypolipidaemic	25 mg/kg for 30 days, oral	In vivo	Kutkin	Khanna et al. 1994
11	Anti-allergic and anti-anaphylactic	6.25–25 mg/kg for 4 days, oral	In vivo	Kutkin	Baruah et al. 1998
12	Ulcerative colitis	12.5 mg/kg for 7 days, oral	In vivo	Kutkin	Zhang et al. 2012

extract of *P. kurroa* leaves due to the stimulation of phagocytosis along with cell-mediated and humoral components of the immune system (Sharma et al. 1994). In addition, experimental investigations showed that *P. kurroa* significantly reduced the lipid content, glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT) in liver injury induced by galactosamine in rats. This was also supported by clinical trials which showed the efficiency of *P. kurroa* in viral hepatitis hepatoprotection in animal model (Vaidya et al. 1996). Further findings reported that administration of hydroalcoholic extract of *P. kurroa* for a period of 4 weeks significantly lowered the lipid content (mg/g) of liver and thus, has the potential for treatment of non-alcoholic fatty liver disease (Shetty et al. 2010). Moreover, the ethanolic extract of *P. scrophulariiflora* prevents diabetic nephropathy via inhibition of redox-sensitive inflammation (He et al. 2009).

Picroside II

P-II showed neuroprotective properties by reducing the expressions of Caspase-3 and PARP and thus inhibited the neuronal apoptosis induced by cerebral ischemia reperfusion injury (Li et al. 2010). Further, it was observed that inhibition of neuronal apoptosis was the result of antioxidant and anti-inflammatory role of P-II. The anti-inflammatory mechanism of P-II showed reduced expressions of Toll-like receptor 4 (TLR4), nuclear factor κB (NFκB) and tumor necrosis factor α (TNFα) (Guo et al. 2010). Moreover, the optimization of therapeutic dose and time window of P-II revealed that 20 mg/kg body weight P-II should be injected intraperitoneally at ischemia 1.5 h in treating cerebral ischemic injury (Pei et al. 2012). Further treatment with 10–20 mg/kg body weight P-II at ischemia 1.5 h recorded the reduced levels of free radicals in cerebral ischemic injury along with the reduction in malonaldehyde

content and an improvement in superoxide dismutase activity (Zhang et al. 2013). This suggests that antioxidant potential of P-II could reduce the oxidative damage induced by cerebral ischemic injury. Further, treatment with P-II significantly decreased the concentrations of malondialdehyde, nitric oxide and hydrogen peroxide in brain tissue along with an increase in the activities of superoxide dismutase, glutathione peroxidase (GSHPx) and catalase (Yang et al. 2014). This implied the neuroprotective role of P-II with the optimal therapeutic dose and time window as injecting 10–20 mg/kg intraperitoneally at 1.5–2.0 h. Further, the neuroprotective effect of P-II was also observed due to increased mRNA and protein expression of myelin basic protein (MBP) and arrangement of myelin fibres in order with optimized therapeutic dose and time window (Zhao et al. 2014). Moreover, the optimized therapeutic dose of 10–20 mg/kg body weight P-II at ischemia 1.5–2.0 h was observed for anti-inflammatory effect (Zhao et al. 2013).

P-II was also observed to relieve hepatocyte injuries induced by carbon tetrachloride, D-galactosamine and acetaminophen in mice. Hepatic injury was associated with high levels of alanine aminotransferase and aspartate aminotransferase which were decreased upon administration of P-II. In addition, P-II scavenged free radicals and modulated the balance of liver energy metabolism by increasing ATPase activity in mitochondria and thereby producing hepatoprotective effects (Gao and Zhou 2005a). Further investigations revealed the role of *bcl-2* and *bax* genes in hepatocytes protection against apoptosis. The study showed up-regulation of *bcl-2* and *bcl-2/bax* ratio on administration of 10 mg/kg P-II in a dose dependent manner (Gao and Zhou 2005b).

Liu et al. (2007) observed that P-II along with nerve growth factor (NGF) (2 ng/mL) significantly reduced reactive oxygen species (ROS) levels, leading to the synergistic protective effect in vitro on PC12 cells against hydrogen peroxide induced oxidative stress. Furthermore, P-II induced reduction of ROS levels along with enhanced activities of antioxidant enzymes (SOD and GSH-Px) and calcium level demonstrated its protective effect against cardiomyocyte injury induced by hypoxia/reoxygenation (Meng et al. 2012a). The P-II treatment increased the expression of *bcl-2* but decreased the expression of *bax* along with the activation of PI3K/Akt and cAMP response element-binding protein (CREB) pathways which inhibited caspase-3 activation and finally attenuated myocyte apoptosis (Meng et al. 2012b). Interestingly, P-II treatment prevents myocardial ischemia reperfusion injury (MIRI) in rats (Wu et al. 2014). It increases the production of nitric oxide mediated by activation of PI3K/Akt/eNOS signalling pathway along with the modulation of *bcl-2* and *bax* genes expression similar to the previous finding of Meng et al. (2012b) in order to mediate anti-apoptotic effect.

Kutkin/picroliv

Picroliv/Kutkin showed promising hepatoprotective activity against hepatotoxic effects induced by paracetamol and galactosamine in adult male albino rats (Ansari et al. 1991). The administration of 12 mg/kg/day picroliv for 7 days protected liver by preventing the biochemical changes in liver and serum of galactosamine-toxicated rats (Dwivedi et al. 1992). Moreover, administration of picroliv along with alcohol for 15 days subsequent to an initial alcohol exposure for 30 days resulted in reduced biochemical parameters in liver and serum of albino rats (Rastogi et al. 1996). Further, picroliv altered biochemical parameters in a dose-dependent manner (36–100 %). Its anticholestatic property results in the prevention of bile flow, reduction in bile salts and bile acids. It also increases the activity of aldehyde dehydrogenase and acetaldehyde dehydrogenase which impairs with acetaldehyde metabolism whose accumulation was mainly responsible for hepatic injury (Saraswat et al. 1999). Further, the hepatoprotective effect of picroliv was revealed against rat hepatotoxicity induced by aflatoxin B1. Picroliv administration resulted in the reversal of increase in tau-glutamyl transpeptidase, 5′-nucleotidase, acid phosphatase and acid ribonuclease activities induced in liver and serum of rats treated with aflatoxin B1 (Rastogi et al. 2000). Further, Singh et al. (2005) revealed prevention of *Entamoeba histolytica* induced hepatic damage by picroliv. It provides protection in the elevated levels of glutamic oxaloacetate transaminase (60.7 %), glutamic pyruvate transaminase (87 %) and alkaline phosphatase (61.37 %); induced by treatment of carbon tetrachloride followed by *E. histolytica* infection. Similarly, the reversal of changes in glutamic oxaloacetate transaminase, glutamic pyruvate transaminase and alkaline phosphatase enzyme activities upon administration of picroliv in dose dependent manner was reported against thioacetamide-induced hepatic damage in the rats (Visen et al. 1991). Further, the efficiency of picroliv has been shown to be comparable with silymarin for preventing galactosamine, paracetamol, thioacetamide and carbon tetrachloride induced hepatic damage (Verma et al. 2009).

The potential of picroliv has also been observed for scavenging oxygen free radicals. Picroliv in addition to P-I and kutkoside, inhibited the xanthine oxidase activity thereby preventing the generation of superoxide anions along with malondialdehyde (MDA) generation in rat liver microsomes (Chander et al. 1992). An investigation of the picroliv potential as immunostimulant revealed that 10 mg/

kg picroliv administration for 7 days showed significant elevation in macrophage migration index, [^{14}C]-glucosamine uptake, chemiluminescence of peritoneal macrophages and higher uptake of [^{3}H]-thymidine in the lymphocytes of treated mice along with providing protection against *Leishmania donovani* infection in golden hamsters (Puri et al. 1992). An enhancement in the inhibition of *L. donovani* parasite was observed when picroliv was administered in combination with antileishmanial drugs viz. paromomycin and miltefosine, which significantly increased the phagocytosis and lymphocyte proliferation responses (Sane et al. 2011).

Picroliv also reduces the serum lipids levels in hyperlipaemia induced by triton WR-1339 and cholesterol. This hypolipidaemic action of picroliv was mediated through cholesterol biosynthesis inhibition along with increase in excretion of faecal bile acid and cholesterol acyltransferase activity (Khanna et al. 1994). Further, picroliv was observed to be associated with augmentation of human T cell response in mycobacterial diseases (Sinha et al. 1998). At a dose of 25 mg/kg p.o., picroliv caused 82 % inhibition of passive cutaneous anaphylaxis in mice and 50–85 % in rats along with the protection of mast cells from degranulation thereby exhibited anti-allergic and anti-anaphylactic properties (Baruah et al. 1998). Picroliv acts as protective agent against injuries induced by hypoxia/reoxygenation by reducing the lactate dehydrogenase release in Hep 3B and Glioma cells. The picroliv treatment inhibited protein tyrosine kinase activity which led to tyrosine dephosphorylation of several proteins along with the reduction in protein kinase C (PKC), indicating the involvement of a novel signal transduction pathway in hypoxia/reoxygenation induced injuries (Gaddipati et al. 1999).

The antioxidant and anti-inflammatory properties of picroliv play a critical role in ulcerative colitis. The administration of picroliv caused subsequent amelioration in disease activity index along with the reduction in enhanced myeloperoxidase activity, MDA concentration, and the IL-1β, TNF-α, and NF-κB p65 expressions in dextran sulfate sodium induced ulcerative colitis in mice (Zhang et al. 2012). Further investigations documented the anticancer potential of picroliv which along with P-I and kutkoside from *P. kurroa* down-regulated MCF-7 cell lines invasion and migration via reduced matrix metalloproteinases (MMPs) expressions (Rathee et al. 2013).

Picroside-I

P-I along with P-II exhibited neuritogenic activity at a concentration of 60 μM in the presence of 2 ng/mL, NGF. This significantly enhanced neurite outgrowth induced by NGF from PC12D cells through amplifying a mitogen-activated protein (MAP) kinase (Li et al. 2000). Further, enhanced neurite outgrowth from PC12D cells was also induced by basic fibroblast growth factor (bFGF), staurosporine and dibutyryl cyclic AMP (dbcAMP) through activation of MAP kinase-dependent signaling pathway upon P-I and P-II treatment (Li et al. 2002).

Catalpol

Catalpol showed anti-leishmanial as well as topoisomerase inhibiting activities (Mandal and Mukhopadhyay 2004). The pharmacological action of catalpol has not been fully established, but it might be involved in stimulation of secondary metabolites production (Kumar et al. 2013).

Conclusions and future directions

The iridoid glycosides derived from *Picrorhiza* genus harbour a wide spectrum of high-value pharmacological potential including antioxidant, anti-inflammatory, antimalarial, antimicrobial, anticancer, anticholestatic and hepatoprotective activities. With so much bioactivity, a meticulous analysis of iridoid glycosides biosynthesis has been observed in *Picrorhiza* genus. The incorporation studies over the years demonstrated the involvement of MEP, MVA, phenylpropanoid and monoterpenoid pathways in biosynthesis of picrosides but the sporadic knowledge of enzymes in the their biosynthesis provenance distinct challenges in pathway analysis. Nevertheless, plant tissue culture has been standardised for picrosides production; however, P-I content is much lower ($\sim$5-fold) and P-II is not detected in tissue cultured plants as compared to plants grown in natural habitats. Thus, metabolic engineering may be exploited to maximize picrosides content in cellular systems by redirecting the carbon flux towards picrosides biosynthesis. Consequently, it is hard to decide the strategy for enhancing the P-I content in *P. kurroa* through metabolic engineering. Hence, the incorporation of remaining enzymes of picroside biosynthetic pathway along with additional constraints such as identification of key steps, metabolic control analysis and thermodynamics would expand the scope of novel drugs development. This will result in the identification of rate limiting enzymes along with the knowledge of mechanisms and regulation of pathway which will benefit the biotechnological production of picrosides.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have any conflict of interest.

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